Effect of β₂-adrenergic receptor polymorphism on response to longacting β₂ agonist in asthma (LARGE trial): a genotype-stratified, randomised, placebo-controlled, crossover trial


Summary

Background Some studies suggest that patients with asthma who are homozygous for arginine at the 16th aminoacid position of the β₂-adrenergic receptor (B16 Arg/Arg) benefit less from treatment with longacting β₂ agonists and inhaled corticosteroids than do those homozygous for glycine (B16 Gly/Gly). We investigated whether there is a genotype-specific response to treatment with a longacting β₂ agonist in combination with inhaled corticosteroid.

Methods In this multicentre, randomised, double-blind, placebo-controlled trial, adult patients with moderate asthma were enrolled in pairs matched for forced expiratory volume in 1 s and ethnic origin, according to whether they had the B16 Arg/Arg (n=42) or B16 Gly/Gly (n=45) genotype. Individuals in a matched pair were randomly assigned by computer-generated randomisation sequence to receive inhaled longacting β₂ agonist (salmeterol 50 μg twice a day) or placebo given in a double-blind, crossover design for two 18-week periods. Open-label inhaled corticosteroid (hydrofluoroalkane beclometasone 240 μg twice a day) was given to all participants during the treatment periods. The primary endpoint was morning peak expiratory flow (PEF). Analysis was by intention to treat. This trial is registered with ClinicalTrials.gov, number NCT00200967.

Findings After 18 weeks of treatment, mean morning PEF in Arg/Arg participants was 21.4 L/min (95% CI 11.8–31.1) higher when participants were assigned to receive salmeterol than when assigned to receive placebo (p<0.0001). In Gly/Gly participants, morning PEF was 21.5 L/min (11.0–32.1) higher when participants were assigned to receive salmeterol than when assigned to receive placebo (p<0.0001). The improvement in PEF did not differ between genotypes (difference [Arg/Arg–Gly/Gly] –0.1, –14.4 to 14.2; p=0.99). In Gly/Gly participants, methacholine PC₂₀ (20% reduction in forced expiratory volume in 1 s; a prespecified secondary outcome) was 2.4 times higher when participants were assigned to salmeterol than when assigned to placebo (p<0.0001). Responsiveness to methacholine did not differ between salmeterol and placebo in Arg/Arg participants (p=0.87). The 2.5 times higher genotype-specific difference in responsiveness to methacholine was significant (1.3 doubling dose difference between genotypes, 0.43–2.21, p=0.0038). Seven Arg/Arg participants (placebo, n=5; salmeterol, n=2) and six Gly/Gly participants (placebo, n=3; salmeterol, n=3) had an asthma exacerbation. Five serious adverse events were reported, one each during the pre-match and run-in phases on open-label inhaled corticosteroid, two during double-blind treatment with salmeterol/inhaled corticosteroid, and one during double-blind treatment with placebo/inhaled corticosteroid. None of the serious events was asthma-related or related to study drugs or procedures.

Interpretation In asthma patients with B16 Arg/Arg and B16 Gly/Gly genotypes, combination treatment with salmeterol and inhaled corticosteroid improved airway function when compared with inhaled corticosteroid therapy alone. These findings suggest that patients should continue to be treated with longacting β₂ agonists plus moderate-dose inhaled corticosteroids irrespective of B16 genotype. Further investigation is needed to establish the importance of the genotype-specific difference in responsiveness to methacholine.

Funding National Institutes of Health.

Introduction Combination therapy with longacting β₂ agonists and inhaled corticosteroids is one of the most widely prescribed treatments for the control of asthma in the world. Some studies suggest that, on average, this combination improves lung function and asthma control, others suggest that a subpopulation of patients with asthma could be at risk for severe exacerbations or death with use of longacting β₂ agonists. β₂ agonists act primarily at the β₂-adrenergic receptor (ADRB2). A common single nucleotide polymorphism in the coding region of ADRB2 codes for arginine
instead of glycine at the 16th aminoacid of the receptor (allele frequency 0·4 in white people).

In retrospective and prospective studies in patients with asthma not taking inhaled corticosteroids, regular use of shortacting β, agonists, such as salbutamol (albuterol), was associated with lower lung function in individuals homozygous for arginine at the 16th aminoacid position (B16 Arg/Arg) than in individuals homozygous for glycine at that position (B16 Gly/Gly). Another study showed increased risk of exacerbations with regular use of salbutamol but not salmeterol in patients with the B16 Arg/Arg genotype.

In view of these genotype-specific findings, we undertook a genotype-stratified retrospective analysis of patients with asthma who had participated in randomised trials of the longacting β, agonist salmeterol. Patients with the B16 Arg/Arg genotype did not benefit from treatment with salmeterol, even when used with a concomitant inhaled corticosteroid. We have therefore examined prospectively whether there is a genotype-specific difference in the response to longacting β, agonists, by undertaking a randomised controlled trial that compared the effects of salmeterol plus inhaled corticosteroid with inhaled corticosteroid alone in B16 Arg/Arg patients with asthma versus B16 Gly/Gly patients with asthma.

Methods

Participants

Seven centres recruited participants for the LARGE trial. Patients with asthma were recruited from the clinical practices of each study site and through community advertising by use of a variety of media. After patients had given written informed consent (approved by participating site institutional review boards), their medical history was reviewed (eg, medication use and history of asthma exacerbations) and they were screened for eligibility on the basis of the inclusion and exclusion criteria shown in the panel. Blood samples were also taken. B16 genotyping by restriction fragment length polymorphism was confirmed by sequencing (see webappendix p 1 for details of genotyping). The study protocol was reviewed and approved by an independent protocol review committee appointed by the study sponsor. A separate data and safety monitoring board, also appointed by the sponsor, oversaw the study and reviewed adverse events as the trial was implemented.

Figure 1 shows the study design. Arg/Arg and Gly/Gly individuals who met the study criteria entered a pool of eligible patients waiting to be matched with a participant with the opposite genotype, stratified by forced expiratory volume in 1 s (FEV1) and ethnic origin. Individuals with the Arg/Gly genotype were excluded because outcomes for such patients had been inconsistent in previous studies and preliminary data with both shortacting and longacting β, agonists were based on differences between individuals with Arg/Arg and Gly/Gly genotypes. Match-eligible participants began treatment with open-label, inhaled corticosteroid (hydrofluoroalkane beclometasone dipropionate [QVAR, Teva, Petach Tikva, Israel] 240 μg twice a day) and salbutamol (as needed), and returned after 3 weeks of treatment for spirometry to establish baseline FEV1 for matching. Participants returned every 4 weeks thereafter for diary review, medication compliance review, spirometry, and safety checks until a match was identified. Pairs of matched participants (ie, an Arg/Arg and a Gly/Gly participant within 10% of predicted FEV1, and of the same ethnic origin [white or non-white]) returned to enter the main study.

Matched participants entered an 8-week run-in period to wash out previous use of longacting β, agonist (which, in previous studies, was seen to last as long as 8 weeks). During this run-in period, participants continued treatment with open-label hydrofluoroalkane beclometasone (240 μg twice a day). Inhaled salbutamol was used...
as rescue therapy. Asthma control was monitored by peak expiratory flow (PEF) via an electronic peak flow meter (AM1 device, Cardinal Health, Yorba Linda, CA, USA), and by spirometric values, morning/evening peak flow variability index, asthma symptoms, and use of rescue therapy. Baseline data were obtained for airway responsiveness (20% reduction in FEV₁ [PC₂₀] in response to methacholine), bronchodilator response to ipratropium, bronchodilator response to salbutamol, exhaled nitric oxide concentration, and pH in exhaled breath condensate.

Randomisation and masking

Individuals in a matched pair were randomly assigned to the same crossover treatment sequence. Randomisation was done by a password-protected, web-based scheme administered by the Asthma Clinical Research Network (ACRN) Data Coordinating Center. A statistician (SJK) at the data coordinating centre wrote the randomisation program in SAS version 9.1. This code was passed to an unmasked database programmer who assigned a new random number generator seed, assigned blinding codes to each of the treatments (ie, placebo or salmeterol), and ran the program to generate the randomisation codes used in the study. The resulting randomisation list was imported into the study database and accessed by the clinic coordinators via a web-based application. The application returned only a drug kit number from which all study drugs were dispensed for the participant who had been randomised. SJK did not have access to the blinding codes during study implementation or analysis. The database programmer was not involved in the rest of the trial other than to provide routine maintenance of the LARGE database.

Clinic personnel, participants, and most data centre personnel (apart from the database programmer and administrative coordinator) remained masked throughout study implementation. Investigators undertaking the data analyses were masked to both genotype and treatment allocation. Success of masking was assessed via questionnaires completed by participants and coordinators every time a participant completed a treatment period. Participants were asked for their guess as to which treatment they had just completed, as well as for any specific sensations they had noted with the study drug (taste, smell, etc). Coordinators were asked for their guess as to which treatment the participant had just completed.

Procedures

In each treatment sequence, there was an 18-week double-blind treatment phase in which participants received either inhaled long-acting β₂ agonist (salmeterol 50 μg twice a day; Serevent 50 μg Diskus, GSK, North Carolina, USA) or matching placebo. Additionally, all participants received open-label, inhaled hydrofluoroalkane beclometasone (240 μg twice a day). Inhaled ipratropium bromide (two actuations [puffs] as needed; Atrovent, Boehringer Ingelheim, Ridgefield, CT, USA) was used as primary rescue therapy to avoid the confounding effects of β₂-adrenergic stimulation on outcome variables. However, if an episode of adverse asthma control responded incompletely to ipratropium, salbutamol was used as a superseding rescue therapy. Patients received the alternative double-blind treatment for the second treatment period.

At the end of each double-blind treatment period, all participants resumed regular use of hydrofluoroalkane beclometasone, with salbutamol (as needed), for a run-out period (figure 1). During the two treatment periods and two run-out periods, asthma control was monitored by the same indicators as in the run-in stage. Although the second run-out period lasted 10 weeks (as opposed to 8 weeks for the first run-out period) to assess whether any potential genotype-specific effects would be seen up to 10 weeks, run-out measurements were taken at the

Figure 1: Schematic diagram of the trial protocol

After screening and genotyping, genotype-eligible and matched participants who received 8 weeks of inhaled corticosteroid (ICS; hydrofluoroalkane beclometasone dipropionate 240 μg twice a day) during the run-in period were randomly assigned to continue ICS with either salmeterol or placebo for 18 weeks, followed by an 8-week run-out period on ICS alone, followed by the alternative treatment and a 10-week run-out period. prn=as needed.
end of 8 weeks and compared with the end of the first 8-week run-out period.

Participant adherence with medication dosing was determined by use of a DOSER device (Meditrack Products; Hudson, MA, USA) attached to each beclometasone metered-dose inhaler (MDI). This device registers each actuation of the MDI and stores a daily history, which was reviewed at each clinic visit. Diskus inhalation counters were used to determine the number of inhalations of salmeterol or placebo. These two devices gave objective measurements of the number of puffs or doses actuated.

Diary cards, on which participants recorded the number of puffs of each drug per day, were used as a secondary source of compliance information. These data were compared with PEF measurements electronically recorded and date/time stamped from the electronic peak flow meter device. Because participants were instructed to take their morning and evening PEF measurements immediately before taking their study drugs, timing of PEF monitoring was used as a surrogate for timing of dosing with study drugs.

The primary endpoint of the study, assessed separately within and between genotypes, was morning PEF at the end of 18-week treatment. Secondary endpoints included evening PEF, peak flow variability (evening PEF–morning PEF), FEV1, airway responsiveness (methacholine PC20), bronchodilator reversibility with four puffs of either ipratropium or salbutamol, exhaled nitric oxide concentration, and pH in exhaled breath condensate, as described elsewhere. Genotype-specific outcomes were examined after stratification by sex and ethnic origin. The asthma control variables analysed were mean daily symptom scores (measured on a scale from 0 [absent] to 3 [severe]), number of actuations of rescue MDI (ipratropium, salbutamol, and the combined total during treatment periods), number of exacerbations, and episodes of adverse asthma control. Variables measured daily from the participant diary cards—eg, PEF and PEF variability were averaged between visits and weighted by the inverse of the squared SE. The purpose of the weighting scheme for diary card data was to assign greater weight to means measured with low variability and less weight to means measured with high variability.

Statistical analysis
With the SD of the primary comparison of interest (ie, comparing the estimate at the end of the placebo treatment period with the estimate at the end of the salmeterol treatment period) with respect to morning PEF from the ACRN BARGE trial (24 L/min), we calculated that a sample size of 24 participants per genotype was required to detect a difference of 25 L/min between Arg/Arg and Gly/Gly with a two-sided, 0·05 significance level test, 90% statistical power, and accounting for a 15% dropout rate. However, to attain

---

**Figure 2: Trial profile**
90% statistical power for the secondary outcome variable of FEV₁, a sample size of 40 participants per genotype (80 total randomised participants) was required for an effect size of 0·15 L with an estimated SD of 0·19 L. With a sample size of 40 participants per genotype, the actual effect size for detecting between-genotype differences with respect to end-of-treatment morning PEF was 15 L/min, and the effect sizes for detecting treatment regimen differences within each genotype were 13·3 L/min for morning PEF and 0·13 L for FEV₁.

Means (SDs) were calculated as descriptive statistics for baseline variables, apart from those variables with skewed distributions, for which medians or geometric means (IQR) were calculated. Paired \( t \) tests, Wilcoxon signed rank tests, and McNemar tests were applied, corresponding with the type of descriptive statistic, to compare genotypic groups with respect to baseline variables while accounting for matched pairs. Because of repeated measurements of primary and secondary response variables over time, we used longitudinal data analysis, incorporating all data from study participants.

For the primary outcome variable (morning PEF) and several of the secondary outcomes, we invoked a mixed-effects linear model that included an intercept and three slopes for the first treatment period (study weeks 8–10, weeks 10–14, and weeks 14–26) and an intercept and three slopes for the second treatment period (study weeks 34–36, weeks 36–40, and weeks 40–52). Because we were mainly interested in the genotypic-stratified response to 18 weeks of treatment with salmeterol versus placebo, data from run-in and run-out periods were not included because treatment altered substantially when participants went from run-in to treatment period and from treatment to run-out period. Inclusion of either of these phases could artificially alter the longitudinal model, and not reflect true treatment-related effects. Model parameters were estimated separately for each genotype and randomised drug sequence (ie, placebo followed by salmeterol or salmeterol followed by placebo). Outcome variables based on daily diary records were averaged over all of the days between clinic visits before data analysis. For most outcome variables, values were estimated from the model for the beginning and end of each treatment period, and appropriate contrasts comparing treatment regimens were calculated. Run-out data were modelled separately, fitting an intercept and one slope for the first run-out (study weeks 26–34) and an intercept and one slope for the second run-out period (study weeks 52–62). Different model specifications were required for secondary outcome variables that were not obtained at all study visits (eg, methacholine PC₂₀ was obtained only at the beginning and end of each treatment period, allowing for estimation of only one treatment period slope). Use of rescue therapy and symptom score data showed scant variability, and values were generally very small (close to 0). Therefore, these outcome variables were analysed via Wilcoxon signed rank tests and Wilcoxon rank sum tests. Hodges–Lehmann estimates for the median paired difference (within genotype) and median difference (between genotypes) with 95% CIs are presented. See webappendix p 1 for more detailed explanations of these statistical analyses.

## Table 1: Baseline characteristics of randomised participants, by genotype

<table>
<thead>
<tr>
<th>Arg/Arg (n=42)</th>
<th>Gly/Gly (n=45)</th>
<th>( p ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>39 (11)</td>
<td>42 (12)</td>
</tr>
<tr>
<td>Male</td>
<td>10 (24%)</td>
<td>16 (36%)</td>
</tr>
<tr>
<td>Ethnic origin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>27 (64%)</td>
<td>31 (69%)</td>
</tr>
<tr>
<td>African-American</td>
<td>8 (21%)</td>
<td>8 (18%)</td>
</tr>
<tr>
<td>Asian</td>
<td>2 (5%)</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Hispanic</td>
<td>4 (10%)</td>
<td>5 (11%)</td>
</tr>
<tr>
<td>Positive allergy skin test</td>
<td>40 (100%)</td>
<td>40 (89%)</td>
</tr>
<tr>
<td>Morning PEF (L/min)</td>
<td>405 (85)</td>
<td>427 (102)</td>
</tr>
<tr>
<td>Evening PEF (L/min)</td>
<td>403 (87)</td>
<td>430 (102)</td>
</tr>
<tr>
<td>FEV₁ (%)</td>
<td>78·6% (13·4)</td>
<td>79·6% (15·3)</td>
</tr>
<tr>
<td>FVC (L)</td>
<td>3·56 (1·03)</td>
<td>3·69 (0·95)</td>
</tr>
<tr>
<td>FEV₁ reversibility with salbutamol (%)</td>
<td>12·2% (6·3)</td>
<td>8·9% (6·6)</td>
</tr>
<tr>
<td>FEV₁ reversibility with ipratropium (%)</td>
<td>13·0% (7·4)</td>
<td>8·9% (5·7)</td>
</tr>
<tr>
<td>Number of puffs of salbutamol per day</td>
<td>0·15 (0·26)</td>
<td>0·18 (0·22)</td>
</tr>
<tr>
<td>Methacholine PC₂₀ (mg/mL)</td>
<td>2·43 (0·57–9·40)</td>
<td>1·92 (0·67–3·90)</td>
</tr>
<tr>
<td>Exhaled nitric oxide (parts per billion)</td>
<td>16·8 (11·7–28·4)</td>
<td>15·8 (11·3–24·9)</td>
</tr>
<tr>
<td>pH in exhaled breath condensate</td>
<td>8·44 (8·35–8·53)</td>
<td>8·44 (8·25–8·59)</td>
</tr>
<tr>
<td>Number of positive skin tests</td>
<td>3·7 (2·4)</td>
<td>3·7 (2·5)</td>
</tr>
<tr>
<td>IgE (IU/mL)</td>
<td>127 (43–308)</td>
<td>152 (81–282)</td>
</tr>
</tbody>
</table>

Data are n (%), mean (SD), or median (IQR) unless otherwise indicated. Baseline data was obtained during the run-in phase, after at least 3 weeks of standard inhaled corticosteroid therapy. PEF=peak expiratory flow. FEV₁=forced expiratory volume in 1 s. FVC=forced vital capacity. PC₂₀=20% reduction in FEV₁. *Paired \( t \) test. †McNemar test. ||Wilcoxon signed-rank test.
Role of the funding sources
A protocol review committee appointed by the sponsor of the study approved the study design. A data and safety monitoring board appointed by the sponsor reviewed the final manuscript. Neither group had any other role in the study. The companies providing the study drugs had no role in study design, data collection, data analysis, data interpretation, writing of the report, or in the decision to submit the report for publication. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results
Figure 2 shows the trial profile. Between 2004 and 2006, 474 patients were screened for the trial. The B16 alleles were in Hardy–Weinberg equilibrium (p=0.95) in this population. 244 patients had eligible genotypes. Several of these patients (Arg/Arg, n=9; Gly/Gly, n=42) withdrew consent after screening because no appropriate match was identified and they no longer wanted to participate in the pre-match protocol. 42 participants with the B16 Arg/Arg genotype and 45 with the B16 Gly/Gly genotype were randomly assigned to study treatment.

Table 1 shows the demographic, clinical, and physiological characteristics of randomised participants at enrolment in the run-in period of the main study (after at least 3 weeks of standard inhaled corticosteroid therapy) by genotype. Matching participants by lung function was successful. Arg/Arg and Gly/Gly participants did not differ significantly at the beginning of each treatment period with respect to any measured baseline characteristic (data not shown).

Of 1910 scheduled main study visits, 1901 (99.5%) were completed. During the double-blind treatment periods, participants recorded their morning PEF on a median of 95.7% (IQR 90.1–98.4) of days. Based on data from the DOSER and the Diskus devices, participants took 95.1% (90.1–97.9) of their scheduled inhaled corticosteroid puffs and 94.9% (88.2–98.6) of their scheduled salmeterol or placebo puffs.

After 18 weeks, mean morning PEF in Arg/Arg participants was 21.4 L/min (95% CI 11.8–31.1) greater when participants were assigned to receive salmeterol than when assigned to receive placebo (p=0.0001; table 2). Similarly, in Gly/Gly participants, morning PEF was 21.5 L/min (11.0–32.1) greater when participants were assigned to receive salmeterol than when they were assigned to receive placebo (p=0.0001). The difference between genotypes (the effect of treatment in Arg/Arg participants minus that in Gly/Gly participants) was –0.1 (–14.4 to 14.2; p=0.99; figure 3). Correspondingly, morning PEF in the run-out periods following treatment with salmeterol or placebo did not differ significantly (data not shown).

Table 2 shows the analyses of the prespecified secondary outcomes. There were within-genotype differences between the salmeterol and placebo treatments with respect to evening PEF, peak flow

<table>
<thead>
<tr>
<th>Morning PEF (L/min)*</th>
<th>Placebo</th>
<th>Salmeterol</th>
<th>Difference (95% CI)</th>
<th>p value</th>
<th>Placebo</th>
<th>Salmeterol</th>
<th>Difference (95% CI)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arg/Arg</td>
<td>401</td>
<td>423</td>
<td>21.4 (11.8 to 31.1)</td>
<td>&lt;0.0001</td>
<td>405</td>
<td>426</td>
<td>21.5 (11.0 to 32.1)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Gly/Gly</td>
<td>394</td>
<td>420</td>
<td>25.2 (16.4 to 34.1)</td>
<td>&lt;0.0001</td>
<td>400</td>
<td>424</td>
<td>23.8 (18.1 to 33.6)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Peak flow variability*</td>
<td>–0.9</td>
<td>–1.0</td>
<td>–0.2 (–1.0 to 0.6)</td>
<td>0.67</td>
<td>0.0</td>
<td>–0.7</td>
<td>–0.7 (–4.4 to –0.0)</td>
<td>0.04</td>
</tr>
<tr>
<td>FEV1 (L)*</td>
<td>2.52</td>
<td>2.60</td>
<td>0.08 (0.03 to 0.13)</td>
<td>0.0022</td>
<td>2.65</td>
<td>2.69</td>
<td>0.04 (–0.00 to 0.09)</td>
<td>0.06</td>
</tr>
<tr>
<td>FVC (L)*</td>
<td>3.57</td>
<td>3.61</td>
<td>0.04 (–0.01 to 0.08)</td>
<td>0.12</td>
<td>3.67</td>
<td>3.71</td>
<td>0.03 (–0.02 to 0.09)</td>
<td>0.29</td>
</tr>
<tr>
<td>Spirometry PEF (L/min)*</td>
<td>409</td>
<td>426</td>
<td>17.2 (7.6 to 26.7)</td>
<td>0.0005</td>
<td>429</td>
<td>446</td>
<td>16.5 (5.8 to 27.3)</td>
<td>0.0027</td>
</tr>
<tr>
<td>Exhaled NO (parts per billion)*</td>
<td>2.68</td>
<td>2.56</td>
<td>–0.12 (–0.24 to 0.00)</td>
<td>0.06</td>
<td>2.70</td>
<td>2.72</td>
<td>0.02 (–0.11 to 0.35)</td>
<td>0.76</td>
</tr>
<tr>
<td>pH in exhaled breath condensate†</td>
<td>7.97</td>
<td>8.07</td>
<td>0.10 (–0.26 to 0.45)</td>
<td>0.60</td>
<td>8.13</td>
<td>8.16</td>
<td>0.03 (–0.31 to 0.37)</td>
<td>0.87</td>
</tr>
<tr>
<td>Methacholine PC20 (log2 mg/mL)‡</td>
<td>1.40</td>
<td>1.35</td>
<td>–0.06 (–0.72 to 0.60)</td>
<td>0.87</td>
<td>1.03</td>
<td>2.30</td>
<td>1.27 (0.66 to 1.87)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>FEV1 reversibility with salbutamol (%)§</td>
<td>10.3%</td>
<td>6.7%</td>
<td>–3.6% (–5.7 to –1.5)</td>
<td>0.0010</td>
<td>10.8%</td>
<td>8.5%</td>
<td>–2.3% (–4.4 to –0.1)</td>
<td>0.04</td>
</tr>
<tr>
<td>FEV1 after salbutamol (L)§</td>
<td>2.82</td>
<td>2.77</td>
<td>–0.04 (–0.10 to 0.01)</td>
<td>0.11</td>
<td>2.93</td>
<td>2.90</td>
<td>–0.03 (–0.08 to 0.03)</td>
<td>0.40</td>
</tr>
<tr>
<td>FEV1 reversibility with ipratropium (%)¶</td>
<td>10.1%</td>
<td>9.4%</td>
<td>–0.8% (–2.9 to 1.4)</td>
<td>0.47</td>
<td>8.4%</td>
<td>7.6%</td>
<td>–0.9% (–2.8 to 1.1)</td>
<td>0.38</td>
</tr>
<tr>
<td>FEV1 after ipratropium (L)¶</td>
<td>2.77</td>
<td>2.82</td>
<td>0.05 (–0.02 to 0.11)</td>
<td>0.14</td>
<td>2.83</td>
<td>2.89</td>
<td>0.05 (–0.01 to 0.12)</td>
<td>0.09</td>
</tr>
<tr>
<td>Daily symptoms</td>
<td></td>
<td></td>
<td>0.06</td>
<td>0.04</td>
<td>–0.04 (–0.12 to 0.00)</td>
<td>0.02</td>
<td>0.01</td>
<td>0.04</td>
</tr>
<tr>
<td>Rescue ipratropium and salbutamol (puffs per day)</td>
<td></td>
<td></td>
<td>0.2</td>
<td>0.0</td>
<td>–0.02 (–0.5 to 0.0)</td>
<td>0.01</td>
<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Data are mean unless otherwise indicated. PEF=peak expiratory flow. FEV1=forced expiratory volume in 1 s. FVC=forced vital capacity. NO=nitric oxide. PC20=20% reduction in FEV1. *Mixed longitudinal model (three-slope treatment period, weeks 0–2, 2–6, 6–18) without run-in or run-outs. †Mixed longitudinal model (two-slope treatment period, weeks 0–10, 10–18) without run-in or run-outs. §Mixed longitudinal model (one-slope treatment period, weeks 0–18) without run-in or run-outs. ¶Mixed longitudinal model (one-slope treatment period, weeks 0–10) without run-in or run-outs. ||Non-parametric analysis.

Table 2: Outcomes after 18 weeks of treatment with inhaled corticosteroid plus placebo or salmeterol in B16 Arg/Arg and B16 Gly/Gly participants.

www.thelancet.com Vol 374 November 21, 2009 1759
variability, FEV₁, FEV₁ reversibility with salbutamol, asthma symptoms, use of rescue therapy, and responsiveness to methacholine (table 2).

In Gly/Gly participants, methacholine PC₂₀ was 2.4 times higher when participants were assigned to salmeterol than when assigned to placebo (p<0.0001); however, in Arg/Arg participants, methacholine PC₂₀ did not differ between treatments (p=0.87; figure 4). The 2.5 times higher genotype-specific difference in responsiveness to methacholine was significant (1.32 doubling dose difference between genotypes, 95% CI 0.43–2.21, p=0.0038; table 2); plots of individual participants are shown in figure 4 (and webappendix p 3). There were no genotype-specific differences for any other outcomes.

Both genotype groups had a fairly high degree of FEV₁ reversibility to four puffs of ipratropium (7.6–10.1%) and to four puffs of salbutamol (6.7–10.8%; table 2). There was no genotype-specific advantage for one bronchodilator over another. However, independent of genotype, the degree of bronchodilation with salbutamol was significantly higher when participants were assigned to receive placebo than when assigned to receive salmeterol (Arg/Arg, p=0.001 and Gly/Gly, p=0.04; table 2). For both genotypes, response to ipratropium was similar for salmeterol and placebo treatments.

Seven Arg/Arg participants (placebo, n=5; salmeterol, n=2) and six Gly/Gly participants (placebo, n=3; salmeterol, n=3) had an exacerbation of asthma. No genotype-specific differences in exacerbation rates were seen. Five serious adverse events were reported: severe postoperative pain after loop electrosurgical excision procedure/cone biopsy for cervical dysplasia needing hospital stay (pre-match inhaled corticosteroid phase); hospital admission after scheduled outpatient surgery for repair of umbilical hernia (run-in inhaled corticosteroid phase); hospital admission for cellulitis of the right leg (double-blind treatment with salmeterol/inhaled corticosteroid); hospital admission for bipolar disorder mood event (manic episode during double-blind treatment with salmeterol/inhaled corticosteroid); and hospital admission for vaginal hysterectomy for treatment of abdominopelvic pain secondary to uterine fibroids (double-blind treatment with placebo/inhaled corticosteroid). None of the serious events was asthma-related or related to study drugs or procedures. All participants recovered. The most prevalent non-serious adverse events reported were expected and respiratory in nature, with 94 events occurring during the pre-match/run-in/run-out phases of the study, 74 occurring during double-blind treatment with placebo/inhaled corticosteroid, and 57 occurring during double-blind treatment with salmeterol/inhaled corticosteroid. Most events were acute nasopharyngitis and acute pharyngitis.

Results in white participants alone mirrored those of the entire study population. However, an exploratory post-hoc subgroup analysis in African-Americans showed that in the eight Gly/Gly participants, mean morning and evening PEF were 29 L/min and 45 L/min higher when participants were assigned to salmeterol than when assigned to placebo, respectively (p=0.013 and p=0.0005, respectively). Morning and evening PEF did not differ between treatments in the nine participants with the Arg/Arg genotype (difference [salmeterol–placebo] –12 L/min, p=0.57 and –2.2 L/min, p=0.92, respectively; figure 5). Mean PEF measured at clinic visits during spirometry was also higher when
participants were assigned to salmeterol than when assigned to placebo in African-American Gly/Gly participants (difference 39 L/min, \(p=0.0016\)) but not in Arg/Arg participants (–4.8 L/min, \(p=0.73\)). Although these subgroups were small, the genotype-specific differences in morning PEF \((p=0.09)\) and evening PEF \((p=0.07)\) approached significance, while the differences in clinic-measured PEF reached significance \((p=0.02)\). Differences in responsiveness to methacholine paralleled the genotype-specific trend seen in the entire study cohort.

There were only two Asian participants with the Arg/Arg genotype (only one of whom completed a phase of the trial), and one with the Gly/Gly genotype. Additionally, there were only four Hispanic Arg/Arg participants and five Hispanic Gly/Gly participants. For such small groups, model-derived estimates could not be obtained. It is more appropriate to assess genotype-specific differences within ethnic groups if sufficient numbers of participants are available for analysis.

There were no other significant genotype-specific differences in other subgroups analysed, including in preplanned subanalyses of those who reversed by greater than or less than 12% with salbutamol, or in post-hoc subanalyses including only those who completed the entire trial, those who were on a controller within 6 weeks of study initiation, or men versus women (data not shown).

**Discussion**

Over the past decade, several studies have investigated the effect of specific mutations of the \(\beta\)-adrenergic receptor gene on response to \(\beta_2\) agonists.\(^6,7\) A previous retrospective analysis of ACRN trials suggested that individuals with the \(\text{ADRB2}\ B16\ Arg/Arg\) genotype might not benefit from treatment with longacting \(\beta_2\) agonists, with or without inhaled corticosteroids.\(^8\) However, in this prospective, randomised controlled trial, the addition of a longacting \(\beta_2\) agonist to inhaled corticosteroid for 18 weeks produced similar improvements in airway function as shown by morning PEF in individuals with the B16 Arg/Arg and B16 Gly/Gly genotypes. Additionally, PEF did not decline during the run-out period, as has been seen in trials with shortacting \(\beta_2\) agonists.\(^5,7\) These findings are reassuring and accord with those from other retrospective studies that generally failed to show a B16 genotype-specific difference in PEF and asthma symptoms with combined use of longacting \(\beta_2\) agonists and inhaled corticosteroids.\(^8,9,19\)

A genotype-specific effect was noted in one of our prespecified secondary outcome variables—methacholine PC\(_{20}\). The discrepant effects of longacting \(\beta_2\) agonists on airway function compared with airway reactivity have been previously noted.\(^20\) Furthermore, B16 Arg has been associated with greater decreases in bronchoprotection in response to regular use of a longacting \(\beta_2\) agonist than has B16 Gly.\(^21\) In all these cases, the bronchoprotective effect of the longacting \(\beta_2\) agonist was assessed at 12 h or less after administration. Somewhat surprisingly, in our study, we found that salmeterol, when added to inhaled corticosteroid, enhanced bronchoprotection in B16 Gly/Gly participants (doubling dose shift in methacholine reactivity) but not in B16 Arg/Arg participants. This effect was unlikely to be caused by persistent bronchodilating effects of salmeterol, since the methacholine challenges were done after withholding salmeterol or placebo for 24 h. Some in-vitro data suggest that longacting \(\beta_2\) agonists and inhaled corticosteroids produce synergistic effects through multiple molecular mechanisms (in-
Inhaled corticosteroids plus longacting β₂ agonists are often used in combination therapy. However, genotype-specific effects on peak expiratory flow, drug use, asthma exacerbations, and asthma symptoms have been shown. These polymorphisms might be in linkage disequilibrium with other polymorphisms that are, in fact, biologically important. Since our study was genotype-stratified, we could not assess whether haplotypic combinations of polymorphisms showed greater associations than those we detected.


diching facilitating the transcription of anti-inflammatory genes and by acting as allosteric modulators, which might modify the conformation of glucocorticoid-bound glucocorticoid receptor or the binding of necessary cofactors and coactivators. Whether such interactions are affected by polymorphisms in ADRB2 and whether such a finding translates into increased efficacy of the drug combination in patients with the Gly/Gly genotype are unclear. Another potential explanation for the observed difference relates to the finding that Arg/Arg human airway smooth muscle cells (as compared with Gly/Gly) can produce increased amounts of proinflammatory mediators in response to β₂-agonist stimulation, which might counteract the increased anti-inflammatory effect of the β₂-agonist/corticosteroid synergy.

In a post-hoc subanalysis of African-American individuals (20% of the cohort), we noted a genotype-specific difference in morning PEF (for both the mean daily home measurements and, separately, PEF measured at clinic visits) and in evening PEF. The Arg/Arg group did not benefit when salmeterol was added to inhaled corticosteroid, whereas the Gly/Gly group did; neither group had any adverse effects. Since our African-American subpopulation was small, this finding might be caused by beta error and should be considered exploratory; however, if confirmed, this finding might have important implications. If African-American individuals do not benefit from the addition of longacting β₂ agonists to inhaled corticosteroids, they might be less likely to comply with such medications. Furthermore, a recent meta-analysis of the US Food and Drug Administration suggested an increased risk of serious adverse outcomes (combined deaths, intubations, and hospital admissions) with use of longacting β₂ agonists, especially in African-Americans.

Therefore, physicians might consider not treating African-Americans with the Arg/Arg genotype with longacting β₂ agonists in the context of a possible increase in serious adverse outcomes.

A recent prospective study in 475 African-Americans found no significant difference in the rate of asthma exacerbations when longacting β₂ agonist was added to inhaled corticosteroid and only a small difference in FEV₁, (3.5% between treatment groups) despite the fact that to enter the study all patients needed to demonstrate a >12% improvement in FEV₁, while on inhaled corticosteroid. Furthermore, although there was a substantial reduction in nocturnal asthma, there was no difference in symptom scores, symptom-free days, salbutamol use, or salbutamol-free days between treatment groups. It is possible to speculate that the Arg/Arg participants (20% of African-Americans) contributed to the small degree of improvement seen in this study.

We used a moderately high dose of inhaled corticosteroid in this study (480 μg hydrofluorouralkane beclometasone per day). It is unclear whether genotype-specific effects become evident at lower doses of inhaled corticosteroid often used in combination therapy. Additionally, it is important to distinguish our study of a longacting β₂ agonist from studies of shortacting β₂ agonists. Retrospective and prospective studies of shortacting β₂ agonists have shown genotype-specific effects on peak flow, drug use, asthma exacerbations, and asthma symptoms suggesting either that there is a differential response between shortacting and longacting β₂ agonists or that inhaled corticosteroid might prevent the detrimental response seen with shortacting β₂ agonists. Additionally, the Arg/Arg associations might not be causative. These polymorphisms might be in linkage disequilibrium with other polymorphisms that are, in fact, biologically important. Since our study was genotype-stratified, we could not assess whether haplotypic combinations of polymorphisms showed greater associations than those we detected.

Thus, the LARGE study showed that B16 Arg/Arg and B16 Gly/Gly patients with asthma had similar and substantial improvements in airway function when salmeterol was added to inhaled corticosteroid therapy. These findings provide reassurance that, in the general population, patients should continue to be treated with longacting β₂ agonists plus moderate-dose inhaled corticosteroids irrespective of B16 genotype. However, we need to further investigate the importance of the genotype-differentiated response in airway reactivity favouring Gly/Gly participants, as well as the finding that African-Americans with the the Arg/Arg genotype might not benefit from treatment with salmeterol.

Contributors
MEW, SJK, VMC, EB, HAB, BTA, MC, TJC, JVF, NJ, SKa, MK, SCL, RFL, RJM, SPP, JR, CAS, ERS, SJS, MJW, SW, and EI participated in the design of the study. MEW, SJK, VMC, EB, HAB, WJC, BTA, MC, JVF, NJ, SKa, SK, SCL, RFL, AM, RJM, PP, SPP, JR, CAS, ERS, SJS, MJW, SW, and EI participated in the running of the study. MEW, SJK, VMC, HAB,
SJS, SW, and EI participated in analysis of the data. MEW, SJK, VMC, EB, WJC, BTA, MC, LD, SKi, MK, SCL, RFL, AM, RJM, PP, SPP, ERS, SJS, SW, and EI contributed to the writing of the report. All authors have seen and approved the final version of the report.

The National Heart, Lung, and Blood Institute's Asthma Clinical Research Network research coordinators and staff

Linda L Engle, Kelly Bixler, Aimee J Merchlinski, Kerrie Sheaff er, Research Network research coordinators and staff.

All authors have seen and approved the final version of the report.

Conflicts of interest

HAB, RFL, RJM, their institutions, and the Brigham and Women's Hospital are inventors on a patent concerning the use of genotype at the β2-adrenergic receptor and effects of regular salbutamol use. MEW reports that he has consulted for or participated in advisory boards or speaker bureaus for AstraZeneca, GlaxoSmithKline, Schering-Plough, Novartis, Genentech, Merck, MedImmune, and Separac; EB reports serving as a consultant, giving presentations and performing clinical trials that were administered by Wake Forest University Health Sciences for AstraZeneca, GlaxoSmithKline, and Novartis. HAB reports research project support from GlaxoSmithKline, participating on a scientific advisory committee for GlaxoSmithKline; ad-hoc consulting for Altana, Boehringer Ingelheim, Genentech, Nanomix, Novartis, Sumitomo, Theravance, and Watermark Research; and honoraria for lectures and presentations from Merck, Novartis, Sanofi-Aventis, and Genentech. BTA has received consulting fees from Separac. TJC reports performing research with Schering, Merck, GlaxoSmithKline, Boehringer Ingelheim, Altana, Genentech/Novartis, and Forrest; grants for investigator initiated research from Viopharma, CSL Mehring, GlaxoSmithKline, and Merck; honorarium for consultative services from Teva, Alcon, Novartis, Genentech, Dyax, CSL Behring, Viopharma, Shire, and Pharmac; and honorarium for speaking from Teva Schering, Merck, AstraZeneca, Sanofi-Aventis, Genentech, and Novartis. LD reports receiving consultation honoraria from Merck. IVF reports providing consultation services for Abgenix, Aerovance, Guard, Biogen, Cytokinetiks, Gilead, Merck, Otsuka, and Roche; grants for research from Aerovance, Roche, Genentech, and Boehringer Ingelheim. NJ reports receiving advisory board honorarium and clinical trial support from GlaxoSmithKline; consulting fees from Asthma; advisory board compensation, lecture honorarium and clinical trial support from Genentech-Novartis; trial support from MedImmune; and clinical trial support and lecture honorarium from Merck. MK reports speaker’s bureau for GlaxoSmithKline; advisory board/consultation for GlaxoSmithKline, Merck, Novartis, and Amira; and research funding from GlaxoSmithKline, Bronchus, GE Healthcare, Asthmas, Novartis, and Genentech. RFL reports receiving speaker honorarium from Merck, AstraZeneca, Medicus Group, Park Nicolet Institute; consultant honorarium from AstraZeneca, MAP Pharmaceuticals, Gray Consulting, Smith Research, Merck Childhood Asthma Network, Novartis, Quintiles/ANOVA, and BCHorowitz & Company; and serving as an author of UpToDate. RJM reports serving as a consultant/litigator for Adelphi, Abbott, Schering, Novartis, Genentech, Nitec, Cividien, AstraZeneca, KaleBio, and Sepracor. SPP reports pharmaceutical trial support as a member of the Wake Forest University Clinical Trials Group sponsored by Abaris, Alanta, Boehringer Ingelheim, Contour, Genentech, GlaxoSmithKline, MedImmune, Novartis, Pfizer, Schering, and Wyeth; consulting under the auspices of Adelphi, Eoxrine, AstraZeneca, Bristol-Meyers Squibb, Cepion Therapeutics, Dey, Dyson, Genentech, Johnson & Johnson, Merck, Novartis, RAD Foundation, Respiratory Medicine, Respiratory Research, Sepracor, and Teva; and participating in Physician Education Programs sponsored by AdvanceMed, AstraZeneca, Creative Educational Concepts, DIME, Merck Pharmaceuticals, Genentech, Novartis, Practiceone, Pri-Med/SCIOS, Respiratory and Allergic Disease (RAD) Foundation, and UpToDate. CAS reports receiving honorarium for speaker’s bureau and consultation from GlaxoSmithKline; and receiving research support from Pharmaxis and Schering. ERS reports serving as an advisor or consultant to Dey, GlaxoSmithKline, and Schering-Plough; and receiving grant funding from National Institutes of Health and Novartis. SJS reports serving as a consultant for GlaxoSmithKline, Genentech, and Merck; and research funding from Ross and GlaxoSmithKline. SW reports grant support from Merck and Schering-Plough; and serving as a consultant for Amlin. Elise Bender, Steve DeMartino, Pamela Kemp (Washington University, School of Medicine, St Louis, MO). Paul Ferguson (University of California, San Diego Medical Center, San Diego, CA). Bob Hmleski, Bill Kahn, Jeffrey Krings, Susan Truhan, Cheryl Wilmoot (Wake Forest University, School of Medicine, Winston-Salem, NC).

Acknowledgments

The study was funded by the National Heart, Lung, and Blood Institute (NHLBI) of the US National Institutes of Health (NIH). An NHLBI-appointed protocol review committee approved the study design and reviewed the final manuscript. Funding references: US NIH/NHLBI: R33-HL607245, U10-HL74217, U10-HL74231, U10-HL74204, U10-HL74212, U10-HL74073, U10-HL74026, U10-HL74208, U10-HL74225, U10-HL74218. Open-label ipratropium bromide was provided by Boehringer Ingelheim Pharmaceuticals (Ridgefield, CT, USA), and open-label beclometasone hydrofluoroalkane was provided by Teva Pharmaceuticals Industries (Petach Tikva, Israel). The ACRN data safety monitoring board reviewed the final version of the report. We thank the Asthma Clinical Research Network research coordinators and study staff.

References


