



Influence of β_2 -adrenergic receptor polymorphism on methacholine hyperresponsiveness in asthmatic patients

Arvind Manoharan, MBChB; William J. Anderson, MBChB; and Brian J. Lipworth, MD

Asthma and Allergy Research Group, Division of Cardiovascular and Diabetes Medicine, Medical Research Institute, University of Dundee, Dundee, Scotland

ARTICLE INFO

Article history:

Received for publication October 30, 2012.
Received in revised form December 4, 2012.
Accepted for publication December 26, 2012.

ABSTRACT

Background: We previously reported that the β_2 -adrenergic receptor (ADRB2) polymorphism had no effect on bronchial hyperresponsiveness (BHR) to methacholine in asthmatic patients. We have now replicated this analysis in a different and larger cohort of patients.

Objective: To assess the effect of the ADRB2 polymorphism in methacholine-responsive patients with asthma.

Methods: We conducted a retrospective analysis of the effects of ADRB2 haplotypes at position 16 (Gly/Arg) and 27 (Gln/Glu) in 449 patients with a physician diagnosis of asthma who were responsive to methacholine (ie, provocation concentration that caused a decrease in forced expiratory volume in 1 second [FEV₁] of 20% [PC₂₀], <8 mg/mL).

Results: No differences were found in age, FEV₁, or inhaled corticosteroid dose among the genotypes or haplotypes. No significant differences were found in methacholine PC₂₀ (ie, <8 mg/mL) between the separate genotypes at position 16 or 27 or between the haplotypes at positions 16/27 combined. In addition, no significant differences were found among individual genotypes when stratified according to severity of BHR using different doubling dilution cutoff points for methacholine PC₂₀ (ie, <4 mg/mL, <2 mg/mL, and <1 mg/mL).

Conclusion: We have confirmed in this replication study that common ADRB2 genotypes or haplotypes at positions 16/27 do not influence BHR in methacholine-responsive patients with asthma.

© 2013 American College of Allergy, Asthma & Immunology. Published by Elsevier Inc. All rights reserved.

Introduction

Bronchial hyperresponsiveness (BHR) is an excessive response of the airways to a provoking stimulus. BHR is not pathognomonic of asthma but is an important feature of asthma that is associated with underlying inflammation and disease severity.^{1,2} BHR may be influenced by genetic and environmental factors.³ The gene governing BHR is located at chromosome 5q31-q33, where the β_2 -adrenergic receptor (ADRB2) gene is located.⁴ Because of its role in the regulation of airway tone, the gene is thought to play a role in BHR. The 2 most common polymorphisms for the ADRB2 gene occur because of the substitution of glycine for arginine at position 16 and glutamate for glutamine at position 27. Both these polymorphisms are in linkage disequilibrium.⁵

In a previous report by our group, we found that ADRB2 genotypes or haplotypes had no effect on BHR to methacholine in patients with asthma.⁶ We have now replicated the previous study

in a different and larger cohort of patients with asthma who were all methacholine responsive as defined by a provocation concentration that caused a decrease in forced expiratory volume in 1 second (FEV₁) of 20% (PC₂₀) less than 8 mg/mL.

Methods

Patients

All patients were screened at the Asthma and Allergy Research Group and had a physician diagnosis of asthma. They were seen for more detailed assessment before potential participation in subsequent clinical research studies. The assessment included details of medication, skin prick testing, spirometry, methacholine challenge, and genotyping for the ADRB2 polymorphism. The Tayside Committee on Medical Research Ethics provided ethical approval for the screening process, including ADRB2 genotyping, and all patients gave their written consent.

Methacholine Challenge

Methacholine was dispensed via a nebulized solution (Methapharm Inc, Brantford, Ontario, Canada), and the challenge was performed in accordance with American Thoracic Society

Reprints: Brian J. Lipworth, MD, Asthma and Allergy Research Group, Division of Cardiovascular and Diabetes Medicine, Medical Research Institute, University of Dundee, Ninewells Hospital and Medical School, Dundee, DD1 9SY, Scotland, United Kingdom; E-mail: brianlipworth@gmail.com.

Disclosures: Authors have nothing to disclose.

guidelines.⁷ A dosimeter (Markos-Mefar, Bovezzo, Italy) was used with doubling concentrations from 0.03 to 64 mg/mL. The PC₂₀ was then calculated by computer-assisted log linear interpolation of the dose response.

Spirometry

A SuperSpiro spirometer (Micro Medical Ltd, Chatham, Kent, England) was used to perform spirometry in accordance with guidelines.⁸

Genotyping

A whole blood DNA sample was collected from patients after written informed consent was specifically given for this. DNA was prepared with the Qiagen Dneasy 96 kit (Qiagen GmbH, Hilden, Germany), and genotypes were determined with the Taqman-based allelic discrimination assays on an ABI7700 sequence detection system.⁹ For the Gly16Arg variant, the primers and Gly 16 probe were used as previously described,¹⁰ and the fluorophore on the Arg 16 probe was either Cal Orange or VIC.

Skin Prick Testing

Patients withheld antihistamine medication for 4 days before skin prick testing. This was performed following a standard protocol (Allegra Pharma Testing Solution; Diagnostics Ltd, Notts, England) using extracts to grass, trees, weeds, house dust mite, *Aspergillus*, feathers, and cat and dog dander in addition to a positive (histamine) and negative control. Results were read after 10 minutes; a positive reaction being defined as a wheal diameter 2 mm greater than the negative control.

Statistical Analysis

Data for the PC₂₀ were log transformed to normalize their distribution before analysis. An analysis of variance (ANOVA) was used to determine whether there was any overall difference between genotypes and haplotypes for methacholine PC₂₀ and other outcomes, followed by Bonferroni-corrected pairwise comparisons in the presence of an overall significant ANOVA ($P < .05$, 2-tailed). Multiple linear regression analysis was performed to assess predictors for methacholine PC₂₀. PC₂₀ was used as the dependent variable, and FEV₁, atopy, polymorphism, inhaled corticosteroid (ICS) dose, and whether the patient was taking a long-acting β -agonist (LABA) were used as independent variables in the analysis. IBM SPSS statistical software, version 20 (SPSS Inc, Chicago, Illinois) was used for all analyses.

Results

A total of 449 participants were genotyped, and a positive methacholine challenge result was defined as a methacholine PC₂₀ of less than 8 mg/mL. Overall 323 patients (72%) were taking ICSs, and 114 (25%) were taking an LABA (Table 1). No significant

differences were found between genotypes or haplotypes in terms of age, ICS dose, FEV₁, or atopy (Table 1).

Allele frequencies for both positions were as follows: Gly16, 64.4%; Arg16, 35.6%; Glu27, 44.6%; and Gln27, 55.4%. The genotype frequencies at position 16 were 11.8% for Arg-Arg, 47.2% for Arg-Gly, and 41% for Gly-Gly. The genotype frequencies at position 27 were 30.5% for Gln-Gln, 50.1% for Gln-Glu, and 19.4% for Glu-Glu. There was an unequal distribution of haplotypes, with no participants having the Arg-Arg/Glu-Glu or the Arg-Gly/Gln-Glu haplotype (Table 1).

No significant differences were found for methacholine PC₂₀ among the different genotypes or haplotypes (Table 2). When comparing the separate genotypes at position 16 and 27, no significant differences were found in PC₂₀ either. Each genotype was also stratified according to the severity of BHR using different doubling dilution cutoff points for methacholine PC₂₀ (ie, <8 mg/mL, <4 mg/mL, <2 mg/mL, and <1 mg/mL), which did not reveal any significant differences between the genotypes at positions 16 or 27 (Tables 3 and 4).

Further subgroup analysis according to treatment (ie, no treatment, taking an ICS only, taking an LABA only, and taking both an ICS and LABA) did not reveal any significant difference in terms of methacholine PC₂₀ among the different haplotypes. Subdividing the cohort into atopic and nonatopic individuals, we could not demonstrate any significant difference for methacholine PC₂₀ among the different haplotypes. The only independent predictor for methacholine PC₂₀ from the multiple linear regression was FEV₁ ($P < .001$).

Discussion

The results of this study demonstrated no association between common ADRB2 genotypes or haplotypes at position 16 and 27 and BHR in a population of asthmatic patients, all of whom were methacholine responsive. It therefore confirms the previous findings of Wilson et al⁶ in a different and smaller cohort, which included asthmatic patients who were responders or nonresponders to methacholine. In this regard the cohort of Wilson et al had 265 BHR-positive (ie, PC₂₀ <8 mg/mL) asthmatic patients compared with the current cohort, which has 449 BHR-positive asthmatic patients.

In the present study, the 4 most common haplotypes at 16/27 were Arg-Gly/Gln-Glu (32%), Gly-Gly/Glu-Glu (19%), Gly-Gly/Gln-Glu (17%), and Arg-Gly/Gln-Gln (15%) (Table 1). These haplotype frequencies are similar to the haplotype frequencies reported in previous studies.^{5,6} We did not identify any patients with the Arg-Arg/Glu-Glu or Arg-Gly/Glu-Glu haplotype. Both these haplotypes are exceedingly rare because of known linkage disequilibrium.⁵

Previous studies have investigated the relationship between the ADRB2 polymorphism and BHR in mainly healthy participants in contrast to our studies. These studies have sought to focus on BHR as an independent trait and not within the context of a known

Table 1
Characteristics of the participants with a methacholine challenge result of less than 8 mg/mL for the different haplotypes

Characteristic	Arg-Arg/Gln-Gln	Arg-Arg/Gln-Glu	Arg-Gly/Gln-Gln	Arg-Gly/Gln-Glu	Gly-Gly/Gln-Gln	Gly-Gly/Gln-Glu	Gly-Gly/Glu-Glu	Total
No. (%) of patients	48 (10.7)	5 (1.1)	69 (15.3)	143 (31.8)	20 (4.5)	78 (17.4)	86 (19.2)	449 (100)
Age, mean (SEM), y	45 (2.2)	45 (1.0)	44 (1.8)	46 (1.3)	45 (3.4)	46 (1.7)	45 (1.6)	45 (0.7)
Taking an ICS, No. (%)	31 (65)	4 (80)	51 (64)	105 (73)	16 (80)	57 (73)	59 (69)	323 (72)
ICS dose, median (range), μ g	400 (200–3,200)	400 (400–1,600)	400 (200–4,000)	400 (200–2,000)	400 (200–1,000)	400 (50–2,000)	400 (200–2,000)	400 (50–4,000)
Taking an LABA, No. (%)	7 (15)	2 (40)	22 (32)	31 (22)	6 (30)	23 (29)	20 (23)	111 (25)
FEV ₁ , mean (SEM), L	2.98 (0.1)	3.10 (0.4)	2.62 (0.1)	2.70 (0.1)	2.75 (0.2)	2.75 (0.1)	2.98 (0.1)	2.81 (0.04)
FEV ₁ , mean (SEM), % predicted	85.7 (2.5)	86.6 (8.0)	83.6 (2.1)	87.4 (1.4)	86.7 (3.9)	85.3 (1.9)	87.5 (1.8)	85.9 (0.8)
Positive SPT result, No. (%)	41 (85)	4 (80)	57 (83)	84 (59)	15 (75)	70 (90)	67 (78)	338 (75)

Abbreviations: FEV₁, forced expiratory volume in 1 second; ICS, inhaled corticosteroids; LABA, long-acting β -agonist; SPT, skin prick test.

Table 2Geometric mean for methacholine PC₂₀ according to haplotype

Haplotype	No. of patients	Methacholine PC ₂₀ , geometric mean (95% CI)
Arg-Arg/Gln-Gln	48	0.66 (0.44–0.99)
Arg-Arg/Gln-Glu	5	1.17 (0.74–1.87)
Arg-Gly/Gln-Gln	69	0.45 (0.33–0.62)
Arg-Gly/Gln-Glu	143	0.67 (0.52–0.85)
Gly-Gly/Gln-Gln	20	0.64 (0.23–1.80)
Gly-Gly/Gln-Glu	78	0.56 (0.40–0.78)
Gly-Gly/Glu-Glu	86	0.76 (0.57–1.01)
Total	449	0.63 (0.55–0.72)

Abbreviations: CI, confidence interval; PC₂₀, provocation concentration that caused a decrease in forced expiratory volume in 1 second of 20%.

diagnosis of asthma. This is an important point to take into consideration when interpreting the findings of prior studies, which have not been able to demonstrate consistent findings and frequently yielded contradictory results. For example, studies by D'Amato et al¹¹ and Ulbrecht et al,¹² examined the association of BHR and ADRB2 haplotypes in healthy populations but came to conflicting conclusions. Whereas D'Amato et al found that the ADRB2 haplotype Gly16/Gln27 was associated with BHR, Ulbrecht et al came to the opposite conclusion that the Gly16/Gln27/Th164 haplotype had a protective effect against BHR. The studies by D'Amato et al and Ulbrecht et al only had 30 and 152 individuals, respectively, who were defined as being BHR positive compared with this study, which has 449 BHR-positive asthmatic patients.

Studies have also investigated the ADRB2 polymorphisms at position 16 and 27 individually and not as haplotypes with regard to their effect on BHR. As in the previous haplotype studies, results have not been consistent. Fukui et al,¹³ in a study of 120 healthy Japanese students, found that Gly16 homozygotes were more responsive to methacholine than individuals with the Arg16 allele. Fowler et al¹⁴ made a similar observation in 158 asthmatic patients. Hall et al¹⁵ concluded that the Glu27 polymorphism was associated with lower airway reactivity based on a study in 65 asthmatic patients. Hence, in addition to analyzing our data according to haplotypes, we also analyzed the genotypes at position 16 and 27 individually but still failed to demonstrate any association between the polymorphisms and BHR, despite looking at different PC₂₀ doubling dilution cutoff values. This would be in keeping with the comprehensive meta-analysis of the association of ADRB2 polymorphisms with asthma phenotypes by Contopoulos-Ioannidis et al.¹⁶

Table 3Analysis of genotype at position 16 stratified according to bronchial hyperresponsiveness severity using different doubling dilution cutoff points for methacholine PC₂₀

Genotype at position 16	No. of patients	Methacholine PC ₂₀ , geometric mean (95% CI)
PC ₂₀ <8 mg/mL		
Arg-Arg	53	0.70 (0.48–1.01)
Arg-Gly	212	0.59 (0.48–0.71)
Gly-Gly	184	0.66 (0.53–0.82)
PC ₂₀ <4 mg/mL		
Arg-Arg	47	0.53 (0.37–0.74)
Arg-Gly	194	0.48 (0.40–0.57)
Gly-Gly	158	0.46 (0.37–0.57)
PC ₂₀ <2 mg/mL		
Arg-Arg	40	0.39 (0.37–0.74)
Arg-Gly	164	0.34 (0.40–0.57)
Gly-Gly	134	0.34 (0.37–0.57)
PC ₂₀ <1 mg/mL		
Arg-Arg	31	0.27 (0.20–0.37)
Arg-Gly	135	0.25 (0.21–0.30)
Gly-Gly	110	0.25 (0.20–0.30)

Abbreviations: CI, confidence interval; PC₂₀, provocation concentration that caused a decrease in forced expiratory volume in 1 second of 20%.

Table 4Analysis of genotype at position 27 stratified according to bronchial hyperresponsiveness severity using different doubling dilution cutoff points for methacholine PC₂₀

Genotype at position 27	No. of patients	Methacholine PC ₂₀ geometric mean (95% CI)
PC ₂₀ <8 mg/mL		
Gln-Gln	137	0.54 (0.42–0.70)
Gln-Glu	225	0.64 (0.53–0.77)
Glu-Glu	87	0.76 (0.57–1.01)
PC ₂₀ <4 mg/mL		
Gln-Gln	127	0.45 (0.35–0.57)
Gln-Glu	198	0.47 (0.39–0.56)
Glu-Glu	74	0.54 (0.41–0.71)
PC ₂₀ <2 mg/mL		
Gln-Gln	106	0.31 (0.24–0.40)
Gln-Glu	170	0.35 (0.30–0.42)
Glu-Glu	62	0.40 (0.31–0.52)
PC ₂₀ <1 mg/mL		
Gln-Gln	89	0.23 (0.18–0.30)
Gln-Glu	138	0.25 (0.21–0.30)
Glu-Glu	49	0.28 (0.22–0.36)

Abbreviations: CI, confidence interval; PC₂₀, provocation concentration that caused a decrease in forced expiratory volume in 1 second of 20%.

However, the authors of this study noted that the Gly16 allele, particularly the homozygotes, had increased risk of nocturnal asthma and asthma severity.

It is possible that the effect of the ADRB2 polymorphism on BHR may have been modified by prior asthma treatment. In our present study, 72% of the participants were taking an ICS, and 25% were taking an LABA. In this regard, meta-analysis has revealed that both ICSs and LABAs attenuate BHR.^{17,18} In a group of 375 asthmatic patients, Rebordosa et al¹⁹ observed that there was an increased risk of BHR among asthmatic patients who were homozygotes for the Arg16 allele compared with Gly16 homozygotes,¹⁹ with the risk being higher among nonusers of ICSs. The risk was no different among the genotypes whether the patient were taking an LABA or not. These results, however, contradict the findings of a prospective genotype stratified trial,²⁰ where adding salmeterol (compared with placebo) to an ICS conferred significant bronchoprotection against methacholine in Gly16 homozygotes but not Arg16 homozygotes. Similar genotype-dependent differences in additive effects of LABAs on BHR have been reported in a meta-analysis by Lee et al.²¹

The ADRB2 polymorphisms at position 16 and 27 are known to be in linkage disequilibrium. Thus, haplotype analysis might be expected to have greater power in demonstrating associations with specific phenotypes compared with analysis of individual polymorphisms. For example, Drysdale et al⁵ found that although there was no association when polymorphisms were analyzed individually, there was a clear association between one of the common haplotypes and β -agonist responders in vivo. Litonjua et al²² found that the Gly16/Glu27 haplotype was negatively associated with BHR in nonsmokers, whereas this association was not apparent when the ADRB2 polymorphisms at position 16 and 27 were analyzed separately. However, Lipworth et al²³ did not find any association between common ADRB2 haplotypes and ex vivo ADRB2 binding density and coupling in peripheral blood mononuclear cells.

In conclusion, we have confirmed in this replication study of a larger cohort of patients that ADRB2 genotype and haplotype 16/27 does not have an effect on BHR in methacholine-responsive patients with asthma.

References

- [1] Boushey HA, Holtzman MJ, Sheller JR, Nadel JA. Bronchial hyperreactivity. *Am Rev Respir Dis*. 1980;121:389–413.
- [2] Chung KF. Role of inflammation in the hyperreactivity of the airways in asthma. *Thorax*. 1986;41:657–662.

- [3] Meyers DA, Postma DS, Stine OC, et al. Genome screen for asthma and bronchial hyperresponsiveness: interactions with passive smoke exposure. *J Allergy Clin Immunol*. 2005;115:1169–1175.
- [4] Postma DS, Bleeker ER, Amelung PJ, et al. Genetic Susceptibility to asthma: bronchial hyperresponsiveness coinherited with a major gene for atopy. *N Engl J Med*. 1995;333:894–900.
- [5] Drysdale CM, McGraw DW, Stack CB, et al. Complex promoter and coding region beta 2-adrenergic receptor haplotypes alter receptor expression and predict in vivo responsiveness. *Proc Natl Acad Sci U S A*. 2000;97:10483–10488.
- [6] Wilson AM, Gray RD, Hall IP, Lipworth BJ. The effect of β_2 -adrenoceptor haplotypes on bronchial hyper-responsiveness in patients with asthma. *Allergy*. 2006;61:254–259.
- [7] Guidelines for methacholine and exercise challenge testing—1999. *Am J Respir Crit Care Med*. 2000;161:309–329.
- [8] Miller MR, Hankinson J, Brusasco V, et al. Standardisation of spirometry. *Eur Respir J*. 2005;26:319–338.
- [9] Bieche I, Olivi M, Champeme MH, Vidaud D, Lidereau R, Vidaud M. Novel approach to quantitative polymerase chain reaction using real-time detection: application to the detection of gene amplification in breast cancer. *Int J Cancer*. 1998;78:661–666.
- [10] Palmer CN, Lipworth BJ, Lee S, Ismail T, Macgregor DF, Mukhopadhyay S. Arginine-16 beta2 adrenoceptor genotype predisposes to exacerbations in young asthmatics taking regular salmeterol. *Thorax*. 2006;61:940–944.
- [11] D'Amato M, Vitiani LR, Petrelli G, et al. Association of persistent bronchial hyperresponsiveness with β_2 -adrenoceptor (ADRB2) haplotypes. *Am J Respir Crit Care Med*. 1998;158:1968–1973.
- [12] Ulbrecht M, Hergeth MT, Wjst M, et al. Association of beta(2)-adrenoreceptor variants with bronchial hyperresponsiveness. *Am J Respir Crit Care Med*. 2000;161(2 pt 1):469–474.
- [13] Fukui Y, Hizawa N, Takahashi D, et al. Association between nonspecific airway hyperresponsiveness and Arg16Gly β_2 -adrenergic receptor gene polymorphism in asymptomatic healthy Japanese subjects. *Chest*. 2006;130:449–454.
- [14] Fowler SJ, Dempsey OJ, Sims EJ, Lipworth BJ. Screening for bronchial hyper-responsiveness using methacholine and adenosine monophosphate. *Am J Respir Crit Care Med*. 2000;162:1318–1322.
- [15] Hall IP, Wheatley A, Wilding P, Liggett SB. Association of Glu 27 β_2 -adrenoceptor polymorphism with lower airway reactivity in asthmatic subjects. *Lancet*. 1995;345:1213–1214.
- [16] Contopoulos-Ioannidis DG, Manoli EN, Ioannidis JPA. Meta-analysis of the association of β_2 -adrenergic receptor polymorphisms with asthma phenotypes. *J Allergy Clin Immunol*. 2005;115:963–972.
- [17] Currie GP, Fowler SJ, Lipworth BJ. Dose response of inhaled corticosteroids on bronchial hyperresponsiveness: a meta-analysis. *Ann Allergy Asthma Immunol*. 2003;90:194–198.
- [18] Currie GP, Jackson CM, Ogston SA, Lipworth BJ. Airway-stabilizing effect of long-acting β_2 -agonists as add-on therapy to inhaled corticosteroids. *QJM*. 2003;96:435–440.
- [19] Rebordosa C, Kogevinas M, Guerra S, et al. ADRB2 Gly16Arg polymorphism, asthma control and lung function decline. *Eur Respir J*. 2011;38:1029–1035.
- [20] Wechsler ME, Lehman E, Lazarus SC, et al. β -Adrenergic receptor polymorphisms and response to salmeterol. *Am J Respir Crit Care Med*. 2006;173:519–526.
- [21] Lee DK, Currie GP, Hall IP, Lima JJ, Lipworth BJ. The arginine-16 beta2-adrenoceptor polymorphism predisposes to bronchoprotective sub-sensitivity in patients treated with formoterol and salmeterol. *Br J Clin Pharmacol*. 2004;57:68–75.
- [22] Litonjua AA, Silverman EK, Tantisira KG, Sparrow D, Sylvia JS, Weiss ST. β_2 -Adrenergic receptor polymorphisms and haplotypes are associated with airways hyperresponsiveness among nonsmoking men. *Chest*. 2004;126:66–74.
- [23] Lipworth B, Koppelman GH, Wheatley AP, et al. Beta2 adrenoceptor promoter polymorphisms: extended haplotypes and functional effects in peripheral blood mononuclear cells. *Thorax*. 2002;57:61–66.