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ORIGINAL ARTICLE

β_2-adrenergic receptor polymorphisms are associated with asthma and COPD in adults

Melanie C. Matheson · Justine A. Ellis · Joan Raven · David P. Johns · E. Haydn Walters · Michael J. Abramson

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Abstract The β_2-adrenergic receptor (β_2AR) is a transmembrane protein expressed by airway smooth muscle cells. In vitro studies have shown that polymorphisms at amino acid positions 16 and 27 alter receptor function. The aim of this study was to examine the associations between the *β_2AR* polymorphisms and risks of asthma, chronic obstructive pulmonary disease (COPD) and respiratory symptoms in a sample of adults. Participants were part of a cross-sectional population-based study of risk factors for respiratory disease. A total of 1,090 Caucasian participants completed a detailed respiratory questionnaire, spirometry, methacholine challenge and measurement of gas transfer. Genotyping for *β_2AR* polymorphisms at positions 16 and 27 was performed using the tetra-primer amplification refractory mutation system–polymerase chain reaction (ARMS–PCR) method. Haplotype frequencies for the two polymorphisms were estimated using the E-M algorithm. We found the Arg16 homozygotes had an increased risk of COPD (OR 5.13; 95% CI 1.40,18.8), asthma (2.44; 1.12,5.31) and symptoms of wheeze (1.84; 1.02,3.35). The Gln27 homozygotes had an increased risk of asthma (2.08; 1.05,4.13) and bronchial hyperreactivity (BHR) (1.92; 1.07,3.46). The Arg16/Gln27 haplotype was associated with asthma (1.63; 1.12,2.38) and COPD (2.91; 1.42,5.94). The Arg16/Gln27 β_2AR haplotype is important in COPD, asthma and BHR, and may be associated with more severe respiratory symptoms in middle-aged and older adults.

Keywords β_2AR (adrenoreceptor) · Asthma · COPD · Haplotypes

M. C. Matheson · M. J. Abramson
Department of Epidemiology and Preventive Medicine, Monash University, Monash, VIC, Australia

J. A. Ellis
Department of Physiology, The University of Melbourne, Melbourne, VIC, Australia

J. Raven
Department of Allergy, Immunology and Respiratory Medicine, The Alfred Hospital, Melbourne, VIC, Australia

D. P. Johns · E. H. Walters
Cardio-Respiratory Research Group, School of Medicine, University of Tasmania, Hobart, Australia

M. C. Matheson (✉)
Centre for Molecular, Environmental, Genetic and Analytic Epidemiology, School of Population Health, The University of Melbourne, Level 2/723 Swanston Street, Carlton, VIC 3053, Australia
e-mail: mcmat@unimelb.edu.au

Abbreviations

ATS	American Thoracic Society
β_2	Beta$_2$
β_2AR	β_2-Adrenergic receptor
BHR	Bronchial hyperreactivity
COPD	Chronic obstructive pulmonary disease
$D_L$co	Diffusing capacity
LHS	Lung health study
MCh	Methacholine
Ors	Odds ratios
PCR	Polymerase chain reaction
RFLP	Restriction fragment length polymorphism
SNP	Single nucleotide polymorphism

Tetra-primer ARMS	Tetra-primer amplification refractory mutation system
95% Cis	95% Confidence intervals

Introduction

Beta$_2$ (β_2)-agonists are one of the most important classes of drugs used in the treatment of asthma and chronic obstructive pulmonary disease (COPD). Their action is mediated by the β_2-adrenergic receptor (β_2AR), a transmembrane protein expressed by airway smooth muscle cells. When stimulated, β_2AR results in cAMP-mediated muscular relaxation that is important to the regulation of airway tone, and therefore airway reactivity and lung function (Turner et al. 2004).

Several polymorphisms within *β_2AR* have been described (Reihsaus et al. 1993). In particular, the amino acid substitutions at positions 16, arginine → glycine (Arg → Gly) and 27, glutamine → glutamate (Glu → Gln) have been shown to alter the receptor function in vitro (Green et al. 1994, 1995). Initial in vivo studies of transfected cell lines and primary cultures of human-airway smooth muscle cells found the Gly16 form of the receptor becomes more down-regulated and desensitised after exposure to β_2-agonist than the Arg16 form (Green et al. 1994, 1995). However, more recent in vivo work in humans has shown the Arg16Gln27 haplotype is the form of the receptor that is associated with agonist-promoted desensitization (Dishy et al. 2001). Genetic studies of these polymorphisms have found them to be associated with asthma severity (Turki et al. 1995; Weir et al. 1998), bronchial hyperreactivity (BHR) (Hall et al. 1995; Ramsay et al. 1999), bronchodilator (BD) response (D'Amato et al. 1998; Lima et al. 1999; Martinez et al. 1997; Ulbrecht et al. 2000) and lung function (Summerhill et al. 2000).

Few studies have investigated *β_2AR* polymorphisms in COPD and related phenotypes. One study in Chinese patients found a decreased prevalence of the Arg16 allele in the COPD cases, and subjects with the Gln27 allele had lower FEV_1 percent predicted (Ho et al. 2001). In contrast a study of Egyptian COPD patients found a decreased prevalence of the Gln27 allele in the COPD patients compared to controls (Hegab et al. 2004). Furthermore, the Lung Health Study (LHS) of current smokers found a protective effect of heterozygosity at position 27 against rapid decline in lung function (Joos et al. 2003).

Most genetic studies have investigated these polymorphisms in isolation. However, work by Drysdale et al. (2000) has shown that the haplotype containing Arg16/Gln27 was associated with a reduced response to β_2-agonist in vivo (Drysdale et al. 2000), highlighting the importance of examining haplotypes. A few studies have investigated *β_2AR* haplotypes and risk of asthma, COPD or related phenotypes with conflicting results. Two studies have found that the Gly16/Gln27 haplotype was protective against BHR (Litonjua et al. 2004; Ulbrecht et al. 2000). Conversely, an Italian study found the Gly16/Gln27 haplotype to be associated with an increased risk of BHR (D'Amato et al. 1998). Furthermore, the LHS found no association between any *β_2AR* haplotypes and bronchodilator response or non-specific BHR, or rate of decline in lung function (Joos et al. 2003). BHR is a known risk factor for chronic respiratory symptoms (Xu et al. 1997) and mortality from COPD (Hospers et al. 2000), so given these conflicting results it is clear that further study of the *β_2AR* gene in asthma, COPD and related phenotypes is warranted.

We recruited 1,232 randomly selected middle-aged and older adults from the general community in Melbourne, Australia. We examined their lung function and bronchial responsiveness, and genotyped them for the *β_2AR16* and *β_2AR27* polymorphisms. The aim of this study was to further examine the associations between the *β_2AR* polymorphisms and haplotypes, and risk of asthma, COPD and related phenotypes.

Methods

Participants

Recruitment and pulmonary-function testing were described in detail elsewhere (Matheson et al. 2005). Briefly, 1,232 subjects (595 females, 637 males) were randomly recruited to be part of a two-stage cross-sectional epidemiological study to investigate risk factors for asthma and COPD in adults aged between 45 and 69 years. From this general Caucasian population, sample cases of respiratory conditions and controls were selected (described in more detail below). The study was approved by the Ethics Committees at Monash University and The Alfred Hospital, Melbourne, Australia. All participants gave written informed consent.

Lung function testing

Spirometry and diffusing capacity ($D_L$co) was performed according to the American Thoracic Society (ATS) criteria. Predicted values for FEV_1, FVC and $FEF_{25–75}$ were calculated from age, height and gender using equations by Gore et al. (1995), and for $D_L$co using the equations by Quanjer et al. (1983). Methacholine (MCh) (USP Methapharm Inc., Brantford, ON, Canada) was delivered by a Mefar 3B dosimeter (Mefar, Bovezzi, Italy) until FEV_1 fell by 20% from the initial value or up to a cumulative dose of 2 mg (PD_{20}). BHR was defined as a PD_{20} FEV_1 < 2 mg MCh (Toelle et al. 1992). Reasons which caused subjects not to complete the MCh challenge included poor-quality spirometry (n=5), being on medication for heart disease including beta-blockers, epilepsy (n=92) or an initial FEV_1 less than 70% predicted or 1.5 l (n=46). For those subjects with an initial FEV_1 less than 70% predicted or 1.5 l, spirometry was repeated 10 min after the administration of a bronchodilator [200 μg (2 puffs) of salbutamol via a spacer]. Significant bronchodilator reversibility was defined as an increase in FEV_1 of at least 12% and 200 mL (Pellegrino et al. 2005).

Definitions

Wheeze was defined as a positive response to: ''Have you had wheezing or whistling in your chest at any time in the last 12 months?''. Nocturnal chest tightness was defined as a positive response to: ''Have you been woken by chest tightness at any time in the last 12 months?''. Dyspnoea was defined as a positive response to: ''Are you troubled by shortness of breath when hurrying on level ground or walking up a slight hill?''. Current asthma was defined as wheeze during the last 12 months, together with BHR to methacholine (Toelle et al. 1992) or significant bronchodilator reversibility (where BHR could not be done). Mild airflow obstruction was defined as FEV_1/FVC ratio less than 70% (Pauwels et al. 2001). Chronic obstructive bronchitis was defined as a positive response to: ''Have you brought up phlegm on most days for as much as 3 months of a year for at least 2 successive years?'' and mild airflow obstruction. Symptomatic emphysema was defined as mild airflow obstruction, a $D_L$co < 80% predicted and dyspnoea. COPD was defined as either chronic obstructive bronchitis or symptomatic emphysema. Pack years were calculated as the average number of cigarettes smoked per day divided by 20 and multiplied by the number of years smoked.

Genotyping

From the 1,232 participants in the study, blood and consent to extract DNA were available for 1,138 participants (92.4%). DNA was successfully extracted from 1,102 subjects (96.8%) using standard phenol chloroform techniques. All analyses were performed blind with respect to diagnosis and patient characteristics. *β_2AR* genotype was determined using the tetra-primer ARMS–PCR method described by Ye et al. (2001). Primers were designed using the computer program created by the authors of the method. Approximately 50 ng of DNA was amplified in a 20-μl reaction mix. This mix contained 1 × reaction buffer, 2.5 mM $MgCl_2$, 250 μM of dNTP (GeneAmp, Applied Biosystems, CA, USA), one unit of AmpliTaq Gold DNA polymerase (Applied Biosystems) and 0.5 μM of each primer. The outer primers were diluted to 1:10 of the concentration of the inner primers to enhance specificity. The PCR cycling conditions for the *β_2AR16* polymorphism were 95°C for 10 min, followed by 35 cycles of 94°C for 1 min, 67°C for 1 min and 72°C for 1 min, and then a final extension for 10 min at 72°C. For *β_2AR27* the conditions were identical, with the exception of an annealing temperature of 85°C. The PCR products were resolved by electrophoresis on a 3% agarose gel stained with ethidium bromide. A random sample of 10% of the total sample was genotyped using the restriction fragment length polymorphism (RFLP) method described by Martinez et al. (1997) to validate the genotyping protocol.

Statistical analysis

The statistical analysis was performed using the statistical package STATA (version 6; STATA Corporation, TX, USA). The haplotype analysis was performed using the haplo.stats package for R (Version 2.0.0; The R Foundation for Statistical Computing). These procedures assume Hardy–Weinberg equilibrium (HWE). HWE was calculated by a χ^2 goodness of fit test.

Multiple regression analysis was used to examine the association between genotype and continuous outcomes. For binary outcomes logistic regression was used, and cases were subjects that reported the symptom or met the criteria for the disease of interest. Controls were subjects who did not report any symptoms and did not have any of the outcomes investigated. The same control group was used for all analyses. Covariates included in the models were age, gender, smoking status (current, past or never smoker), and pack years. Modification of the effect of genotype by smoking was assessed by including an interaction

term (genotype*ever smoking) in the models. All subjects in the analysis presented were of Caucasian descent.

Haplotype frequencies were estimated using the E-M algorithm for genetic markers measured on unrelated subjects with unknown phase. The *β2AR16* and *β2AR27* SNPs were used in the haplotype analysis. Those traits significant in the genotype analysis, for either single nucleotide polymorphism (SNP), were further explored using haplotype analysis using haplo.score. The haplo.glm function was used to compute the regression of a trait on haplotypes, and to adjust for covariates (Hawley and Kidd 1995). For binary traits, this procedure fitted logistic regression models which provided odds ratios (ORs) and 95% confidence intervals (95% CIs). For continuous traits, linear regression models were fitted giving differences of means between groups and 95% CIs. For all analyses the significance level was set at $P<0.05$.

Results

Subject characteristics

The clinical characteristics of the subgroups are presented in Table 1. The control group was significantly younger than all other clinical subgroups. There was a significantly greater proportion of males in the control group than in the groups with asthma, BHR, dyspnoea or nocturnal chest tightness. The prevalence of current and past smokers was significantly greater in all the clinical subgroups compared to the controls. Wheeze was the most frequently reported symptom, followed by dyspnoea when walking on level ground or up a slight hill (dyspnoea grade 2), and then nocturnal chest tightness (Table 1). Subjects with COPD had the worst lung-function parameters, but all clinical subgroups had worse lung function than the controls.

Genotype and haplotype frequencies

Genotyping was successfully completed for 1,090 subjects (98.9% of available samples) for the β_2AR16 and β_2AR27 polymorphisms. For *β2AR16*, the prevalence of the Arg16 homozygotes was 13.1% (n=143), for Arg16/Gly16 heterozygotes 46.2% (n=504) and for Gly16 homozygotes 40.6% (n=443). For *β2AR27*, the prevalence of the Gln27 homozygotes was 32.0% (n=349), for Gln27/Glu27 heterozygotes 47.6% (n=519) and for Glu27 homozygotes 20.4% (n=222). In the controls, both polymorphisms were in HWE (β_2AR16 P=0.45; β_2AR27 P=0.85). There was strong linkage disequilibrium between the two polymorphisms According to Ď measures, there appeared to be strong LD between the two SNPs (Ď 0.996; 95% CI 0.97, 1.0 $P<0.0001$); however, r2 measures (0.447) suggest that LD is more moderate. The haplotype frequencies were estimated using the E-M algorithm. As found by other investigators, the Arg16 segregated more commonly with Gln27 and Gly16 with Glu27. The Gly16/Glu27 haplotype (0.44) was the most common, with Arg16/Gln27 (0.36) the next most frequent, Gly16/Gln27 at 0.197 and the Arg16/Glu27 haplotype very rare in our population (0.00057).

Table 1 Clinical features of the study population

Characteristics	Wheeze	Asthma	COPD	Nocturnal chest tightness	BHR	Dyspnoea	Controls
N[a]	336	123	39	171	214	225	221
Age (mean ± SD)	58.4±7.52	58.2±7.23	62.7±6.20	58.6±7.26	59.1±7.66	60.1±7.26	56.9±6.85
Males, n (%)	178 (53.0)	57 (46.3)	21 (53.9)	78 (45.6)	94 (43.9)	81 (36.0)	128 (57.9)
Smoking status							
Non-smokers, n (%)	143 (42.6)	52 (42.3)	11 (28.2)	79 (46.2)	104 (48.6)	95 (42.2)	132 (59.7)
Past-smokers, n (%)	125 (37.2)	35 (28.5)	13 (33.3)	68 (39.8)	65 (30.4)	89 (39.6)	76 (34.4)
Current smokers, n (%)	68 (20.2)	36 (29.3)	15 (38.5)	24 (14.0)	45 (21.0)	41 (18.2)	13 (5.9)
Pack years							
Past smokers (Med and IQR)	18 (6, 36)	16 (2, 27)	39 (27, 43)	16 (5, 31)	16 (4, 30)	18 (6, 45)	9 (4, 16)
Current smokers (Med and IQR)	32 (17, 47)	34 (19, 47)	36 (18, 47)	32 (18, 44)	27 (18, 44)	36 (17, 47)	33 (6, 36)
Lung function parameters							
FEV_1, l (mean ± SD)	2.89±0.84	2.60±0.76	2.10±0.79	2.79±0.79	2.67±0.74	2.61±0.76	3.58±0.74
FEV_1 % predicted (mean ± SD)	95.3±19.7	89.0±19.6	71.2±20.9	94.8±20.4	92.6±18.2	95.0±22.0	113.6±12.3
FEV_1/FVC % (mean ± SD)	71.3±10.2	67.5±10.0	56.5±10.8	71.4±9.61	69.3±9.01	71.8±10.3	79.0±4.28
FVC, l (mean ± SD)	4.06±1.05	3.88±1.10	3.63±1.12	3.93±0.99	3.87±1.05	3.64±0.91	4.53±0.93
FVC % predicted (mean ± SD)	105.5±15.4	104.4±15.9	97.0±17.4	105.4±16.9	105.8±15.9	104.8±17.0	113.2±11.8
$D_L$co ml/min/mmHg (mean ± SD)	22.8±6.18	22.5±6.50	20.8±6.56	22.4±5.82	22.2±6.02	20.4±5.45	25.9±4.71
$D_L$co % predicted (mean ± SD)	94.6±16.8	96.6±18.1	85.3±19.9	95.5±15.6	96.3±15.8	93.0±18.3	103.8±11.1

[a] With the exception of the controls, the outcome groups may not be mutually exclusive

Respiratory symptoms, current asthma and single β_2AR polymorphisms

The associations between individual polymorphisms and risk of current asthma and respiratory symptoms are reported in Tables 2 and 3. A significantly increased risk of wheeze, asthma and COPD was found for individuals with the Arg16/Arg16 genotype.

For the β_2AR27 polymorphism we found a higher prevalence of BHR and asthma. There was also a marginally increased risk of the respiratory symptoms of nocturnal chest tightness and dyspnoea in individuals homozygous for the Gln27 genotype. Also a marginally lower $FEF_{25–75}$ % predicted in individuals homozygous for the Gln27 genotype was found (regression coefficient –5.54; 95% CI –11.6, 0.48, P=0.07). There was no evidence of an interaction between either polymorphism and ever smoking for any of the outcomes investigated.

Haplotype analysis

In order to investigate the combined effect of the two SNPs, haplotype analysis was also performed. The results of the haplotype analyses are presented in Table 4. The Arg16/Gln27 haplotype was associated with an increased risk of asthma and COPD. A marginal association between the Arg16/Gln27 haplotype and symptoms of wheeze, and dyspnoea, and BHR was found. The Arg16/Gln27 haplotype was also associated with a lower $FEF_{25–75}$ % predicted, but this did not quite reach statistical significance (regression coefficient –2.95; 95% CI –6.12, 0.22, P=0.07).

Discussion

The Arg16/Gln27 haplotype was associated with current asthma, BHR and COPD in this sample of middle-aged and older adults. Our study is one of the largest of older adults from the general population, and one of the first to investigate β_2AR haplotypes in this age group.

For the SNPs individually, the association with asthma was strongest in the Arg16/Gly16 heterozygotes. Our results are consistent with a Chinese study that reported an association between the Arg16 allele and asthma in cigarette smokers (Wang et al. 2001). However, a study of childhood asthma found an association with the Glu27 allele (Hopes et al. 1998), and another study found nocturnal asthma to be associated with the Gly16 allele (Turki et al. 1995). Furthermore, many other studies have reported no association with asthma at all (Dewar et al. 1998; Martinez et al. 1997; Ramsay et al. 1999; Reihsaus et al. 1993; Summerhill et al. 2000; Turner et al. 2004). Only one study has looked at β_2AR haplotypes and risk of asthma, which found the Gly16/Gln27 haplotype was more prevalent

Table 2 Association between *β_2AR*16 genotypes and respiratory symptoms and conditions

*β_2AR*16 genotype	Cases *n* (%)	Controls *n* (%)	OR (95% CI)[a]	*P*
Wheeze				
Arg16/Arg16	52 (15.5)	21 (9.5)	1.84 (1.02, 3.35)	0.04
Arg16/Gly16	165 (49.1)	102 (46.2)	1.18 (0.80, 1.73)	0.41
Gly16/Gly16	119 (35.4)	98 (44.3)	1.0	
Asthma				
Arg16/Arg16	18 (14.6)	21 (9.5)	2.44 (1.12, 5.31)	0.02
Arg16/Gly16	69 (56.1)	102 (46.2)	1.72 (1.01, 2.92)	0.05
Gly16/Gly16	36 (29.3)	98 (44.3)	1.0	
COPD				
Arg16/Arg16	9 (23.1)	21 (9.5)	5.13 (1.40, 18.8)	0.01
Arg16/Gly16	21 (53.9)	102 (46.2)	2.12 (0.78, 5.77)	0.14
Gly16/Gly16	9 (23.1)	98 (44.3)	1.0	
Nocturnal chest tightness				
Arg16/Arg16	22 (12.9)	21 (9.5)	1.41 (0.69, 2.86)	0.35
Arg16/Gly16	89 (52.1)	102 (46.2)	1.22 (0.77, 1.92)	0.40
Gly16/Gly16	60 (35.1)	98 (44.3)	1.0	
BHR				
Arg16/Arg16	30 (14.0)	21 (9.5)	1.67 (0.86, 3.24)	0.13
Arg16/Gly16	108 (50.5)	102 (46.2)	1.18 (0.76, 1.82)	0.46
Gly16/Gly16	76 (35.5)	98 (44.3)	1.0	
Dyspnoea				
Arg16/Arg16	31 (13.8)	21 (9.5)	1.29 (0.60, 2.48)	0.59
Arg16/Gly16	120 (53.3)	102 (46.2)	1.20 (0.76, 1.88)	0.44
Gly16/Gly16	74 (32.9)	98 (44.3)	1.0	

[a] Adjusted for sex, age, smoking and pack years

Table 3 Association between β_2AR27 genotypes and respiratory symptoms and conditions

β_2AR27 genotype	Cases *n* (%)	Controls *n* (%)	OR (95% CI)[a]	*P*
Wheeze				
Gln27/Gln27	114 (33.9)	59 (26.7)	1.30 (0.79, 2.13)	0.30
Gln27/Glu27	150 (44.6)	109 (49.3)	0.88 (0.56, 1.39)	0.58
Glu27/Glu27	72 (21.4)	53 (24.0)	1.0	
Asthma				
Gln27/Gln27	46 (37.4)	59 (26.7)	2.08 (1.05, 4.13)	0.04
Gln27/Glu27	56 (45.5)	109 (49.3)	1.17 (0.61, 2.24)	0.64
Glu27/Glu27	21 (17.1)	53 (24.0)	1.0	
COPD				
Gln27/Gln27	24 (32.9)	59 (26.7)	1.64 (0.69, 3.87)	0.26
Gln27/Glu27	37 (50.7)	109 (49.3)	1.09 (0.49, 2.43)	0.84
Glu27/Glu27	12 (16.4)	53 (24.0)		
Nocturnal chest tightness				
Gln27/Gln27	55 (32.2)	59 (26.7)	1.77 (0.96, 3.26)	0.07
Gln27/Glu27	87 (50.9)	109 (49.3)	1.41 (0.80, 2.48)	0.24
Glu27/Glu27	29 (17.0)	53 (24.0)	1.0	
BHR				
Gln27/Gln27	76 (35.5)	59 (26.7)	1.92 (1.07, 3.46)	0.03
Gln27/Glu27	104 (48.6)	109 (49.3)	1.37 (0.80, 2.37)	0.25
Glu27/Glu27	34 (15.9)	53 (24.0)	1.0	
Dyspnoea				
Gln27/Gln27	84 (37.3)	59 (26.7)	1.65 (0.91, 3.01)	0.10
Gln27/Glu27	104 (46.2)	109 (49.3)	1.01 (0.57, 1.77)	0.98
Glu27/Glu27	37 (16.4)	53 (24.0)	1.0	

[a] Adjusted for sex, age, smoking and pack years

Table 4 Association of β_2AR16 and 27 haplotypes and risk of respiratory symptoms and conditions

Symptom	Haplotype[a]	Cases	Controls	OR (95% CI)[b]	*P*
Wheeze	Arg16/Gln27	0.40030	0.32579	1.28 (0.97, 1.70)	0.08
	Gly16/Gln27	0.16220	0.18778	0.95 (0.67, 1.35)	0.78
	Gly16/Glu27	0.43750	0.48643	1.0	
Asthma	Arg16/Gln27	0.42683	0.32579	1.63 (1.12, 2.38)	0.01
	Gly16/Gln27	0.17480	0.18778	1.22 (0.75, 1.99)	0.43
	Gly16/Glu27	0.39837	0.48643	1.0	
COPD	Arg16/Gln27	0.50000	0.32579	2.91 (1.42, 5.94)	0.004
	Gly16/Gln27	0.17949	0.18778	2.07 (0.89, 4.83)	0.093
	Gly16/Glu27	0.32051	0.48643	1.0	
Nocturnal chest tightness	Arg16/Gln27	0.38889	0.32579	1.30 (0.92, 1.83)	0.137
	Gly16/Gln27	0.18713	0.18778	1.37 (0.90, 2.07)	0.141
	Gly16/Glu27	0.42398	0.48643	1.0	
BHR	Arg16/Gln27	0.39252	0.32579	1.35 (0.98,1.86)	0.07
	Gly16/Gln27	0.20561	0.18778	1.41 (0.94,2.11)	0.09
	Gly16/Glu27	0.40187	0.48643	1.0	
Dyspnoea	Arg16/Gln27	0.40444	0.32579	1.26 (0.89, 1.77)	0.19
	Gly16/Gln27	0.20000	0.18778	1.46 (0.98, 2.18)	0.06
	Gly16/Glu27	0.39556	0.48643	1.0	

[a] Rare haplotype (Arg16/Glu27) excluded due to very low prevalence

[b] Adjusted for sex, age, smoking and pack years

in moderate asthmatics than in mild asthmatics (Weir et al. 1998). This is different to our reported association with Arg16/Gln27, which may be because our subjects had milder asthma. Unlike Weir et al. (1998), we were able to resolve β_2AR haplotypes for subjects heterozygous at both polymorphisms, because we used the E-M algorithm to infer phase in all subjects.

An increased risk of COPD was associated with Arg16 homozygous genotype, and COPD and $FEF_{25–75}$ were associated with possession of the Arg16/Gln27 haplotype. The measurement of $FEF_{25–75}$ is a feature of small airways disease, which has been shown to progress to COPD (Hogg 2004). These results are in contrast to two previous studies, one from Taiwan that found the Arg16 allele to be less prevalent in COPD cases (Ho et al. 2001) and the other from Egypt that found the Gln27 allele to be less prevalent in COPD cases (Hegab et al. 2004). The LHS found a protective

effect of the Glu27/Gln27 genotype on rapid decline in lung function, but no association with any of the β_2AR haplotypes (Joos et al. 2003). Further studies of β_2AR haplotypes in COPD are needed to confirm our association in other populations.

We found a significant association between respiratory symptoms of wheeze and dyspnoea with the Arg16 genotype and the Arg16/Gln27 haplotype. These associations suggest that the Arg16/Gln27 haplotype may be associated with more severe respiratory symptoms. A previous Australian study also reported an association between wheeze with a cold and the Arg16 allele (Ramsay et al. 1999). As mentioned previously, however, Weir et al. (1998) found a higher prevalence of the Gly16/Gln27 haplotype in subjects with moderate to severe asthma. The difference from our results may be because our community-based subjects had milder asthma.

Studied in vitro, polymorphisms at positions 16 and 27 have been shown to alter expression of the β_2AR protein. The Gly16 form experiences enhanced degradation of the receptor during agonist exposure compared to the Arg16 form, as does the Gln27 form compared with the Glu27 form (Liggett 1997). Drysdale et al. (2000) investigated multiple SNPs in β_2AR and found the haplotype combination of Arg16/Gln27 was associated with a reduced response to β_2-agonist (Drysdale et al. 2000). They also found that the β_2AR mRNA and protein levels of this haplotype were significantly reduced compared to other haplotypes. In vivo work with humans has also shown Arg16 subjects have more rapid decline in FEF_{50} during exercise than Gly16 subjects (Snyder et al. 2006). These results support our association with the Arg16/Gln27 haplotype, and suggest subjects with this haplotype experience more respiratory symptoms due to their reduced response to endogenous catecholamines and β_2-agonists.

Furthermore, in vivo studies of the β_2AR polymorphisms and response to β_2-agonist therapy have been conducted, and there is accumulating evidence that the Arg16 allele is associated with worse response to β_2-agonist (Israel et al. 2000, 2004; Lee et al. 2004; Martinez et al. 1997). Conversely, a study by Tan et al. (1997) found significantly greater bronchodilator desensitisation in Gly16 homozygotes. However, some studies have not found any evidence of an effect of the β_2AR polymorphisms on response to β_2-agonist therapy (Hancox et al. 1998; Lipworth et al. 1999). These discrepancies are most likely due to the small sample sizes in most studies, and highlight the need for larger studies to determine more accurately the effect of these polymorphisms on bronchodilator response.

We found that Gln27 homozygotes and individuals with the Arg16/Gln27 haplotype had a higher prevalence of BHR. This is consistent with previous studies that have found individuals with the Glu27 allele to be less responsive to histamine (Ramsay et al. 1999) and methacholine (Hall et al. 1995) than those with the Gln27 allele. Furthermore, an American study that used haplotype estimation also found the Arg16/Gln27 haplotype was positively associated with BHR (Litonjua et al. 2004). However, our results are contrary to those of two other haplotype studies of BHR. One study observed an increased frequency of the Gly16/Gln27 haplotype among BHR-positive subjects, but only studied males, and found a higher overall frequency of the Gly16/Gln27 haplotype in their population than we did in ours (D'Amato et al. 1998). The second study found the Gly16/Gln27/Thr164 haplotype to be underrepresented in the BHR cases compared to the controls, resulting in a protective effect of this haplotype (Ulbrecht et al. 2000). Despite the inconsistencies, there is still mounting evidence to suggest that the β_2AR haplotype influences BHR.

One potential limitation of our study is that we did not determine haplotype directly, but instead inferred phase using the E-M algorithm. There have been some concerns regarding the accuracy of this method; however, recent studies have demonstrated that estimated haplotype frequencies accurately reflect the true frequencies for common polymorphisms (Tishkoff et al. 2000). In line with other studies of Caucasian populations, we have found the Arg16/Glu27 haplotype to be extremely rare. In three previous haplotype studies the Arg16/Glu27 was not found at all, or—if using the E-M algorithm to infer phase—present at extremely low frequencies (D'Amato et al. 1998; Joos et al. 2003; Litonjua et al. 2004; Ulbrecht et al. 2000). The prevalence of the other haplotypes in our study was very similar to those reported in other Caucasian populations (Litonjua et al. 2004; Ulbrecht et al. 2000).

Association studies are susceptible to bias as a result of population stratification caused by ethnic variation within the sample. We have attempted to minimise this bias by analysing only those subjects of Caucasian descent. Due to the number of analyses conducted with many different outcomes, it might be argued that adjustment for multiple comparisons is necessary. However, for both SNPs there was an a priori hypothesis of an individual association with the outcomes studied. This assumption was based on the important role of this gene in modulating response to endogenous and exogenous catecholamines. The associations were consistent across both individual SNPs and the haplotype analysis, which suggests a genuine

association. Therefore, the *P* values for the statistical tests completed are presented as they were performed.

In conclusion, we have shown the Arg16/Gln27 haplotype is associated with COPD, asthma and BHR in middle-aged and older adults. We have provided the first evidence that β_2AR haplotype may be important in COPD. We have provided further evidence that haplotype analysis is important when assessing multiple polymorphisms with functional effects on the receptor. Our results would suggest that β_2AR genotype may be an important modulating factor in risk of COPD, asthma and related phenotypes. This study provides good evidence that further randomised clinical trials of β_2-agonist response in specific haplotype groups are needed to fully elucidate the importance of β_2AR haplotypes in modulating response to therapy. In the future, genetic screening for β_2AR genotype may be worthwhile in patients prescribed β_2-agonists, to predict those who will most benefit from this type of therapy. This may be particularly relevant to individuals with COPD, and further work is needed to determine what effect β_2AR has on COPD bronchodilator responsiveness.

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