

Association of β_2 -adrenoreceptor polymorphisms with nocturnal cough among atopic subjects but not with atopy and nonspecific bronchial hyperresponsiveness

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Background: The reports from in vitro studies that β_2 -adrenoreceptor (*B2AR*) polymorphisms are associated with agonist-promoted downregulation have evoked considerable research interest for the roles of these polymorphisms to the pathogenesis of asthma.

Objective: We sought to evaluate the association between asthma phenotypes and *B2AR* polymorphisms at 2 sites (Arg16 → Gly16 and Gln27 → Glu27) in the general population.

Methods: Four hundred forty unrelated Korean adults were randomly selected, and asthma phenotypes were determined with a questionnaire, immunoassay, skin prick testing, and methacholine bronchial provocation testing. Genotypes of *B2AR* polymorphisms were determined with PCR-based methods.

Results: No significant association was found between *B2AR* alleles and haplotypes and total IgE levels, skin test responses to aeroallergens, and bronchial responsiveness to methacholine. Among the atopic subjects, however, numbers of both Arg16 alleles and Arg16-Gln27 haplotypes were negatively associated with nocturnal cough, and in contrast, Gly16-Gln27 was positively associated with it.

Conclusion: *B2AR* polymorphisms may play an important role in the expression of nocturnal cough in atopic subjects but not in the expression of atopy and bronchial hyperresponsiveness in a general population. (*J Allergy Clin Immunol* 2002;109:630-5.)

Key words: β_2 -Adrenoreceptor, asthma phenotype, polymorphism, nocturnal cough

Abbreviations used

B2AR: β_2 -Adrenoreceptor gene

BHR: Bronchial hyperresponsiveness

DRS: Slope of dose-response curve

The chromosome 5q31-33 contains several candidate genes, including the β_2 -adrenoreceptor (*B2AR*) gene, which have been implicated in the pathogenesis of asthma.¹ β_2 -Adrenoreceptors are G protein-coupled receptors that mediate the actions of catecholamines in multiple tissues.² All β_2 -adrenoreceptors have an extracellular amino terminus, 7 transmembrane-spanning domains, 3 intracellular and 3 extracellular loops, and an intracellular carboxyl terminus. Nine single-nucleotide polymorphisms have been found in the *B2AR* gene,³ of which 2 are more frequent and gave rise to amino acid exchanges in the putative extracellular aminoterminal region of the gene: *B2AR* at codon 16, with replacement of Arg for Gly, and *B2AR* at codon 27, with replacement of Gln for Glu. The polymorphisms of *B2AR* have been reported to be associated with asthma severity⁴⁻⁶; Glu27 allele is associated negatively with bronchial hyperresponsiveness (BHR) in asthmatic subjects,⁷ and Gln27 allele is associated positively with IgE levels.⁸ In addition, Gly16-Gln27 haplotype is suggested to be positively associated with BHR in a population study.⁹

Recurrent wheezing and nocturnal cough are the characteristic symptoms of asthma. The genetic contribution to the development of these symptoms can be separated from the inherited tendency to generate excessive IgE.¹⁰ In addition, wheezing and cough are segregated separately in asthmatic families, and the nocturnal phenotype of asthma was reported to be associated with the Gly16 allele of the *B2AR* gene.¹¹ However, no information is available on the correlation between the haplotypes of the *B2AR* gene and these asthmatic symptoms in the general population.

The aim of this study was to evaluate, in the general population, the association between the *B2AR* polymorphisms and asthma phenotypes, such as asthma symptoms, total serum IgE levels, skin responses to common aeroallergens, and bronchial responsiveness to methacholine.

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METHODS

Subjects

Seven hundred eighteen adults were randomly recruited for a cross-sectional study to evaluate the prevalence of adult asthma, which was performed from June to August 1999, on the basis of age and sex profile of the general population from metropolitan Seoul, Korea, and 440 of the 718 were randomly selected for the association study. The mean age of the 440 subjects was 35.5 years (range, 20-75 years). There were 219 male and 221 female subjects. All the subjects responded to a questionnaire, and 9.1% and 19.6% of the questionnaire responders had experienced recurrent wheezing and nocturnal cough during the last 12 months, respectively. Four hundred nineteen of the 440 subjects gave serum for total IgE levels. Four hundred thirty-three underwent skin prick tests to 10 common aeroallergens, and 424 underwent methacholine bronchial provocation tests. Positive skin test responses to one or more aeroallergens and BHR to methacholine were demonstrated in 24.5% and 12.7% of the subjects tested, respectively. The subjects gave written informed consent for the study, and the study protocol was approved by the Ethics Committee of Seoul National University Hospital.

Clinical phenotyping

A questionnaire developed by the American Thoracic Society¹² was modified and translated into Korean. The questionnaire included questions regarding demographic data and asthma symptoms. The questions on asthma symptoms concentrated on recurrent wheezing and nocturnal cough without respiratory tract infection experienced during the previous 12 months.

None of the subjects had received oral or inhaled bronchodilators for 5 days preceding methacholine bronchial provocation testing. Subjects with upper respiratory tract infections during the last 2 weeks were excluded from the methacholine challenge. A total of 424 subjects underwent the methacholine challenge, according to the methods described by Chai et al.¹³ Spirometric lung function was measured with a portable spirometer (Micro Spirometer, Micro Medical), and the largest value of triplicate FEV₁ and forced vital capacity measurements was adopted. Concentrations of 1.25, 2.5, 6.25, 12.5, and 25 mg/mL methacholine were prepared by means of dilution with buffered saline, and 5 inspiratory capacity breaths of the increasing methacholine concentrations were sequentially inhaled until FEV₁ fell to less than 80% of its baseline value or until the highest concentration was reached. A Rosenthal-French dosimeter (Laboratory for Applied Immunology) was used to deliver the aerosol generated by a nebulizer (DeVilbiss). Compressed air at 20 psi was connected to the input value of the dosimeter, which was set at 0.6 seconds per inhalation. The largest value of triplicate FEV₁ at 90 or 180 seconds after each inhalation was adopted for analysis. Results were expressed as PC20, and the bronchial responsiveness to methacholine was regarded as positive in this respect if the PC20 was lower than 25 mg/mL. The slope of the dose-response curve (DRS) was also calculated as follows: percentage fall of FEV₁ divided by log scale of the last concentration (in milligrams per milliliter) of methacholine.

Total serum IgE levels were determined in 419 subjects by using an ELISA, as previously described.¹⁴ The subjects who received antihistamines orally during the 5 days preceding the skin prick testing and had dermatographism were excluded from the testing. A total of 433 subjects underwent the testing. The skin testing was performed with a panel of 10 common aeroallergens (Allergopharma Co), which included *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, cat fur, *Aspergillus fumigatus*, *Alternaria alternata*, tree pollen mixture, grass pollen mixture, mugwort pollen, ragweed pollen, and cockroach. A positive control of histamine (1 mg/mL), along with a negative diluent control, was included in all tests. Fifteen minutes after the prick test, the mean diameter of a

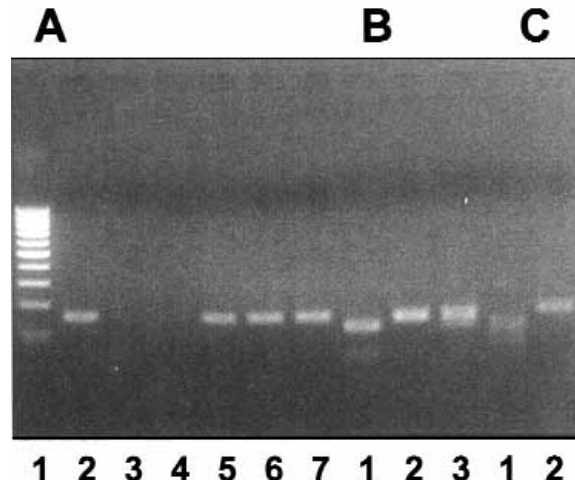


FIG 1. Allele-specific PCR and *B2AR* PCR-restriction fragment length polymorphism for the determination of *B2AR* alleles and haplotypes at codons 16 and 27. **A**, lanes 1 and 2, Arg16 homozygote; lanes 3 and 4, Gly16 homozygote; lanes 5 and 6, heterozygote. **B**, lane 1, Gln27 homozygote; lane 2, Glu27 homozygote; lane 3, heterozygote. **C**, lanes 1 and 2, Arg16/Gln27 and Gly16/Glu27 haplotypes.

wheel formed by the allergen was compared with that formed by histamine. If the former was the same or larger than the latter (allergen/histamine ratio of 1.0), the reaction was defined as positive.

B2AR genotyping

Genomic DNA was extracted from peripheral blood with a Wizard Genomic DNA Purification Kit (Promega Corp). A 219-bp fragment of the *B2AR* gene was amplified with the following primers designed by using Oligo Primer Analysis Software Version 5 (Molecular Biology Insights, Inc): 5'-AGC CAG TGC GCT TAC CTG CCA GAC T-3' (sense) and 5'-GCT CGA ACT TGG CAA TGG CTG TGA-3' (antisense). PCR was performed in a GeneAmp 2400 thermal cycler (Perkin Elmer) in a reaction volume of 25 μ L by using 200 ng of DNA, 1.5 mmol/L MgCl₂, 200 mmol/L deoxyribonucleoside triphosphates, 10 pmol of each primer, and 0.25 U Taq polymerase (Boehringer Mannheim) in standard PCR buffer (10 mmol/L Tris-HCl [pH 8.3] and 50 mmol/L KCl). PCR involved an initial denaturation at 95°C for 2 minutes, followed by 35 cycles of denaturation at 95°C for 40 seconds, annealing at 60°C for 40 seconds, and extension at 72°C for 40 seconds, followed by a final extension at 72°C for 5 minutes. Five microliters of PCR product was electrophoresed on a 2% agarose gel and visualized with ethidium bromide staining and UV illumination to check PCR amplification.

Allele-specific PCR was performed to assess polymorphisms at nucleic acid 46, which results in a change in the encoded amino acid at position 16. Five microliters of primary PCR product was used as the template for allele-specific PCR. The primer pairs (upstream for Arg16, 5'-CTT CTT GCT GGC ACC CTA TA-3'; upstream for Gly16, 5'-CTT CTT GCT GGC ACC CTA TG-3'; downstream, 5'-GGC AAT GGC TGT GAT GAC C-3') were used to delineate the polymorphism at nucleic acid 46 (amino acid 16). A 151-bp fragment of PCR product was obtained by using the following conditions: 30 cycles of 95°C for 30 seconds, 62°C for 50 seconds, and 72°C for 30 seconds. Five microliters of the PCR product was then electrophoresed on 2% agarose gels (Fig 1, A).

PCR-restriction fragment length polymorphism was used to assess polymorphisms at nucleic acid 79, which results in a change in the encoded amino acid at position 27. PCR reaction was carried

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TABLE I. Association between qualitative phenotypes of asthma and *B2AR* genotypes

Genotypes	No. (%)	Asthma symptoms		BHR	SPT
		Wheezing	Cough		
Arg16 (or Arg16-Gln27)		NS	NS	NS	NS
Nil	94 (21.4)	7.4	18.3	9.7	23.7
One	223 (50.7)	11.2	20.5	11.7	28.3
Two	123 (28.0)	6.6	19.0	16.9	18.2
Gln27		NS	NS	NS	NS
Nil	3 (0.7)	0	0	0	0
Hetero	85 (19.3)	9.4	22.9	14.6	27.1
Homo	352 (80.0)	9.1	19.0	12.4	24.1
Gly16-Gln27		NS	NS	NS	NS
Nil	177 (40.2)	8.0	20.1	15.4	21.7
One	206 (46.8)	10.2	19.7	12.6	26.2
Two	57 (13.0)	8.8	17.5	5.4	26.8

Data were expressed as percentage of positive responses. *BHR* indicates a PC20 methacholine value of 25 mg/mL or less. *SPT* indicates positive skin prick test responses (allergen/histamine ratio of ≥ 1.0) to one or more of 10 common aeroallergens.

NS, Not significant ($P > .05$, χ^2 trend test for linear association within groups).

TABLE II. Association between quantitative phenotypes of asthma and *B2AR* genotypes

Phenotypes/genotypes	DRS	Log IgE
Arg16 (or Arg16-Gln27)	NS	NS
Nil	7.85 $\pm$ 7.37	1.87 $\pm$ 0.75
One	7.85 $\pm$ 8.06	1.98 $\pm$ 0.75
Two	8.65 $\pm$ 8.04	1.98 $\pm$ 0.85
Gln27	NS	NS
Nil	6.39 $\pm$ 4.45	1.82 $\pm$ 0.6
Hetero	9.29 $\pm$ 8.38	1.97 $\pm$ 0.75
Homo	7.79 $\pm$ 7.79	1.96 $\pm$ 0.79
Gly16-Gln27	NS	NS
Nil	8.64 $\pm$ 7.84	1.99 $\pm$ 0.81
One	8.00 $\pm$ 8.45	1.95 $\pm$ 0.77
Two	6.60 $\pm$ 5.64	1.89 $\pm$ 0.74

Data were expressed as means $\pm$ SD. DRS indicates percentage fall of FEV₁ divided by log (last concentration of methacholine, in milligram per milliliter). Log IgE indicates log scale of total IgE levels (in international units). NS, Not significant ($P > .05$, Student *t* test and ANOVA between groups).

out by using 5 μ L of primary PCR product as a template. A 139-bp fragment of PCR product was obtained by using the following primers: GCC ATG CGC CGG ACC ACG AC-3' (sense) and 5'-CGC TCG AAC TTG GCA ATG GCT GTG A-3' (antisense). Thirty cycles of PCR were carried out 95°C for 30 seconds, 65°C for 30 seconds, and 72°C for 30 seconds. The PCR products were digested with *Ita1* restriction enzyme (Roche), run on a 2% agarose gel, and stained with ethidium bromide. The samples were compared with undigested (139 bp) or digested (112 bp) fragments (Fig 1, B). Heterozygous samples at both positions were further characterized with PCR-restriction fragment length polymorphism to determine haplotype. Briefly, amplified products by allele-specific PCR were digested with the addition of *Ita1* restriction enzyme (Fig 1, C). Primary PCR products were sequenced with AmpliCycle Sequencing kit (Perkin Elmer) to confirm haplotype detection, according to manufacturer's instructions, in 30 randomly selected products from each haplotype.

Statistical analysis

Allele and haplotype frequencies were estimated by counting. For statistical significance of qualitative dependent variables, the χ^2 test

and the χ^2 test for trend were used for 2 and for more than 2 independent variables, respectively. For quantitative dependent variables, the Student *t* test and ANOVA were used for 2 and for more than 2 independent variables, respectively. Risk factor analysis of asthma phenotypes was assessed by using odds ratios with 95% confidential intervals. A *P* value of .05 or less was regarded as significant.

RESULTS

A total of 440 subjects underwent *B2AR* genotyping by using a PCR-based method that allowed identification of all 4 possible haplotypic combinations of relevant polymorphism at codons 16 and 27. Table I shows the frequency of *B2AR* alleles and haplotypes. The allele frequencies at codon 16 were as follows: homozygous Arg, 28.0%; ArgGly, 50.7%; and homozygous Gly, 21.4%. The allele frequencies at codon 27 were 80.0% of homozygous Gln, 19.3% of GlnGlu, and 0.7% of homozygous Glu. *B2AR* haplotypes were defined by codons 16 and 27, and 3 different haplotypes were present in the studied population: Arg16/Gln27, Gly16/Glu27, and Gly16/Gln27. Haplotype pairs of heterozygous ArgGln/GlyGln, homozygous ArgGln and GlyGln, and heterozygous ArgGln/GlyGlu, in order of decreasing frequency, were observed in more than 10% of the subjects. As for Gly16Gln27 haplotype, 57 (13.0%) of the subjects had 2, 206 (46.8%) had 1, and 177 (40.2%) had none of the haplotypes.

Table I shows association between *B2AR* alleles and haplotypes and qualitative phenotypes of asthma phenotypes, such as asthma symptoms, methacholine BHR, and skin responses to aeroallergens, in the general population. No significant association was observed between the *B2AR* alleles at codons 16 and 27 and recurrent wheezing and nocturnal cough during the last 12 months, skin test responses to aeroallergens, and methacholine BHR. No significant association was observed between the number of Gly16-Gln27 haplotype and these qualitative phenotypes. Table II shows association between *B2AR* alleles and haplotypes and the quantitative phenotypes of asthma phenotypes, such as DRS and total

TABLE III. Association between asthma phenotypes and *B2AR* genotypes according to skin responses to allergens

Genotypes	Positive skin responses				Negative skin responses			
	No. (%)	Wheezing	Cough	BHR	No. (%)	Wheezing	Cough	BHR
Arg16 (Arg16-Gln27)		<.05*	<.05†	NS		NS	NS	NS
Nil	22 (20.8)	18.2	40.9	4.8	71 (21.7)	4.2	11.4	11.3
One	62 (58.5)	12.9	21.0	15.3	157 (58.5)	10.2	20.6	10.7
Two	22 (20.8)	0	9.0	22.7	99 (30.3)	8.1	21.4	15.8
Gln27		NS	NS	NS		NS	NS	NS
Nil	0	—	—	—	3 (0.9)	0	0	0
Hetero	23 (21.7)	17.4	21.7	13.6	62 (19.0)	6.5	23.3	15.0
Homo	83 (78.3)	9.8	23.2	15.0	262 (80.1)	8.8	18.1	11.9
Gly16-Gln27		NS	<.05†	NS		NS	NS	NS
Nil	38 (35.8)	8.1	10.5	21.6	137 (41.9)	8.0	23.0	13.7
One	53 (50.0)	11.3	26.4	11.8	149 (45.6)	9.4	17.7	13.2
Two	15 (14.2)	20.0	40.0	7.1	41 (12.5)	4.9	9.8	4.9

Data were expressed as percentages of positive responses. *BHR* indicates PC20 methacholine value of 25 mg/mL or less.

NS, Not significant ($P > .05$, χ^2 test between groups of alleles and χ^2 trend test for linear association within groups).

* $P < .05$, χ^2 test between subjects with both and neither of Arg16 or Arg16-Gln27.

† $P < .05$, χ^2 test between groups of alleles or haplotypes and χ^2 trend test for linear association within groups.

serum IgE levels in the general population. No significant association was observed between the *B2AR* alleles at codons 16 and 27 and DRS and total serum IgE levels. No significant association was observed between the number of Gly16-Gln27 haplotypes and these quantitative phenotypes.

The subjects were subdivided into those with positive and negative skin responses to evaluate the association between *B2AR* gene polymorphisms and the expression of the qualitative phenotypes of asthma according to skin test responses to common aeroallergens (Table III). The prevalence of 12 months of wheezing was higher in subjects with positive skin responses with no Arg16 allele (or no Arg16Gln27 haplotype) than in those with 2 Arg16 alleles (or homozygous Arg16Gln27 haplotype), although it was associated with Gln27 allele and Gly16Gln27 haplotype. The prevalence of recurrent nocturnal cough during the last 12 months was negatively associated with numbers of Arg16 allele and Arg16Gln27 haplotype in subjects with positive skin responses ($P < .05$, χ^2 test between groups of alleles or haplotypes and χ^2 trend test for linear association within groups, respectively). In contrast, the prevalence of nocturnal cough was positively associated with numbers of Gly16Gln27 haplotype in subjects with positive skin responses ($P < .05$, χ^2 test between groups and χ^2 trend test for linear association within groups, respectively). The prevalence of nocturnal cough was not associated with numbers of Gln27 alleles. Meanwhile, the prevalence of methacholine BHR was not associated with the *B2AR* alleles and haplotypes in subjects with positive skin responses. Among subjects with negative skin responses, however, the prevalences of wheeze, nocturnal cough, and methacholine BHR were not significantly associated with the *B2AR* alleles and haplotypes.

As for other risk factors for nocturnal cough, as shown in Table IV, the prevalence of nocturnal cough in the subjects with positive skin responses was the same according to age, sex, and smoking status.

TABLE IV. Prevalence of nocturnal cough by age, sex, smoking status, and *B2AR* haplotypes in subjects with positive skin test responses to common aeroallergens

Risk factors	No.	Cough*	OR	95% CI
Age				
≤39 y	68	12 (17.6)	1	
≥40 y	37	12 (32.4)	2.24	0.80-6.26
Sex				
Female	52	11 (21.2)	1	
Male	53	13 (24.5)	1.21	0.44-3.32
Smoking status				
Nonsmoker-ex-smoker	74	16 (21.6)	1	
Current smoker	29	7 (24.1)	1.15	0.37-3.52
Arg16 (or Arg16-Gln27)†				
Nil	22	9 (40.9)	1	
One	62	13 (21.0)	0.38	0.11-1.23
Two	22	2 (9.0)	0.15	0.01-0.96
Gly16-Gln27†				
Nil	38	4 (10.5)	1	
One	53	14 (26.4)	2.96	0.79-11.8
Two	15	6 (40.0)	5.50	1.04-30.8

OR, Odds ratio; 95% CI, 95% confidence interval.

*Data were expressed as numbers of positive responses (%).

†Odds ratio adjusted for age, sex, and smoking status.

DISCUSSION

Our previous family studies demonstrated that intermediate phenotypes of asthma, such as total IgE, skin test responses to common aeroallergens, and bronchial responsiveness, are important in the development of nonatopic, as well as atopic, asthma,¹⁵ and a genetic marker of chromosome 5q31-33, in which the *B2AR* gene is located, was significantly linked to asthma phenotypes, such as skin test responses to aeroallergens and bronchial responsiveness.² On the basis of this background, the present study was undertaken to evaluate the association between the *B2AR* polymorphisms and asthma phenotypes at a population level. In this study, however, we did not find any evidence that the *B2AR* alleles

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and haplotypes at codons 16 and 27 were associated with total IgE levels, skin responses to common aeroallergens, or bronchial responsiveness to methacholine. These findings suggest that the *B2AR* polymorphisms do not account for our previously observed linkages between gene markers of the IL-4 cytokine gene cluster (5q31-q33) and asthma phenotypes, such as bronchial responsiveness to methacholine and skin responses to common aeroallergens.

A number of common polymorphisms of the *B2AR* gene have recently been described that alter the behavior of the receptor after agonist exposure.³ Studies on the *B2AR* gene identified a total of 9 different polymorphisms, all of which differ from the accepted wild-type sequence by a single base change at different positions in the coding sequence of the gene.³ Because of redundancy in the amino acid code, a number of these polymorphisms are clinically silent. However, 4 polymorphisms resulting from single base changes were identified that altered the amino acid sequence of the receptor protein. Two of these polymorphisms (Arg16 → Gly16 and Gln27 → Glu27) are common and have been studied in some detail. They appear to alter the functional properties of the receptor, such that the airways of individuals with these forms of the receptor might be expected to behave differently when exposed to circulating catecholamines or exogenously applied β_2 -agonists.¹⁶

Bronchial responsiveness to a number of stimuli is enhanced in many asthmatic subjects, and BHR represents a risk factor for the development of asthma. Although a heritable component of BHR has been demonstrated in family study¹⁷ and segregation analysis,¹⁸ environmental allergen exposure, viral respiratory infections, and air pollutants, including smoking, play a major role in the expression of BHR.¹⁹⁻²¹ The association studies between the *B2AR* polymorphisms and bronchial responsiveness in white populations have reported inconsistent results in case-control and cohort studies.^{9,22-24} D'Amato et al⁹ reported a significant association between the Gly16Gln27 haplotype and persistent BHR. Other investigators have not confirmed this association.^{8,22} The present study revealed that *B2AR* haplotypes were not associated with bronchial responsiveness to methacholine in the general population. To the contrary, a study from a German population reported that the Gly16Gln27Thr164 haplotype had a protective effect on the expression of BHR.²⁴ The reasons for these conflicting results are not clear but may be the result of heterogeneity between the samples studied or differences in definition of BHR.

An interesting finding of the present study is that the frequencies of *B2AR* alleles at codons 16 and 27 and *B2AR* haplotypes in Koreans are different from those already published in white and Hispanic populations.^{9,16,24} The allele frequencies of Arg16 and Gln27 reported by Martinez et al¹⁶ were 38% and 61% in unrelated white subjects, respectively, which is similar to other reports in white populations.^{9,24} The present study reveals that the frequency of the Arg16 allele in Koreans

was higher, whereas that of the Glu27 allele was lower than that seen in white subjects. The meaning of ethnic differences in the frequencies of *B2AR* polymorphisms has yet to be elucidated.

The beneficial effects of β -agonists and the detrimental effects of β -antagonists have justifiably suggested a role for *B2AR* dysfunction in bronchial asthma. Well-known effects of *B2AR* stimulation include bronchodilation, decreased mast cell degranulation, and increased mucus and water secretion.²⁵ Recurrent nocturnal cough without viral respiratory tract infection is a characteristic symptom in asthmatic subjects. This symptom is also common in nonasthmatic subjects, such as in subjects with postnasal drip syndrome and chronic bronchitis. Each cough involves a complex reflex arc, beginning with the stimulation of sensory nerves in the airway epithelium that function as cough receptors. Afferent impulses from these receptors are conducted to a cough center in the brainstem, the function of which is to receive these impulses and to produce a cough by activating efferent nervous pathways. Stimulation of *B2AR* inhibits both cholinergic and noncholinergic excitatory neurotransmission,²⁶ and blockade of these receptors may mediate β -antagonist-induced bronchospasm in asthmatic subjects,²⁷ suggesting that *B2AR* dysfunction may be related to the expression of asthma symptoms, such as cough and wheezing. Interestingly, the present study showed that the number of Arg16 alleles (or Arg16Gln27 haplotype) was negatively associated with the prevalence of nocturnal cough and wheezing in atopic subjects, whereas that of Gly16Gln27 was positively associated with the prevalence of nocturnal cough. Direct comparison of the wild-type and mutant forms of the *B2AR* gene in vitro indicate that the Gly16 and Gln27 receptors each undergo enhanced downregulation compared with the Arg16 and Glu27 receptors, respectively, whereas the agonist- and antagonist-binding affinities and coupling efficiencies to adenylate cyclase of the Gly16 and Glu27 receptors are similar to those of the Arg16 and Gln27 receptors.^{28,29} Taken together, Gly16 allele or Gly16Gln27 haplotype of the *B2AR* gene may increase the expression of respiratory symptoms, especially nocturnal cough, wheezing, or both, by means of enhanced downregulation when IgE-mediated inflammation is present in airway mucosa.

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