β 2-Adrenoreceptor Polymorphisms in Asthmatic Patients

Saeed Binaei, BS,¹ Sahar M. Rashed, PharmD, PhD,¹ and Michael L. Christensen, PharmD^{1,2}

¹Department of Pharmacy, ²Center for Pediatric Pharmacokinetic Therapeutics and Pediatric Pharmacology Research Unit, The University of Tennessee Health Science Center, and LeBonheur Children's Medical Center, Memphis, Tennessee

β 2-adrenergic receptors (β 2AR) are GTP-binding protein (G-protein) coupled receptors widely distributed in human tissue. Inhaled β 2-agonist drugs exert their primary effect on the β 2AR of bronchial smooth muscles, causing relaxation and bronchial dilatation. Polymorphisms in the β 2AR gene have been identified, which may affect responsiveness to β 2-agonists and disease severity in asthmatics. Nine single nucleotide polymorphisms (SNPs) within the coding region and eight SNPs within the 5' upstream region of the β 2AR gene have been identified. The two most studied polymorphisms are mutations in the coding region at codon 16, Arg to Gly (Arg16Gly) and at codon 27, Gln to Glu (Gln27Glu). Evidence suggests that carriers of Gly16, as well as carriers of Gln27, are prone to down-regulation of β 2AR. Patients who are homozygous for Arg16 and/or Glu 27 may be more susceptible to tachyphaxis with chronic use of β 2-agonists. Although β 2AR polymorphism is not related to the severity of asthma, patients with nocturnal asthma have higher frequency of Gly16. A polymorphism in the 5' upstream region, 5' leader cistron (5'LC), encodes for a protein that regulates mRNA transcription. The Cys19 polymorphism in the 5'LC is associated with higher expression of β 2AR. More recent studies have focused on combinations of polymorphisms across the gene region (haplotypes). The interaction of multiple SNPs within a haplotype may control β 2AR function resulting in different phenotypic response in patients with asthma. β 2AR polymorphism may have a significant implication in the pathophysiology of asthma and therapeutic response.

Keywords: β 2-Adrenoreceptor, polymorphisms, asthma, β 2-agonist

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INTRODUCTION

Inhaled selective β 2-agonist drugs are the most commonly used first-line bronchodilator therapy in the management of asthma.¹ β 2-agonists exert their action through interacting with β 2-adrenergic receptors (β 2AR) in bronchial smooth muscles causing bronchial relaxation.² The regular use of inhaled β 2-agonists has been associated with diminished responsiveness due to the development of tolerance (e.g., tachyphaxis or desensitization) or receptor down-regulation that may be a factor in the interpatient variability.³ Genetic variation (polymorphism) in

the β 2AR may also contribute to the variability response to inhaled β 2-agonists and also disease severity in asthmatic patients. This review will focus on the polymorphisms of the β 2AR and its potential implications in asthmatic patients.

β -ADRENERGIC RECEPTOR

β AR have been classified into β 1, β 2 and β 3 subgroups, and there is a 65–70% homology among them.⁴ β AR are important targets for endogenous catecholamine substances (e.g., epinephrine and norepinephrine), and exogenous agents (e.g., β -agonists and β -antagonists). β 1ARs are found in the heart, kidneys and adipose tissues. The endogenous catecholamines and β 1-agonists increase the heart rate, inotropy, renin and lipolysis.⁴ β 3AR are found in adipose tissue where their activation increases lipolysis. β 2AR are widely distributed and play an important role in regulating pulmonary, vascular, metabolic and cardiac functions. The endogenous

Address correspondence and reprint requests to Michael L. Christensen, PharmD; LeBonheur Children's Medical Center; 50 North Dunlap Room 301; Memphis, TN 38103
e-mail: mchristensen@utmem.edu
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catecholamines and β2-agonists induce bronchodilation of smooth muscle cells in lungs, increase constriction of blood vessels, dilate vascular smooth muscles, relax uterine smooth muscle, increase glycogenolysis, increase gluconeogenesis, increase heart rate and increase the force of contraction of heart muscle.

The human β2AR is a seven-transmembrane cell surface protein that is coupled to GTP-binding protein (G-protein). The binding of β2-agonist drugs to the β2AR results in coupling of the receptor to G-protein, forming a complex.⁵ The β2-agonist-β2AR -G-protein complex activates adenylyl cyclase, which mediates conversion of adenosine triphosphate (ATP) to 3-5 cyclic adenosine monophosphate (cAMP). cAMP acts as a second messenger in the signal transduction cascade by activating protein kinase A (PKA). The activation of PKA results in the cascade of protein phosphorylations, mostly enzymes, receptors, or channels which may be activated or deactivated upon phosphorylation. The specific response of the agonist signal depends on the nature of the phosphorylated proteins.⁵ For example, in the heart, activation of β2AR results in the increased rate and force of cardiac muscle contractility, while in the lungs bronchodilation occurs.

Bronchodilator responsiveness to β-agonists is highly variable among asthmatics due to factors such as age, gender, ethnicity, disease severity, and overuse of β2-agonists. Continuous or chronic administration of β-agonists results in the desensitization of the receptors and attenuation or abolishment of the signal transduction cascade in the biological system.⁶ There are two mechanisms responsible for desensitization. First, tachyphylaxis, (i.e., loss of signal transduction) occurs through a negative feedback mechanism that interferes with β2AR binding to G-protein. Second, receptor down-regulation, which decreases the number of active receptors, occurs as a result of receptor sequestration, decreased synthesis or enhanced β2AR degradation.

POLYMORPHISMS OF THE β2-ADRENORECEPTOR

There has been an intense interest in the structure and function of the β2AR since it was first characterized by Kobilka and colleagues in 1987.⁷ There have been nine single nucleotide

polymorphism (SNPs) in the coding region of the β2AR gene and eight SNPs in the 5' upstream region of the β2AR gene identified.⁸⁻¹⁰ Five polymorphisms in the coding region are silent (synonymous or degenerate) SNPs, because changing the nucleotide base does not alter the amino acid sequence of the β2AR. The other four polymorphisms are non-synonymous SNPs and result in changes in amino acids at positions 16, 27, 34 and 164 in the receptor protein. The "wild-type" human β2AR for these positions are Arg16, Gln27, Val34 and Thr164. The amino acid substitution at three of these polymorphisms (Arg16 to Gly16, Gln27 to Glu27 and Thr164 to Ile164) cause a change in functional properties of β2AR (Table 1). β2AR cellular expression is controlled in part by the 5' upstream region. Within the upstream region is a short open reading frame (5' leader cistron, 5'LC), which encodes a 19-amino acid peptide that regulates mRNA translation. One SNP has been identified in the 5'LC, Cys19Arg, and may cause changes in β2AR expression. The functional consequence of β2AR polymorphisms have been evaluated in transfected fibroblasts and cultured human bronchial smooth muscle cells.^{11,12}

Arg16Gly (Wild type Arg16). Arg16 is considered the wildtype, because it was the genotype that was first sequenced by Kobilka and colleagues.⁷ The most common polymorphism, in both asthmatic and non-asthmatic populations, is the Arg16 to Gly16 (Arg16Gly). β2AR with Gly16 and Arg16 polymorphisms have shown no difference in terms of their binding affinity to β2-agonists or alteration in signal transduction. Conversely, *in vitro* studies have noted that the Gly16 is associated with greater down-regulation of the β2AR following exposure to an endogenous catecholamine or β2-agonist than Arg16 in both transfected cell systems and in primary cultured human bronchial smooth muscle cells.^{11,12}

Gln27Glu (Wild type Gln27). The second most common polymorphism, in both asthmatic and non-asthmatic populations, occurs from Gln27 to Glu27. The frequencies of Gln and Glu are about the same at residue 27. Gln27 is associated with down-regulation of the β2AR in response to catecholamines or β2-agonists *in vivo*. The Glu27 β2AR is relatively resistant to down-regulation when it is co-expressed with Arg16.

Arg16Gly + Gln27Glu. The effect of Gly16 is independent of the β2AR 27 polymorphism co-

Table 1. Polymorphism of the $\beta 2$ -Adrenergic Receptors

Nucleic Acid	Amino Acid	$\beta 2$ Agonist Affinity	$\beta 2$ ARs Expression	$\beta 2$ ARs Alleles	Frequency in Normal	Frequency in Asthmatics	Frequency in Nocturnal Asthmatics
46A $\Rightarrow$ 46G	Arg16 $\Rightarrow$ Gly16	$\Leftrightarrow$	$\Downarrow$	Arg16/Arg16	14%	15%	7%
				Arg16/Gly16	29%	32%	21%
				Gly16/Gly16	57%	53%	74%
79C $\Rightarrow$ 79G	Gln $\Rightarrow$ Glu27	$\Leftrightarrow$	$\Uparrow$	Gln27/Gln27	28%	26%	26%
				Gln27/Glu27	44%	50%	45%
				Glu27/Glu27	28%	24%	29%
100G $\Rightarrow$ 100A	Val34 $\Rightarrow$ Met34	$\Leftrightarrow$	$\Leftrightarrow$	Val34/Val34	ND	ND	ND
				Val34/Met34	ND	ND	ND
				Met34/Met34	ND	ND	ND
491C $\Rightarrow$ 491T	Thr164 $\Rightarrow$ Ile164	$\Downarrow$	ND	Thr164/Thr164	95%	98%	100%
				Thr164/Ile164	5%	2%	0
				Ile164/Ile164	0	0	0

$\Leftrightarrow$ = no effect, $\Downarrow$ = decreased, $\Uparrow$ = increased, ND = not determined, $\beta 2$ AR = $\beta 2$ -Adrenergic Receptors

expression; however, $\beta 2$ AR 27 is greatly affected by the Gly16 polymorphism when they are co-expressed. The Arg16/Glu27 polymorphism combination has complete absence of $\beta 2$ -agonist down-regulation.¹¹ In contrast, Gly16/Glu27 and Gly16/Gln27 have an equal down-regulation of $\beta 2$ AR. The presence of Gly16 enhances down-regulation of $\beta 2$ AR regardless of whether the amino acid at position 27 is Gln or Glu.

Thr164Ile (Wild type Thr164). The third polymorphism occurs at amino acid 164 and is rare (~5% of the population) compared to the polymorphism at amino acid 16 and 27.⁸ *In vitro* studies of $\beta 2$ AR expressed with Ile164 showed a decrease in binding affinity of catecholamine.¹³ Thr164 is adjacent to Ser165, which is essential for interacting with the b carbon of $\beta 2$ -agonists. This polymorphism results in decreased binding affinity $\beta 2$ -agonists compared to "wild type" $\beta 2$ AR and produces a decrease in coupling to G_s protein. However, it is not affected by down-regulation of $\beta 2$ AR.

Arg19Cys 5' leader cistron (Wild type Arg19). $\beta 2$ AR mRNA expression is controlled by a 19 amino acid peptide in the 5'LC called $\beta 2$ AR-upstream peptide (BUP).⁹ The terminal amino acid of the peptide is either Arg or Cys. Human airway smooth muscle cells transfected with 5'LC-Cys19 have a higher $\beta 2$ AR density than cells transfected with 5'LC-Arg19. Linkage disequi-

librium analysis has been observed between the 5'LC-Arg19 allele and the Glu27 allele. Seven additional SNPs have been identified in the 5' upstream region of the gene, the functional consequences of these polymorphisms are not known.¹⁴ Combinations of the polymorphism in the promoter region as well as the coding region may be responsible for interindividual variation in $\beta 2$ AR responsiveness.

Haplotype analysis

Assessments of the phenotypes of individual SNPs fails to consider the potential interaction with other SNPs in the coding region of the gene or promoter region. Eight SNPs have been identified in the promoter region in addition to the 9 SNPs in the coding region.¹⁴ Haplotype studies have documented that a strong linkage disequilibrium exists in the gene region resulting in only a small number of common haplotypes. Although *in vitro* analysis has suggested a role for different haplotypes in $\beta 2$ AR responsiveness, studies in patients with asthma have not shown different functional effects or receptor expression for any given haplotype.

STUDIES IN PATIENTS WITH ASTHMA

The clinical significance of $\beta 2$ AR polymorphisms on the clinical response to $\beta 2$ -agonists

and disease severity in asthmatics is being actively investigated. A number of clinical studies have assessed individual SNPs or haplotypes regarding their association with asthma, the severity of asthma, the responsiveness to β₂-agonists, and differences among ethnic groups.

There has been a lack of association between individual β₂AR SNPs or haplotypes in asthmatics and nonasthmatics (Table 1).^{8,10,15,16}

One reason is the high frequency of Gly16 allele (~85%) and Gln27 allele (~76%) carriers in the general population. If there were any correlation between asthma and β₂AR polymorphism, then large numbers of asthmatics would have to be studied to determine a difference. In patients with asthma, the Gly16 polymorphism was associated with more severe asthma,¹³ bronchial hypersensitivity,¹³ and nocturnal worsening of asthma.¹⁷ In nocturnal asthma, two factors may contribute to the down-regulation of the β₂AR; first, both nocturnal and non-nocturnal asthmatics have peak plasma catecholamine concentrations in the late afternoon to early evening due to diurnal variation. The peak in endogenous catecholamines may induce down-regulation in asthmatics with the Gly16 polymorphism thereby contributing to nocturnal asthma. Second, the regular use of β₂-agonists during the day would further down-regulate the β₂AR expression such that they may be susceptible to worsening of their nocturnal asthma.

Several studies have shown that asthmatic patients who are homozygous Arg16 have a better response to β₂-agonists than patients with asthma who are.¹⁸⁻²⁰ These studies, along with the *in vitro* studies previously discussed, indicate that β₂AR with Gly16 is down-regulated by β₂-agonists and endogenous catecholamines while the β₂AR with Arg16 is not. In contrast, a study in asthmatic patients who used albuterol on a regular basis and were homozygous Arg16 had a

greater decline in peak expiratory flow than homozygous Gly16 patients.²¹ Furthermore, this same population of patients showed a decline in their peak expiratory flow compared to homozygous Arg16 who used albuterol on an as-needed basis.²¹ Patients who were homozygous for Gly16 showed no difference in response following regular use or as-needed use of albuterol. These results may appear inconsistent with previous reports; however, patients who were homozygous for Gly16 likely had their β₂AR down-regulated and would be unaffected by the frequency of drug administration. Patients who were homozygous for Arg16 and used albuterol on a regular basis had a change in peak expiratory flow. Homozygous Arg16 patients, whose β₂AR were not down-regulated by endogenous catecholamines, likely had a decline in peak expiratory flow due to desensitization (tachyphylaxis). Therefore, for a patient who is a carrier of a Gly16 allele, down-regulation factor would dominate over tachyphylaxis, and the patient would have a lower FEV₁. This suggests that β₂-agonists may not be as beneficial for patients with Gly16 relative to Arg16. The first line of therapy for patients who are homozygous for Arg16 is as-needed β₂-agonists. However, inhaled corticosteroid therapy with as-needed β₂-agonists should be considered in individuals who fail to respond to β₂-agonists due to the severity of their asthma. These patients should **not** be considered for β₂-agonists therapy on a regular basis because they are prone to developing tachyphylaxis.

There is considerable controversy as to whether adjuvant therapy with inhaled corticosteroids would provide a protective effect against the development of desensitization or down-regulation to β₂-agonists.^{22,23} Although the frequency of Gly16 polymorphism is about the same in those with and without asthma, asthmatic pa-

Table 2. Racial/Ethnic Variability for β₂-Adrenergic Receptors Polymorphism

	Arg 16/Arg 16	Gly 16/Gly 16	Gly 16/Gly 16	Gln 27/Gln 27	Gln 27/Gln 27	Gln 27/Gln 27
Caucasian American ²⁸	27%	38%	35%	46%	39%	15%
Hutterite ⁶	20%	50%	30%	40%	47%	13%
French Canadian ²⁸	17%	39%	44%	38%	41%	21%
Turks ²⁸	15%	50%	35%	47%	42%	10%
Black South African ¹⁸	26%	49%	25%	59%	34%	7%
Black American ²⁶	24%	50%	26%	63%	32%	4%
Japanese ²⁷	27%	50%	23%	90%	10%	0%
Chinese ²⁶	35%	46%	18%	86%	14%	0%

tients with the Gly16 polymorphism require larger dose of oral corticosteroids.²⁴ This could be attributed to the fact that these individuals are more prone to nocturnal asthma, and their β 2ARs are down-regulated secondary to their plasma catecholamine peak in late afternoon. Asthmatic patients with the Gly16 allele (especially homozygous), who have developed tachyphylaxis with long-term use of β 2-agonists, are more likely to be steroid dependent and to be given immunotherapy in comparison to patients who are homozygous Arg16.⁸ Although corticosteroids up-regulate β 2AR mRNA and decrease inflammation, patients might still require β 2-agonists for relief of symptoms.¹³

The Glu27 form of the receptor is strongly associated with an increase concentration of IgE in families with asthma and shows greater sensitivity to inflammatory stimuli.^{12,25} This leads to a higher degree of bronchial inflammation and lower airway reactivity in subjects with mild or moderate asthma.¹³ These patients have a greater risk to develop an allergy upon exposure to an allergen. In addition, Gln27 is responsible for down-regulation of β 2ARs provided Arg16 is homozygous. Except for patients who are homozygous for Arg16 and Glu27, all asthmatics are either affected by down regulation if they have Gly16 and/or Gln27 polymorphism or increased IgE production if they have the Gln27 polymorphism alone. For these patients, treatment with anti-inflammatory corticosteroids may decrease inflammation, decrease IgE production and up-regulate β 2ARs mRNA.⁸ Patients who are homozygous for Arg16 and Glu 27 might benefit from first-line therapy with as-needed β 2-agonists; however, these patients represent <1% of the asthmatic population.

There are only a limited number of studies evaluating the influence of haplotype and response to therapy. Differences in response to albuterol has been observed in asthmatics among the most commonly observed haplotypes.¹⁰

RACIAL STUDIES

A number of studies have evaluated the frequency of β 2ARs alleles and polymorphism in ethnic groups, including Caucasian-Americans,²⁶ African-Americans,²⁶ Chinese,²⁶ Japanese,²⁷ Turks,²⁸ Hutterites,¹⁶ Black South Africans,¹⁶ and French Canadians.²⁹ Japanese and Chinese popu-

lations have a very high frequency (~89%) of homozygous Gln27 and a very low frequency of homozygous Glu27 (~0.5%) (Table 2).^{26,27} There are marked divergences in the distribution of certain haplotypes in different ethnic groups.¹⁰

FUTURE PROSPECTS OF β 2AR POLYMORPHISM IN ASTHMATICS

Future studies of β 2AR polymorphisms need to focus on the relationship between haplotypes, rather than individual SNPs, and their responses to β 2-agonists. In order to better understand the influence of polymorphisms on the pathophysiology of asthma, studies of β 2AR polymorphisms need to classify patients according to the NIH guidelines (i.e., mild, moderate and severe asthma). Finally, polymorphisms are likely not limited to β 2AR, but could exist in proteins involved in the β 2AR signal transduction pathway or possibly other receptors within the respiratory system and contribute to the variability in asthma severity and in therapeutic response.³⁰

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