

The pharmacogenetics of β_2 -adrenergic receptors: Relevance to asthma

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The β_2 -adrenergic receptor (β_2 AR) is the molecular target for β -agonists used in the treatment of asthma. In the human population, 4 polymorphisms of the β_2 AR coding block have been found, 3 of which result in receptors that have different properties compared with wild-type. To date, clinical studies suggest that these β_2 AR polymorphisms may alter asthmatic phenotype and the response to β -agonist therapy, making these variants the first of undoubtedly several genetic loci that will ultimately be found that will provide for individualized therapy in asthma. (J Allergy Clin Immunol 2000;105:S487-92.)

Key words: Adenylyl cyclase, β -agonist, tachyphylaxis, pharmacogenetics

In the human population the β_2 -adrenergic receptor (β_2 AR) gene displays heterogeneity. In our original study of 51 patients with asthma and 56 normal subjects, we delineated 9-point mutations in the DNA sequence of the coding region.¹ Four of these resulted in changes in the amino acid sequence as shown in Fig 1. Because some of these are common and are not clearly causative of disease, these variants are heretofore referred to as polymorphisms. The polymorphisms were originally named using the following convention: AAn $\rightarrow$ AA where the *first* AA is the reference amino acid of the wild-type, as designated with the original cloning of the human β_2 AR. It was necessary to use this as a reference because the majority of structure/function work has been carried out with the use of this sequence, although, as will be discussed later, the wild-type sequence in some cases is not the most common. The *n* in the nomenclature represents the amino acid number beginning with the initiator methionine at the amino terminus of the receptor. The *second* AA represents the change in the amino acid. Thus the polymorphisms found were Arg16 $\rightarrow$ Gly, Gln27 $\rightarrow$ Glu, Val34 $\rightarrow$ Met, and Thr164 $\rightarrow$ Ile. In subsequent reports we have also referred to these polymorphisms simply by their alleles (ie, Arg16, Gly16). The 2 most common polymorphisms are at positions 16 and 27. Less common is the Ile164 polymorphism, and the Met34 variant is rare (<1%). The frequency of these polymorphisms was no different between the asthmatic group and the normal group, so at least in this heterologous population of patients with asthma, genetic variability of the β_2 AR did not appear to play a major

Abbreviations used

β_2 AR: β_2 -adrenergic receptor
CHW: Chinese hamster fibroblast
PEFR: Peak expiratory flow rate

causative role. However, we did note clustering of the Gly16 allele with patients severe chronic asthma that required corticosteroid and those patients receiving immunotherapy. This suggested that, although not causative, these polymorphisms may modify the disease.

The frequencies of these polymorphisms in a larger group of subjects are provided in Table I. From the more frequent polymorphisms, calculations of the expected frequencies of the various alleles can be performed on the basis of the Hardy-Weinberg equilibrium. The distribution of alleles in the population is consistent with what is predicted. Thus there is no evidence to suggest that any particular polymorphism at position 16 or 27 results in differential survival or differential reproduction.

CONSEQUENCES OF β_2 AR VARIABILITY

To assess whether these variations altered receptor function, we performed site-directed mutagenesis of wild-type cDNA to mimic each polymorphism.²⁻⁵ These were then subcloned into the mammalian expression vector pcDNA 1/NEO and transfected into Chinese hamster fibroblast cells (CHW cells) to establish permanent cell lines, each expressing a different β_2 AR variant.^{2,3} (Of note, CHW cells do not natively express any adrenergic receptors.) The phenotypes of these receptors are summarized in Table II and in Figs 2 through 4. The Ile164 receptor displays several abnormalities, the most striking being a substantial functional uncoupling from activation of the stimulatory G protein.² Thus the maximal isoproterenol-stimulated adenylyl cyclase activities in membranes from cells expressing this receptor are approximately 50% of those from cells expressing the Thr164 (wild-type) receptor (Fig 2). This inherent dysfunction has also been demonstrated at the intact organ level by expressing these receptors in the hearts of transgenic mice.⁶ In these animals, cardiac responsiveness to infused isoproterenol was substantially depressed in animals expressing the Ile164 receptor as compared with the wild-type. Thus, although this polymorphism is somewhat rare, we would expect that individuals harboring this polymorphism would have dysfunctional baseline bronchodilator responses to inhaled β -agonists.

In contrast to this extensive signaling defect with the

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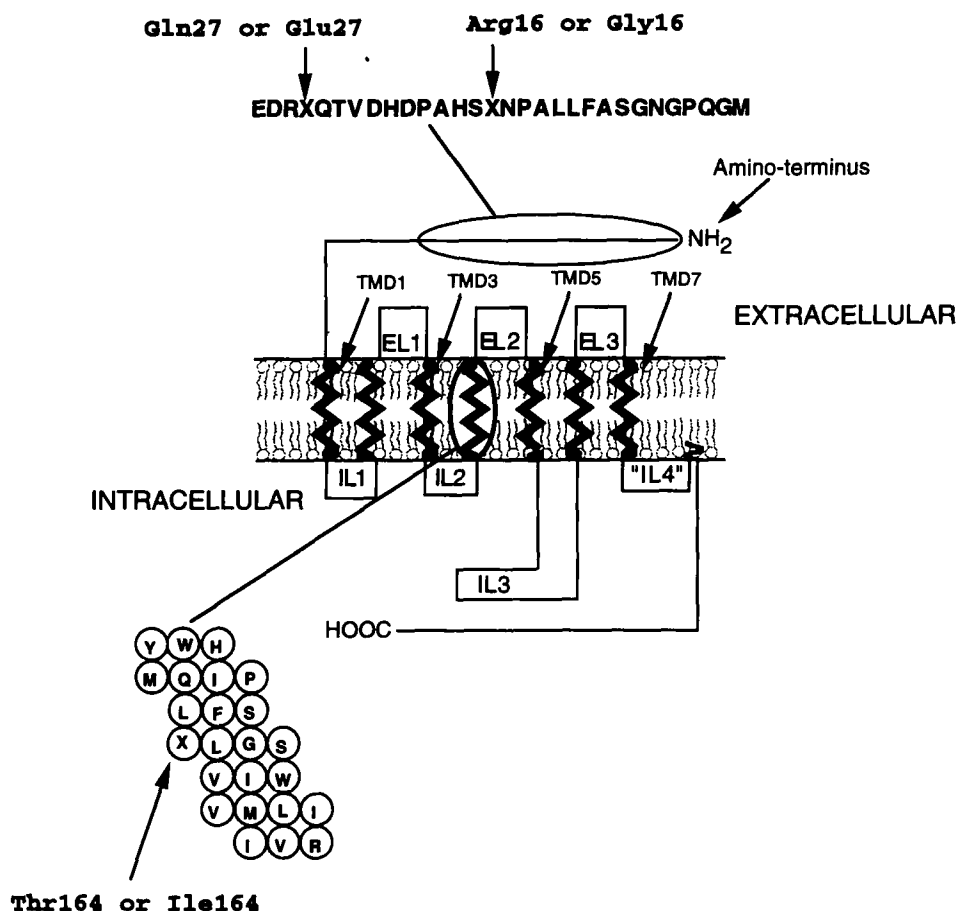


FIG 1. Localization of β_2 AR polymorphisms in the human population. TMD, Transmembrane domain; IL, intracellular loop; EL, extracellular loop.

TABLE I. Allelic frequencies of β_2 AR polymorphisms in the human population

Polymorphism	Allele frequency
Arg16	39.6
Gly16	60.4
Gln27	52.7
Glu27	47.2
Thr164	95.0
Ile164	5.0*

Data compiled from genotyping results of 700 individuals. *Heterozygous frequency (the frequency of Met34 is <1%).

TABLE II. Phenotypes of the β_2 AR genotypic variants as determined in cell and transgenic systems

Nucleic acid no.	Amino acid no.	Designation	Phenotype
46	16	Arg16	Wild-type
46	16	Gly16	Enhanced downregulation
79	27	Gln27	Wild-type
79	27	Glu27	Absent downregulation
100	34	Val34	Wild-type
100	34	Met34	Normal
491	164	Thr164	Wild-type
491	164	Ile164	Decreased coupling, binding, & sequestration

Ile164 receptor, the variants at position 16 and 27 appeared to bind agonists with wild-type affinities and to functionally couple to stimulation of adenylyl cyclase with a wild-type phenotype. However, the regulation of these receptors as the result of prolonged exposure to agonists was markedly altered depending on the residues present at positions 16 and 27.³ As shown in Fig 3, the Gly16 receptor undergoes enhanced agonist-promoted downregulation (loss of receptor number) after 24 hours of exposure to agonist. In contrast the Glu27 form of the receptor (with Arg being at position 16) exhibits no agonist-pro-

moted downregulation. How these specific substitutions alter receptor downregulation has not been fully elucidated. However, it appears that the degradation step that occurs during prolonged agonist exposure is markedly perturbed depending on which residue is present in the amino terminus at positions 16 and 27. The results of our work with the trafficking of these allelic variants is described elsewhere.³ We also performed additional studies with these 2 polymorphic forms using bronchial

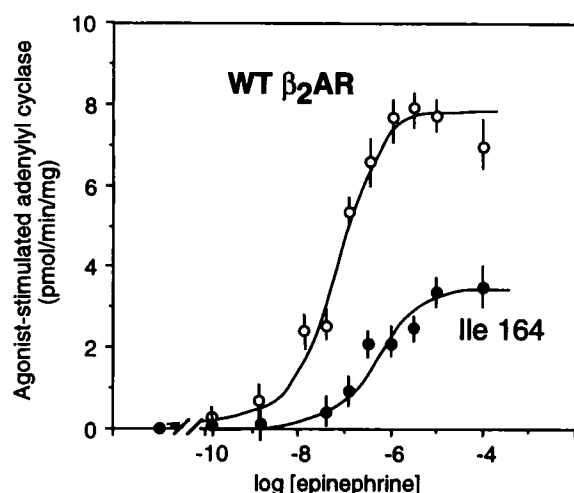


FIG 2. Phenotype of the Ile164 β_2 AR expressed in CHW cells. WT, Wild-type β_2 AR.

smooth muscle cells that natively express different polymorphic forms.⁷ This was done for several reasons. In our recombinant studies, β_2 AR expression was being driven by promoter elements in the expression vector that was used for the transfections. Thus the effects of polymorphisms on downregulation when expression is under control of the natural promoter was not known. In addition, it was not clear that events in fibroblasts would necessarily be reflective of what might happen in a relevant cell type such as smooth muscle. Finally, because the cells used in recombinant studies did not natively express β AR, we were concerned that they may lack cell-specific components that may be involved in the process of receptor downregulation. We thus obtained smooth muscle cells from the bronchi of patients who died from trauma or ischemic heart disease but who had normal lungs. Primary cultures were established, and the genotypes of the receptors in each line were determined. These were then studied with the same techniques used in the recombinant experiments. As shown in Fig 4, again a difference in agonist-promoted downregulation is observed between the different polymorphic forms of the receptor. After 24 hours of agonist exposure, wild-type receptor underwent an approximately 77% downregulation in these bronchial smooth muscle cells. The Gly16 form of the receptor displayed substantially greater downregulation, which amounted to approximately 96% loss of receptor expression. On the other hand the Glu27 form of the receptor displayed much less downregulation, amounting to approximately 30% loss of receptor after 24 hours of agonist exposure. The results from these studies with bronchial smooth muscle cells thus confirmed what was found in the recombinant studies: the Gly16 form of the receptor displays enhanced agonist-promoted downregulation, whereas the Glu27 form of the receptor is resistant to such downregulation.

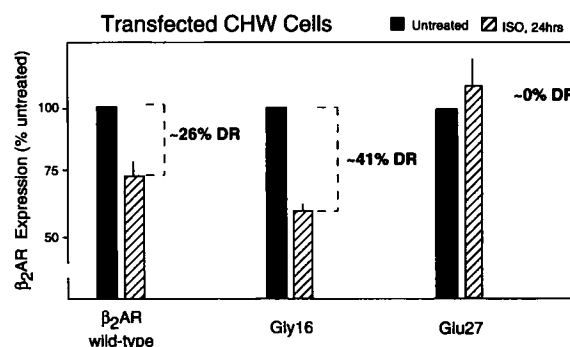


FIG 3. Downregulation (DR) phenotypes of polymorphic β_2 AR recombinantly expressed in CHW cells. Cells were exposed to vehicle alone (dark bars) or 10 μ mol/L isoproterenol (hatched bars) for 18 hours; receptor expression was determined by radioligand binding.

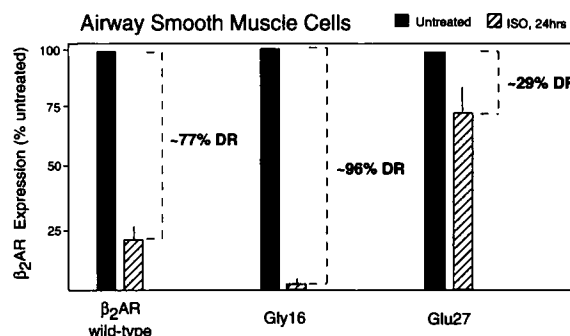


FIG 4. Downregulation of endogenously expressed β_2 AR variants in human airway smooth muscle. For conditions see Fig 3. ISO, isoproterenol. DR, downregulation.

CLINICAL STUDIES

Having established the receptor variant phenotypes in cells, several groups have now begun to examine the role of β_2 AR polymorphisms in asthma. We have considered that such polymorphic variants may act as disease modifiers and thus may dictate certain asthmatic phenotypes, alter baseline airway function, or define the response to β -agonists.

Regarding asthmatic phenotypes, one of the first issues we addressed was that of nocturnal asthma, where a decrease in β_2 AR expression at 4:00 AM as compared with 4:00 PM has been demonstrated and thought to be part of the basis of nocturnal symptoms.⁸ Although there is evidence that additional factors may be involved in the development of nocturnal asthma, we felt that because we had a β_2 AR genotype that displayed enhanced downregulation it was prudent to consider whether this receptor was associated with the nocturnal asthmatic phenotype.⁹ To address this, we screened patients with mild-to-moderate asthma who were not taking oral corticosteroids until we had collected 2 cohorts that differed only in their overnight decrements of peak expiratory flow rates (PEFRs), as assessed on 5 consecutive days. Individuals with nocturnal PEFR decrements of less than 10% for all

	Pre-trial Regulation*	Pre- PFT	During Trial Regulation	Post- PFT	Tachyphylaxis
Dynamic Baseline Model					
Arg16	none	REF	↓↓	↓↓	Observed
Gly16	↓↓	↓	↓↓	↓	Minimal or Not Observed
Static Baseline Model					
Arg16	None	REF	↓	REF/ or ↓	Minimal or Not Observed
Gly16	None	REF	↓↓	↓↓	Observed

* by endogenous catecholamines
or occasional β -agonists

FIG 5. Potential results of β -agonist-tachyphylaxis trials based on receptor phenotype. Two models are shown: The dynamic baseline model shows Gly16 that is already downregulated before a β -agonist trial; therefore pulmonary function testing (PFT) would show tachyphylaxis for the Arg16 variant only. The static baseline model shows Gly16 that is not downregulated before the trial; with the enhanced downregulation evoked by prolonged agonist with this genotype, tachyphylaxis is observed.

5 days were considered patients with nonnocturnal asthma; those patients with nocturnal PEFR decrements of greater than 20% for all 5 days were considered patients with nocturnal asthma. The 23 patients with nocturnal asthma and 22 patients with nonnocturnal asthma were then genotyped at β_2 AR polymorphic loci 16, 27 and 164. The allele frequency for Gly16 was found to be higher in the patients with nocturnal asthma (80%) as compared with the patients with nonnocturnal asthma (52%; $P = .007$; odds ratio, 3.8). In contrast, the frequencies for the alleles at 27 and 164 were not different between the 2 groups. When we analyzed only patients who were homozygous for Arg16 or Gly16, the results also indicate overrepresentation of Gly16 in the nocturnal asthmatic group. In this group of patients with nocturnal asthma, 16 of 18 were Gly16; in the patients with nonnocturnal asthma, 8 of 15 were Gly16 ($P = .046$; odds ratio, 7.0). Further support that the Gly16 polymorphism is associated with nocturnal asthma was noted when patients were segregated on the basis of the history of frequent nocturnal asthma rather than the PEFR criteria. Of 31 patients with asthma with a history of nocturnal worsening, 23 patients (74%) had the homozygous Gly16 polymorphism, although among those without a nocturnal history only 1 of 14 patients (7%) was homozygous for Gly16 ($P < .0001$; odds ratio, 33). We have concluded from these studies that the Gly16 polymorphism is strongly associated with the presence of nocturnal asthma.⁹ The mechanism that may account for this is related to the downregulation phenotype of the Gly16 receptor. As previously indicated, in recombinant and smooth muscle cell studies,

the Gly16 receptor undergoes an enhanced downregulation as compared with the other genotypes.

We have also assessed whether these polymorphisms correlated with bronchial hyperreactivity as assessed in methacholine challenge testing.¹⁰ For this study, 65 patients with asthma were enrolled. Subjects were withdrawn from β -agonists before testing. No patients were taking oral corticosteroids. Genotyping was performed at the 16 and 27 loci. Two hypotheses were tested. First, we considered that individuals with the Glu27 form of the receptor would have experienced little downregulation of β_2 AR because of exogenous or endogenous agonists. Thus these individuals would display less reactive airways as compared with those patients with the Gln27 (wild-type) receptors. Second, individuals with Gly16 would have more reactive airways because of enhanced downregulation as compared with individuals with the Arg16 polymorphism. The geometric mean PD₂₀ for subjects homozygous for Gln27 was 0.86 μ mol, which was lower (ie, indicative of greater hyperreactivity) than the PD₂₀ for those homozygous for Glu27, which was 3.23 μ mol [$P = .035$]. Individuals who were heterozygous had an intermediate PD₂₀. Thus these findings were consistent with a protective effect provided by the Glu27 polymorphism. (Only 8 individuals were found to be homozygous for the Gly16 polymorphism, thus potentially limiting our ability to assess its role in bronchial hyperreactivity. With this smaller population, we found no differences in the PD₂₀ with those homozygous at Arg16 or Gly16).

A recent family study has also been completed that

assessed the relationship between β_2 AR polymorphisms at position 16 and 27 and the presence of asthma, atopy, and IgE levels.¹¹ A significant association ($P = .009$) between the Glu27 form of the β_2 receptor and log serum IgE was found. In addition, linkage was observed between IgE levels and this β_2 AR variant. However, there was no association between variants at position 27 or position 16 and an increased risk of asthma or atopy per se. These studies confirmed what was observed in our initial case control study that β_2 AR polymorphisms are not a causative factor in asthma. However, the fact that a very strong association between one of the variants and IgE levels suggests that β_2 AR polymorphisms may act to modify the asthmatic phenotype in some way. Indeed, serum IgE levels are known to be affected by the activation of β_2 AR.¹²

FUTURE DIRECTIONS

From the work performed in the cell models and transgenic animal models, it is clear that the β_2 AR genotype may have its greatest impact on the response to therapy. If there is no significant prior regulation by endogenous catecholamines or β -agonists, then one can predict several clinical correlates. With the Arg16-Gln27-Thr164 receptor, a given level of cyclic adenosine monophosphate responsiveness to agonist occurs in the resting state because of the presence of a certain level of receptors. After chronic β -agonist exposure, the number of receptors decreases and a depressed cyclic adenosine monophosphate responsiveness is expected. If arginine is substituted by glycine in position 16, the same level of initial responsiveness is present (because the substitution does not alter stimulatory G protein coupling), but there is enhanced downregulation; thus there is a greater loss of responsiveness. With the Gly16-Gln27-Ile164 receptor, there is depressed coupling in the resting state, so there is decreased responsiveness to acute agonist. With the aforementioned combination, not only is there depressed initial responsiveness, but because of the enhanced downregulation seen because of Gly16, there is an even further loss of function and a subsequent very small level of responsiveness after chronic β -agonist. With Glu present at position 27, with the other positions being wild-type, there is very little loss of receptor number, thus the desensitization is not as significant as that seen with the Gln27 receptor.

These scenarios are all based on the notion that there is no baseline regulation of these receptors by endogenous catecholamines or prior β -agonist use. If the Gly16 variant is very sensitive to downregulation and is already downregulated substantially by endogenous or exogenous agonists, then once a trial is begun no additional tachyphylaxis will be observed with continuous β -agonist therapy; thus the responsiveness will not have changed. This, then, is the opposite from what would be expected, based on the cultured cell results. In a like manner, the effect of polymorphisms at position 27 may have a different effect in vivo. Thus it may well be that the Glu27 form of the receptor is resistant to regulation

by endogenous or exogenous agonists in the so-called resting state, but once a trial is begun some degree of downregulation occurs and would therefore be seen as a receptor that does undergo some degree of tachyphylaxis. These concepts of the two different types of steady state conditions (dynamic baseline model and static baseline model) and how clinical trial results would differ are illustrated in Fig 5. Studies to address these questions are currently underway; early evidence suggests that the tachyphylaxis response to β -agonist is dictated by the β_2 AR genotype.

CONCLUSIONS

In the human population, the β_2 AR shows significant genetic variability in its structure. These differences in structure result in differences in the way the receptor functions or is regulated. Early studies have suggested that these different polymorphic forms of the receptor may influence the severity of asthma, certain asthmatic phenotypes, or baseline airway hyperresponsiveness. Based on the in vitro studies it is also predicted that responsiveness to β -agonists could be affected by the β_2 AR genotype.

Note: Since this conference, several additional articles have been published that assess the role of β_2 AR polymorphisms in the response to β -agonist treatment^{13,14} and bronchial hyperreactivity¹⁵ and asthma control.¹⁶

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