JCI The Journal of Clinical Investigation

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J Clin Invest. 1997;100(12):3184-3188. https://doi.org/10.1172/JCI119874.

Research Article

The beta2-adrenergic receptor (beta2AR) agonists are the most widely used agents in the treatment of asthma, but the genetic determinants of responsiveness to these agents are unknown. Two polymorphic loci within the coding region of the beta2AR have been recently described at amino acids 16 and 27. It has been reported that glycine at codon 16 (Gly-16) is associated with increased agonist-promoted downregulation of the beta2AR as compared with arginine-16 (Arg-16). The form of the receptor with glutamic acid at codon 27 (Glu-27), on the other hand, has been shown to be resistant to downregulation when compared with glutamine-27 (Gln-27), but only when coexpressed with Arg-16. To assess if different genotypes of these two polymorphisms would show differential responses to inhaled beta2AR agonists, we genotyped 269 children who were participants in a longitudinal study of asthma. Spirometry was performed before and after administration of 180 microg of albuterol, and a positive response was considered an increase of >15.3% predicted FEV1. There was marked linkage disequilibrium between the two polymorphisms, with 97.8% of all chromosomes that carried Arg-16 also carrying Gln-27. When compared to homozygotes for Gly-16, homozygotes for Arg-16 were 5.3 times (95% confidence interval 1.6-17.7) and heterozygotes for beta2AR-16 were 2.3 times (1.3-4.2) more likely to respond to albuterol, respectively. Similar trends were observed for asthmatic and nonasthmatic children, […]

Association between Genetic Polymorphisms of the β_2-Adrenoceptor and Response to Albuterol in Children with and without a History of Wheezing

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Abstract

The β_2-adrenergic receptor (β_2AR) agonists are the most widely used agents in the treatment of asthma, but the genetic determinants of responsiveness to these agents are unknown. Two polymorphic loci within the coding region of the β_2AR have been recently described at amino acids 16 and 27. It has been reported that glycine at codon 16 (Gly-16) is associated with increased agonist-promoted downregulation of the β_2AR as compared with arginine-16 (Arg-16). The form of the receptor with glutamic acid at codon 27 (Glu-27), on the other hand, has been shown to be resistant to downregulation when compared with glutamine-27 (Gln-27), but only when coexpressed with Arg-16. To assess if different genotypes of these two polymorphisms would show differential responses to inhaled β_2AR agonists, we genotyped 269 children who were participants in a longitudinal study of asthma. Spirometry was performed before and after administration of 180 μg of albuterol, and a positive response was considered an increase of >15.3% predicted FEV_1. There was marked linkage disequilibrium between the two polymorphisms, with 97.8% of all chromosomes that carried Arg-16 also carrying Gln-27. When compared to homozygotes for Gly-16, homozygotes for Arg-16 were 5.3 times (95% confidence interval 1.6–17.7) and heterozygotes for β_2AR-16 were 2.3 times (1.3–4.2) more likely to respond to albuterol, respectively. Similar trends were observed for asthmatic and nonasthmatic children, and results were independent of baseline lung function, ethnic origin, and previous use of antiasthma medication. No association was found between the β_2AR-27 polymorphism and response to albuterol. These results may explain some of the variability in response to therapeutic doses of albuterol in children. (*J. Clin. Invest.* 1997. 100:3184–3188.) Key words: genetics • β_2-adrenergic receptor • β_2-agonists • asthma

Introduction

The β_2-adrenergic agonists are the most potent bronchodilators presently available for the treatment of asthma (1). These drugs are not only widely prescribed for mild asthmatic symptoms, but are also the main pharmacologic tool used to relieve bronchoconstriction during acute, life-threatening asthmatic attacks. Moreover, immediate response to inhaled β_2-adrenergic agonists is often used in clinical practice to differentiate asthma from other conditions (2) and, in the emergency room setting, to make decisions regarding hospital admissions during exacerbation (3). Genetic factors controlling β_2 adrenoceptor function may be very important determinants of response to bronchodilator therapy and thus, of severity and duration of asthmatic symptoms.

Reihsaus et al. (4) recently described nine polymorphisms in the β_2-adrenergic receptor (β_2AR)[1] gene, of which two were more frequent and gave rise to amino acid exchanges in the putative extracellular amino-terminus region of the gene: β_2AR-16, with replacement of arginine (Arg-16) for glycine (Gly-16); and β_2AR-27, with replacement of glutamine (Gln-27) for glutamic acid (Glu-27). There was no relation between β_2AR polymorphisms and asthma prevalence, but the Gly-16 variant was apparently associated with a more severe form of the disease (4). Subsequently, Turki et al. (5) reported that the Gly-16 allele was found more frequently among subjects with nocturnal asthma than among nonnocturnal asthmatics (odds ratio = 3.8). They showed no difference in the frequency of polymorphisms at β_2AR-27 between nocturnal and nonnocturnal asthmatics. Green et al. (6) used site-directed mutagenesis and recombinantly expressed each polymorphism in mammalian cells that normally do not express any adrenergic receptors. They showed that Gly-16 β_2AR undergoes significantly enhanced agonist-promoted receptor downregulation, whereas Glu-27 β_2AR is relatively resistant to such downregulation, but only when coexpressed with Arg-16 β_2AR (6).

Based on these clinical and biochemical studies, we hypothesized that subjects carrying different combinations of the two main β_2AR polymorphisms would show differential responses to inhaled β_2-adrenergic agonists.

Methods

Subjects. The subjects of this report were participants in the Tucson Children's Respiratory Study, a large longitudinal study of asthma and allergies in an unselected population sample enrolled at birth (7). At a mean age ± SD of 10.8±0.6 yr 496 children who had at least one non-Hispanic, White (Caucasian) parent or whose parents were both Hispanic were assessed for response to a bronchodilator. Parents were instructed to stop any bronchodilator therapy 6 h before the scheduled time for the bronchodilator test. At the time of the bronchodilator test, parents answered a questionnaire regarding respiratory symptoms in their children. Specifically, they were asked if their child had experienced episodes of wheezing during the previous year. They were also asked how many such episodes had occurred during

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Received for publication 9 June 1997 and accepted in revised form 7 October 1997.

J. Clin. Invest.

0021-9738/97/12/3184/05 $2.00
Volume 100, Number 12, December 1997, 3184–3188
http://www.jci.org

1. *Abbreviations used in this paper:* β_2AR, β_2-adrenergic receptor; FEV_1, forced expiratory volume in 1 s.

the previous year, if a doctor had ever diagnosed asthma in the child, and about the antiasthma medication that the child was on, if any. Questionnaire responses were not known by the nurse performing the bronchodilator test. Skin tests to eight local aeroallergens (*Alternaria alternata*, bermuda grass, olive tree, mesquite tree, careless weed, mulberry tree, cat dander, and *Dermatophagoides farinae*) were performed at the time of the bronchodilator test as described in detail elsewhere (8). Children were considered to be atopic if they had at least one skin test measuring 3 mm or more in diameter. A total of 269 out of 496 tested children were genotyped for variants in the β_2AR gene. Genotyped children did not differ significantly from nongenotyped children in frequency of wheezing episodes during the previous year or in distribution of ethnic background (data not shown).

Molecular methods. Genomic DNA was prepared from peripheral blood obtained at $\sim$ 11 yr of age using standard techniques. β_2AR genotypes were determined by a combination of primer-induced restriction site assay and restriction fragment assay. A PCR product which includes the region of the β_2AR-16 and the β_2AR-27 polymorphisms was generated using the primers 5′-GCCTTCTTGCTG-GCACCCCAT-3′ and 5′-CAGACGCTCGAACTTGGCCATG-3′. The underlined bases were modified from the reported sequence to create NcoI restriction sites. The 5′-primer creates a NcoI restriction enzyme site on PCR product generated from the Gly-16 allele but not from the Arg-16 allele. The 3′-primer contains a complete restriction site and thus NcoI digests the PCR product from both alleles, which serves as a control for assessing whether digestion was complete. PCR reactions were carried out in a vol of 35 μl containing $\sim$ 55 ng of genomic DNA, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.005% gelatin, 200 μM of each deoxynucleotide triphosphates, and 35 ng of each primer. The DNA was denatured at 94°C for 2 min, then 0.8 unit Taq DNA polymerase (Promega Corp., Madison, WI) was added. Temperature cycling was 94°C for 40 s, 64°C for 40 s, and 72°C for 50 s for 40 cycles then a final extension for 5 min at 72°C. The size of the PCR product generated was 168 bp. For detection of the β_2AR-16 polymorphism, 8 μl of PCR product was digested with 2 U of NcoI (New England BioLabs, Boston, MA) in 6 μl of 25 mM potassium acetate, 10 mM Tris acetate (pH 7.9), 5 mM magnesium acetate, 0.5 mM DTT, and 16.7 mM $MgCl_2$ at 37°C for 2 h. NcoI cuts 22 bp from the 3′-end of both alleles and 18 bp from the 5′-end of the Gly-16 allele. The restriction digests were electrophoresed on 4% NuSieve agarose gels and visualized with ethidium bromide staining and ultraviolet illumination (see Fig. 1).

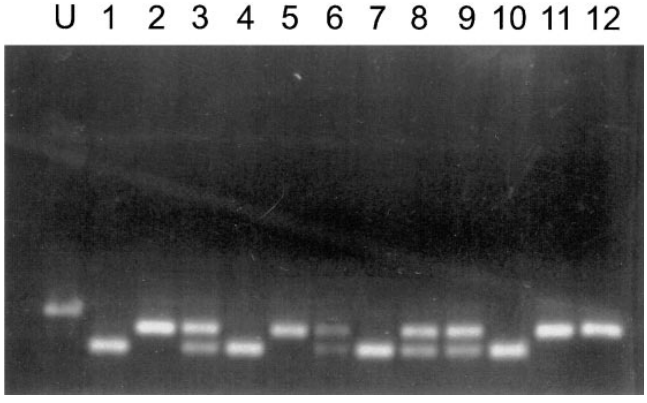

Figure 1. Identification of a polymorphism in amino acid residue 16 of the β_2-adrenoceptor. Lane *U* is undigested polymerase chain reaction product: homozygotes for the glycine 16 allele are in lanes *1*, *4*, *7*, and *10*, homozygotes for the arginine 16 allele are in lanes *2*, *5*, *11*, and *12* and heterozygotes are in lanes *3*, *6*, *8*, and *9*.

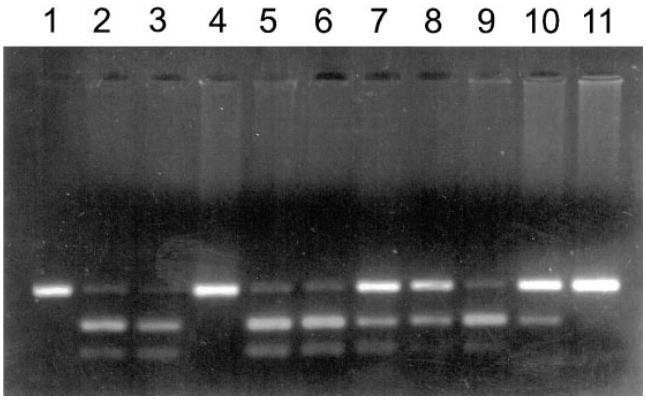

Figure 2. Identification of a polymorphism in amino acid residue 27 of the β_2-adrenoceptor. Homozygotes for the glutamic acid 27 allele are in lanes *1*, *4*, and *11*, homozygotes for the glutamine 27 allele are in lanes *2*, *3*, *5*, *6*, and *9* and heterozygotes are in lanes *7*, *8*, and *10*.

The β_2AR-27 polymorphism was identified in a second restriction digest using another aliquot of the same PCR product. 6 μl of the PCR product were digested with 0.5 U of BbvI (New England BioLabs) in 4 μl 25 mM NaCl, 5 mM Tris-HCl (pH 7.9), 5 mM $MgCl_2$, 0.5 mM DTT at 37°C for 2 h. BbvI digests only the Gln-27 allele to produce 105- and 63-bp fragments which are separated from the uncut Glu-27 alleles on 4% NuSieve gels (see Fig. 2).

The assays were verified by direct dideoxy sequencing of a larger PCR product generated with primers located outside of the modified PCR primers. The primers used were 5′-CAGCCAGTGCGCTTAC-CTGC-3′ and 5′-CACAGCACATCAATGGAAGTC-3′. PCR conditions were as described for the modified primers. PCR products from eight subjects with different genotypes were sequenced using the dye terminator cycle sequencing kit (Perkin-Elmer Corp., Norwalk, CT) and the Applied Biosystems 373 Sequencer (Foster City, CA). In all cases, results obtained by sequencing confirmed those obtained with the primer-induced restriction site assay for β_2AR-16 and with the restriction fragment assay for β_2AR-27.

Bronchodilator responsiveness. To assess response to a bronchodilator, two inhalations (180 μg) of the β_2-adrenergic agonist albuterol were administered using a metered dose inhaler and a spacer. Spirometry was performed before and 15 min after the albuterol dose using a standardized pneumotachygraphic method (9). Response to albuterol was expressed as (10)

$$\%\text{predicted postbronchodilator } FEV_1 - \%\text{predicted prebronchodilator } FEV_1$$

predicted FEV_1 being calculated using equations proposed by Knudson et al. (11) and % predicted values calculated as 100× observed FEV_1/predicted FEV_1. In assessing changes in lung function after a bronchodilator challenge, there is an inherent problem in distinguishing between a true response and the intrinsic variability in the measurement which, if not considered, would bias any comparisons between groups towards the null. To avoid this pitfall, arbitrary thresholds have been proposed for a "significant" response (10, 12). We determined the 95th percentile of the distribution of bronchodilator response among all tested subjects without a history of wheezing during the previous year and changes above this value (15.3% increase) were considered positive. This threshold is very similar to that proposed for FEV_1 by Nickerson et al. (15%, reference 12) based on the measured coefficient of variation of FEV_1.

Statistical analyses. Proportions were compared using Mantel-Haenzel odds ratio statistics (13). Logistic regression was used to adjust the effects of other variables on those of any given variable (13). Linkage disequilibrium between the two variants in the β_2AR gene was measured using Levin's δ (14). This test yields values of 1 for

complete disequilibrium (i.e., one of the alleles of a certain polymorphism is always linked in the chromosome that carries it with a certain allele of the other polymorphism) and 0 for random distribution of the two polymorphism (i.e., the frequency distribution of the alleles of one polymorphism is independent of which allele of the other polymorphism is present in the same chromosome). The test was chosen because results are independent of allele frequency (15). It requires knowledge of the haplotypes for both variants, which was possible for all but double heterozygotes. Standard error and confidence interval for δ was calculated as suggested by Walter (16). Maximum likelihood estimates of allele frequency were done using the formula:

(frequency of homozygotes) + 1/2 (frequency of heterozygotes).

Standard errors for allele frequency were calculated using the binomial distribution (13).

Results

We studied 188 subjects whose parents were both Caucasian, 40 who had one Caucasian parent, and 41 whose parents were both Hispanic. In the group with mixed ethnicity, most children had one Caucasian and one Hispanic parent, but in 11 cases the ethnicity of the non-Caucasian parent was either mixed Hispanic/other or unknown. Allele frequencies for Gly-16 were not significantly different across ethnic groups: estimates ± SD were 0.625±0.025, 0.613±0.055, and 0.586±0.054 for Caucasians, mixed, and Hispanic children, respectively. Hispanic children carried the Gln-27 allele significantly more frequently than Caucasian children (0.732±0.049 vs. 0.612± 0.025, $P = 0.03$), with children of mixed ethnicity showing intermediate frequency (0.663±0.053).

There was marked linkage disequilibrium between the two polymorphisms and this was true for the whole group (Levin's δ = 0.48, 95% confidence interval 0.38–0.56, Table I), and for each ethnic group studied separately (data not shown). No subjects were ascertained who were concomitantly homozygotes for both Arg-16 and Glu-27. Out of the 136 chromosomes carrying Arg-16 and for which the haplotype could be determined, 133 (97.8%) also carried Gln-27.

78 tested children (29.2%) had episodes of wheezing reported during the previous year, but only one-third (26/78) had more than three such episodes in this general population sample. Of the 78 wheezing children, 31 (39.7%) had a diagnosis of asthma, 64 (82.1%) were atopic, and nine (11.5%) had recently required β_2-adrenergic therapy or were receiving theophylline. No child was on chronic treatment with inhaled corticosteroids. We classified children as having asthma if they had wheezed during the previous year and had required bronchodilator therapy or had a physician's diagnosis of asthma. Children were also classified as asthmatics if they had more than three episodes of wheezing during the previous year regardless of therapy or a physician's diagnosis. Prevalence of asthma so defined was 14.1% (37/269) among tested children.

Table I. Association between Genotypes of the Polymorphisms in Amino Acid Residues 16 and 27 of the β_2 Adrenoceptor

	Polymorphism 27			
Polymorphism 16	GlnGln*	GlnGlu	GluGlu	Total
ArgArg	38	2	0	40
	(95.0)‡	(5.0)	(0.0)	(14.9)
ArgGly	55	70	1	126
	(43.7)	(55.6)	(0.8)	(46.8)
GlyGly	18	49	36	103
	(17.5)	(47.6)	(35.0)	(38.3)
Total	111	121	37	269
	(41.3)	(45.0)	(13.7)	

*The polymorphisms are defined by the amino acid residues associated with them: Arg and Gly for residue 16 and Gln and Glu for residue 27, respectively. ‡Numbers in parentheses are row percentages, with the exception of the last column, which shows column percentages.

Table II. Prebronchodilator FEV_1 (as % Predicted) and Prevalence of Wheezing (in %) during the Previous Year at a Mean Age of 11 yr by Genotypes of the Polymorphisms in Amino Acid Residues 16 and 27 of the β_2 Adrenoceptor

	N	Mean±SEM % predicted	Prevalence of asthma	Prevalence of nonasthmatic wheezing
		FEV_1	%	%
Polymorphism 16				
ArgArg	40	103.2±2.1	13.2	13.2
ArgGly	126	102.0±1.0	14.3	11.1
GlyGly	103	102.9±1.1	14.6	20.4
P for trend		0.8	0.6	0.1
Polymorphism 27				
GlnGln	111	103.2±1.2	14.5	13.6
GlnGlu	121	101.3±1.0	14.2	14.2
GluGlu	37	104.4±1.8	13.5	21.6
P for trend		0.3	0.9	0.3

*Two subjects had no information about wheezing at age 11 and are not included in prevalence calculations. The polymorphisms are defined by the amino acid residues associated with them: Arg and Gly for residue 16 and Gln and Glu for residue 27, respectively.

There was no significant relation between either β_2-adrenoceptor polymorphism and prebronchodilator FEV_1, prevalence of asthma, or prevalence of wheezing without asthma during the previous year (Table II). Children with asthma were most likely to respond to bronchodilators (26.3%). Children with reported episodes of wheezing during the previous year but no asthma had an intermediate prevalence of response (12.5%), and children with no episodes of reported wheezing and no asthma had the lowest prevalence of response (5.9%): *P* for trend = 0.0001.

There was a linear association between the number of Arg-16 alleles carried by each subject and the prevalence of bronchodilator responsiveness (Table III). This trend was observed for asthmatic children, children with wheezing episodes but who were not classified as having asthma, and for normal children, although this trend did not reach statistical significance for nonasthmatic wheezers probably because of the small number of subjects. After adjusting for asthma and for wheezing status, children who were homozygous for the Arg-16 allele were 5.3 times more likely to show a positive response to bronchodilators than homozygotes for the Gly-16 allele, with heterozygotes having an intermediate value. There was a trend for homozygotes for Gln-27 to have higher prevalence of positive responses to albuterol than homozygotes for Glu-27, but

*Table III. Prevalence of a Positive Bronchodilator Response (> 15.3% of Predicted FEV_1) by Genotypes of the Polymorphisms in Residues 16 and 27 of the β_2 Adrenoceptor and by Current Asthma Wheezing at 11 yr**

	% Positive (number) asthmatics‡	% Positive (number) nonasthmatic wheezers	% Positive (number) normals	Odds ratio (95% CI) adjusted for asthma and wheezing
Polymorphism 16				
ArgArg	60.0	20.0	14.3	5.3
	(5)	(5)	(28)	(1.6–17.7)
ArgGly	27.8	14.3	6.4	2.3
	(18)	(14)	(94)	(1.3–4.2)
GlyGly	13.3	9.5	3.0	1.0
	(15)	(21)	(67)	
P for trend	0.05	0.5	0.04	0.007
Polymorphism 27				
GlnGln	31.3	20.0	8.9	3.1
	(16)	(15)	(79)	(0.8–11.4)
GlnGlu	29.4	5.9	4.6	1.8
	(17)	(17)	(87)	(0.9–3.4)
GluGlu	0	12.5	4.2	1.0
	(5)	(8)	(24)	
P for trend	0.5	0.5	0.27	0.08

*Two subjects had no information about wheezing at age 11 and are not included in prevalence calculations. The polymorphisms are defined by the amino acid residues associated with them: Arg and Gly for residue 16 and Gln and Glu for residue 27, respectively. CI, confidence interval.
‡Definition of asthma is explained in text.

the results did not reach statistical significance. Similar trends for both polymorphisms were observed in all three ethnic groups (data not shown).

To assess if the effect of each polymorphism on β_2-adrenergic agonist responsiveness was independent of prebronchodilator percentage-predicted FEV_1, asthma/wheezing status, ethnic group, and previous requirement of bronchodilator therapy, a logistic regression was performed in which the number of Arg-16 alleles and Gln-27 alleles were introduced into the model together with all the above variables. Results showed that β_2AR-16 ($P = 0.02$), asthma/wheezing status ($P = 0.006$), baseline lung function ($P = 0.002$), and requirement of bronchodilator therapy ($P = 0.01$) were all significantly and independently associated with increased β_2-adrenergic agonist responsiveness, whereas β_2AR-27 was not ($P = 0.8$).

Discussion

In this paper we report for the first time that subjects with different genotypes for a polymorphism originally reported by Liggett et al. (17) in amino acid residue 16 of the β_2AR show marked differences in the prevalence of positive responses to bronchodilators. These differences were observed in asthmatic and in nonasthmatic children, and were independent of ethnic background and baseline lung function. No significant differences in β_2-adrenergic agonist responsiveness were observed among subjects carrying different genotypes for a polymorphism in amino acid 27 of the β_2AR gene. Our observations confirm and extend those of Reihsaus et al. (4) who reported that asthmatic subjects who were homozygous for the Gly-16 allele were significantly more likely to be steroid dependent and to be referred for immunotherapy when compared with patients without this genotype. Because asthmatic subjects with the Gly-16 allele (especially homozygotes) should show less improvement when treated with β_2-adrenergic agonists, they should be expected to require more antiinflammatory therapy than carriers of the Arg-16 allele, as observed by Reihsaus et al. (4).

The mechanisms by which the variants of the β_2AR gene may alter receptor function have been studied by Green et al. (6, 18). These authors performed site-directed mutagenesis and recombinantly expressed combinations of homozygous forms of the β_2AR-16 and the β_2AR-27 polymorphisms in Chinese hamster fibroblasts, which normally do not express any adrenergic receptors (6). They showed no difference in agonist binding between genotypes, but the Gly-16 variant showed markedly increased agonist-promoted downregulation of receptor expression when compared with the Arg-16 variant. These effects of Gly-16 were independent of the β_2AR-27 variant with which the β_2AR-16 variant was coexpressed. On the contrary, the β_2AR-27 variants showed remarkable heterogeneity of effects depending on the β_2AR-16 variant with which they were coexpressed; the Arg-16/Glu-27 combination showed complete absence of agonist-promoted receptor downregulation, whereas Gly-16/Glu-27 showed the same level of enhanced downregulation as Gly-16/Gln-27. These results suggested that the enhanced downregulation associated with the Gly-16 variant prevails over the opposite effect of Glu-27 when both variants are concomitantly expressed in homozygous form. Green et al. (18) also studied β_2AR function in primary cultures of human airway smooth muscle. The authors confirmed that Gly-16 was associated with markedly increased agonist-driven downregulation of β_2AR. Homozygotes for Glu-27 showed very little agonist-driven downregulation of β_2AR, but only one, rare haplotype (ArgGly/GluGlu, see Table I) was studied.

We found a marked linkage disequilibrium between the two β_2AR gene polymorphisms. As a result, almost all chromosomes that carried the Arg-16 allele in our data also carried the Gln-27 allele, and no subjects in this population were homozygous for the Arg-16/Glu-27 haplotype. A likely consequence of the observed linkage disequilibrium between the two polymorphisms and of the preponderance of the effects of Gly-16 over those of Glu-27 on receptor downregulation (6) should be a very limited physiologic role for the β_2AR-27 polymorphism. This may explain why both Reihsaus et al. (4) and Turki et al. (5) observed an association of β_2AR-16, but not β_2AR-27, with more severe asthma and nocturnal asthma, respectively. Our data also showed that carriers of Gly-16 had decreased β_2-adrenergic responsiveness when compared with carriers of the Arg-16 allele, but that β_2AR-27 was unrelated to β_2-adrenergic responsiveness. If anything, Glu-27 homozygotes showed lower prevalence of positive responses to albuterol than Gln-27 homozygotes, which is in apparent contradiction with the observation that airway smooth muscle cells of a carrier of the ArgGly/GluGlu haplotype showed decreased agonist-promoted downregulation of β_2AR (18). However, 36 out of 37 Glu-27 homozygotes in our sample were also homozygotes for Gly-16, a combination that, as explained earlier,

is associated with marked agonists-driven receptor downregulation in vitro (6).

We found the same trends for the relation between β_2AR-16 polymorphisms in subjects who were classified as having asthma, in subjects with a history of wheezing during the previous year but who were not classified as having asthma, and in nonwheezing subjects. This, in spite of the fact that subjects with a history of asthma and/or wheezing were more likely to respond to bronchodilators than nonwheezing subjects, as has been observed by others (10). This result suggests that the differences in response to β_2-adrenergic agonists in subjects with different β_2AR-16 genotypes are independent of the factors that are determining the increased responsiveness to β_2-adrenergic agonists observed in subjects with asthma-like symptoms, most likely airway inflammation and bronchial hyperresponsiveness. The association between β_2AR-16 and bronchodilator response was also independent of any antiasthma treatment that symptomatic subjects were receiving at the time they were tested. The results therefore cannot be explained by changes in β_2AR expression or function associated with such treatment (19).

Interestingly, we found that the β_2AR-16 polymorphism is probably codominant, with heterozygotes showing intermediate levels of response to β_2-adrenergic agonists when compared with both β_2AR-16 homozygote genotypes. Similar results were reported by Turki et al. (5) for the association between β_2AR-16 and prevalence of nocturnal asthma. It is thus likely that receptors with different downregulation properties may be expressed in the cell surface of subjects who are heterozygotes for β_2AR-16. Naturally, this contention requires experimental confirmation.

Our results are in apparent contradiction with a report by Hall and coworkers (20) who showed that adult asthmatic subjects who were homozygotes for Glu-27 had significantly less methacholine hyperresponsiveness than asthmatic subjects who were homozygotes for Gln-27. These authors also reported that β_2AR-16 was unrelated to bronchial hyperresponsiveness to methacholine. It is possible that the mechanisms by which polymorphisms in the β_2AR gene determine responsiveness to methacholine in adult asthmatic subjects may be different from those through which they determine response to β_2-adrenergic agonists in children. Also, the distribution of β_2AR-16/β_2AR-27 haplotypes was not reported in the paper by Hall et al., and it is thus not possible to determine if the haplotype distribution of Glu-27 homozygotes was different from that observed in our population.

In the current study, we used a single dose (180 μg) of albuterol to assess β_2-adrenergic agonist responsiveness. This is the dose usually recommended to relieve mild asthmatic symptoms in children (3), and our results thus suggest that genetic determination of responsiveness to β_2-adrenergic agonists may be clinically relevant at these rather low doses. Studies assessing dose–response relationships in subjects with different β_2AR polymorphisms may help to clarify the precise role of these polymorphisms in determining the whole range of potential response to β_2-adrenergic agonists, and may thus be more relevant for clinical settings such as the emergency room or the intensive care unit, where much higher doses of these drugs are used.

In conclusion, our data demonstrate that the β_2AR-16 polymorphism has a significant physiologic role in regulating responses to exogenous and presumably endogenous β_2AR agonists. Since these drugs are the most widely used agents in the treatment of asthma, our findings, together with those of Liggett et al. (17), may have profound implications for our understanding of the genetic factors determining asthma severity and response to asthma therapy.

Acknowledgments

The authors wish to thank Maureen Cameron for preparing the manuscript.

This study was funded by grants HL14136 and HL56177 from the National Heart, Lung, and Blood Institute (NHLBI). Dr. Martinez was also funded by a Research Development Award for Minority Faculty, HL03154 from NHLBI.

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