

β_2 -Adrenoreceptor Polymorphisms in Asthmatic and Non-Asthmatic Schoolchildren From Colombia and Their Relationship to Treatment Response

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Summary. Asthma is a chronic and recurrent disease. Its high prevalence around the world is the result of a complex interaction between genetic and environmental factors. The genetic aspects of susceptibility, severity, and response to treatment in asthma are of great scientific interest. The purpose of the study was to establish the relationship between the Gln27Glu and Arg16Gly alleles of the β_2 -adrenergic receptor (*ADRB2*) gene with respect to the susceptibility to and severity of asthma, as well as the response to treatment in mestizo schoolchildren. 109 schoolchildren with asthma diagnosis and 137 asymptomatic controls were genotyped for the Arg16Gly and Gln27Glu alleles of the *ADRB2* gene by minisequencing. Allele, genotype, and haplotype frequencies of the *ADRB2* gene between asthmatic and non-asthmatic as well as demographic, clinical, and spirometric variables among asthmatic patients according to their genotype were compared. *ADRB2* gene expression was determined by real-time quantitative PCR. No statistical differences were found in allele, genotype, and haplotype frequencies of the *ADRB2* gene between cases and controls. We did not find differences between asthmatic patients classified according to their *ADRB2* genotypes and haplotypes when evaluating demographic, clinical, and spirometric variables. The *ADRB2* genotype and haplotype are not associated with spirometric responses or *ADRB2* gene expression after administration of a β_2 -agonist plus a glucocorticoid. These results suggest that in the group of mestizo schoolchildren studied, the Arg16Gly and Gln27Glu polymorphisms are not markers of susceptibility or severity of asthma and do not affect *ADRB2* gene expression during the rescue therapy. **Pediatr Pulmonol.** © 2012 Wiley Periodicals, Inc.

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INTRODUCTION

Asthma is a chronic disease whose prevalence has been increasing, particularly in children.¹ Given the magnitude of the problem, international health authorities have recommended increasing health research on asthma, including investigation into topics such as genetic susceptibility, severity and drug response.² Asthma has a polygenic nature due to the interaction between genetic and environmental factors and it has been estimated that the genetic contribution to asthma varies from 48% to 79%.³ Candidate genes in the pathogenesis of asthma have been the subject of intense research and constitute promising targets for therapeutic interventions.⁴ Additionally, research has shown that the wide variability between individuals in response to anti-asthmatic drugs appears to be related to allelic variants of several genes, including the polymorphisms

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of both the β_2 -adrenergic receptor gene and the glucocorticoid receptor gene. Up to 70% of the variation in the response to drug treatment could be attributed to genetic diversity.⁵ Genetic variations at the β_2 adrenoreceptor (*ADRB2*) gene may explain the ethnic differences in asthma severity and response to β_2 -agonists.^{6,7} A family-based genetic association study in Puerto Ricans and Mexicans demonstrated that the Arg16 allele is associated with asthma severity and β_2 -agonists responsiveness in Puerto Ricans but not in Mexicans.⁸

The β_2 -adrenoreceptor is subjected to a physiological mechanism of signal regulation under which the agonist-occupied β_2 receptor initiates a series of events leading to the attenuation of the response as a result of internalization, sequestration, and downregulation of receptors on the cell membrane; this phenomenon is reversed when the agonist is removed.⁹ Nevertheless, the extent of the downregulation is genotype-dependent, because the Gly16 allele of the gene encoding the β_2 adrenoreceptor is a variant with an increased extent of downregulation compared to the native Arg16 allele, so that when the β_2 receptor is subjected to the action of the agonist, the downregulation results in a lower density of β_2 receptors and subsequently in a diminished response. On the other hand, the Glu27 allele reduces the downregulation induced by β_2 agonists, which gives this variant resistance to desensitization and increased response to β_2 -agonists compared with the native Gln27 allele.¹⁰ Until now, the relation of these alleles to both susceptibility and severity of asthma as well as response to anti-asthmatic drugs has not been investigated in the Colombian population.

Expression of the *ADRB2* gene is also related to glucocorticoid activity because the *ADRB2* gene is positively regulated by glucocorticoids, as these agents increase the density of β_2 receptors in the cell membrane, causing upregulation in normal individuals, or blocking the downregulation induced by continuous exposure to β_2 agonist drugs.^{11,12} Consequently, by combining glucocorticoids and β_2 agonist drugs in the treatment of asthma, one adds the ability to restore the sensitivity of the β_2 receptors downregulated by β_2 agonists to the anti-inflammatory property of glucocorticoids, which would explain the synergism observed in medical practice.^{13–16} Some patients, however, are resistant to glucocorticoids, and improving our knowledge of the mechanisms involved in this resistance has been the subject of great scientific interest due to the enormous clinical implications.¹⁷ Our hypothesis was that the *ADRB2* genotype is related to asthma and asthma severity in Colombian Schoolchildren. In addition, we sought to determine whether stimulation of the *ADRB2* gene by glucocorticoids is genotype dependent.

METHODS

Identification and Recruitment of Patients

We conducted a case-control study of asthmatic and non-asthmatic Colombian children. After the administration of the ISAAC phase II survey (to detect respiratory symptoms consistent with asthma) to 1,234 school children of both genders, aged between 6 and 17 years, motivational and informative meetings were held with the parents of any schoolchildren who had ever had a diagnosis of asthma or respiratory symptoms. The survey used a Spanish version of ISAAC Phase II core and supplementary questionnaires developed according to ISAAC translation protocol.¹⁸ The core questionnaires consisted of two parts: demographic characteristics and ISAAC core questionnaire on wheezing, rhinitis, and eczema. The supplementary questionnaires consisted of three parts: (i) additional respiratory questions regarding cough with phlegm and wheeze with breathlessness, (ii) questions on disease management for asthma rhinitis and eczema, and (iii) questions on risk factors.

Of the 506 students evaluated by specialists in pneumology, 217 were diagnosed as asthmatics. After receiving signed informed consent forms from parents and the assent of the schoolchildren, 109 samples from patients and 137 samples from asymptomatic controls, unrelated to the cases and matched for age, gender, degree of admixture, education and socioeconomic status, were collected from oral mucosa and genotyped for the Arg16Gly and Gln27Glu alleles of the *ADRB2* gene. Allele, genotype, and haplotype frequencies of the *ADRB2* gene were first compared between asthmatics and non-asthmatics, and later between asthmatics categorized according to their *ADRB2* genotypes.

To determine whether stimulation of the *ADRB2* gene by glucocorticoids depends on the Arg16Gly or Gln27Glu genotypes, clinical changes in spirometric parameters and the expression of the β_2 -adrenoreceptor gene before and after treatment with a β_2 -agonist and systemic glucocorticoid were evaluated.

A subset of 36 asthmatic children underwent a second phase of the study, which included: (i) determination of the degree of genetic admixture by using multiplex ancestry-informative SNPs, according to the SNaPshot technique;¹⁹ (ii) a wash out period without asthma medication for at least 4 days; (iii) baseline assessment of the clinical, spirometric, and *ADRB2* gene expression parameters; (iv) 4 days of treatment with prednisolone (1 mg/kg/day in a single morning dose) and salbutamol (6 mg/day split into three portions); and (v) post-treatment assessment of the clinical, spirometric, and *ADRB2* gene expression parameters. This research was approved by the Bioethics Committee of the Universidad Tecnológica de Pereira, in the category of “minimal risk” research.

Assessment of Patients and Pulmonary Function Tests

The clinical and spirometric evaluation of patients and controls were performed by pneumologists according to the criteria of the European Respiratory Society (ERS) and the American Thoracic Society (ATS) using a DATOSPIR-70 (Sibelmed, Barcelona, Spain) portable spirometer that had been recently calibrated. The parameters selected for analysis were FVC (forced vital capacity), FEV₁ (forced expiratory volume in 1 sec), FVC/FEV₁ ratio and FEF_{25–75%} (forced expiratory flow). The values obtained were compared with the Knudson reference values predicted for normal populations.²⁰

Genotyping

Determination of the genotypes was performed using a mini-sequencing technique or “ddNTP primer extension.” Following extraction of genomic DNA from cells obtained from buccal brushes, using an extraction method on FTA cards, we proceeded to amplify a 709 bp fragment of the *ADRB2* gene, in which the SNPs to be studied are located. PCR was carried out on a total volume of 20 μ l that contained 15–20 ng of genomic DNA, 1 $\times$ buffer, 0.31 mM dNTPs, 4 μ M of each primer, 3.13 mM MgCl₂, 1.5% DMSO, and 1 U of Taq DNA polymerase (Invitrogen, Carlsbad, CA). The amplification was carried out on an automatic thermal cycler (PTC 100 MJ Research, Watertown, MA) with the following parameters: an initial denaturing step at 94°C for 3 min, followed by 40 cycles of 94°C for 45 sec, 60°C for 30 sec and 72°C for 45 sec, and a final elongation step at 72°C for 5 min. The resulting PCR product was analyzed by electrophoresis and visualized in a 2% agarose gel. Next, the samples were purified with 1 U EXOSAP IT (Amersham Pharmacia Biotech, Uppsala, Sweden). Using this product as template, reactions to detect native and mutated forms of the gene were performed. Mini-sequencing was performed on the ABI 3100 Avant, using the ABI Prism SNaPshot kit (PE Applied Biosystems, Foster City, CA), following the manufacturer’s instructions. The extension reactions of the primers were done in a total volume of 10 μ l, containing between 0.01 and 0.4 pmol of the amplicon, 0.15 pmol/ μ l of each primer and 2.5 μ l of a SNaPshot reaction mix that contains AmpliAq DNA polymerase FS and fluorescently labeled ddNTPs. The tubes containing the mixture were placed in a thermal cycler to be amplified for 30 cycles using the following conditions: denaturing at 96°C for 10 sec, annealing at 50°C for 5 sec, and extension at 60°C for 30 sec. The test includes a positive and a negative control and quality standards. The resulting product was injected electrokinetically over 5 sec to 15 kV and put under

electrophoresis for 40 min to 15 kV, 9 μ A, 60°C in a 36 cm long capillary, using the performance optimum polymer 4 (POP-4) with a laser to a constant power of 9.9 mW. The data was analyzed by the color of the curves and the size of the fragments using GeneMapper Analysis Software (PE Applied Biosystems).

RNA Isolation, Reverse Transcription, and Real-Time PCR

Five-milliliter blood samples were collected in EDTA vacutainer tubes, diluted 1:2 in phosphate buffered saline and centrifuged at 400 $\times$ g for 30 min over a Ficoll–Hypaque gradient to isolate peripheral blood mononuclear cells (PBMC, >98% lymphocytes as determined by microscopy). Total RNA was extracted from PBMCs (5 $\times$ 10⁶ cells) using the RNAeasy Mini-kit (Quiagen Inc, Valencia, CA). The mRNA expression of the *ADRB2* gene and the β -actin and *GAPDH* house-keeping genes were determined by Real-time quantitative PCR (RT-qPCR), using the SmartCycler Real-Time System (Cepheid, Sunnyvale, CA). The qRT-PCR for *ADRB2* and β -actin was carried out in a reaction volume of 25 μ l, using the Power SYBR Green RNA-to-C_T 1-Step Kit (Applied Biosystems), following the manufacturer’s instructions, using 200 nM of primers for β -actin (Actin gene Internal Stds, Ambion, Austin TX) and 200 nM of the primers 5'-TGCGCCGGAC-CACGACGTCACG-3' (forward) and 5'-GCTCGAAC-TTGGCAATGGCTG-3' (reverse) to amplify a 133 bp product of the *ADRB2* gene. The qRT-PCR for *GAPDH* was carried out in a reaction volume of 25 μ l, using the Taqman RNA-to-C_T 1-Step Kit (Applied Biosystems) and Taqman *GAPDH* Control Reagent (Applied Biosystems) following the manufacturer’s recommendations. The thermal profile for the qRT-PCR was 30 min at 48°C for cDNA synthesis, 10 min at 95°C for reverse transcriptase inactivation and AmpliTaq Gold DNA polymerase activation, with 40 cycles of 15 sec at 95°C for denaturing and 1 min of annealing/extension at 60°C for amplification. At the end of each PCR run, the data were analyzed automatically by the system and amplification plots were generated for each RNA sample. We calculated the relative amount of each transcript in the samples and normalized to the two control genes (β -actin and *GAPDH*) as internal reference. The magnitude of *ADRB2* gene expression was calculated using the comparative Δ C_T method (C_T *ADRB2* gene—average C_T β -actin and *GAPDH* genes). All experiments were performed in duplicate.

Statistical Analysis

Parametric tests were used to compare genotype distributions between asthmatic and non-asthmatic children and to establish Hardy–Weinberg equilibrium.

Non-parametric tests were used to compare demographic and spirometric variables in asthmatics who had undergone treatment and to determine the significance of the differences in *ADRB2* gene expression and lung function tests before and after treatment. We used 95% confidence intervals and all statistical tests were two-tailed. A P -value < 0.05 was considered to be significant. All statistical analyses were performed using SPSS for Windows, version 15 (SPSS Inc., Chicago, IL) and Structure Software v.2.0 to identify the percentage of ancestry of each individual.²¹

RESULTS

Phase I

No differences in age (11.6 ± 5.4 years vs. 11.8 ± 5.2 years, $P = 0.98$) or gender distribution (78/31 vs. 96/41 M/F, $P = 0.46$) were found between asthmatic schoolchildren and non-asthmatic controls. Based on the allele, genotype and haplotype distributions of the *ADRB2* gene shown in Table 1, we did not observe statistically significant differences between asthmatics ($n = 109$) and non-asthmatics ($n = 137$) with regard to the *ADRB2* genotypes studied. The haplotype pairs Arg16Arg-Glu27Glu and Arg16Gly-Glu27Glu were not found in any of the groups. For the subsequent analysis, the haplotypes with the lower frequency, Gly16Gly-Glu27Glu ($n = 4$) and Arg16Arg-Gln27Glu ($n = 2$), were discarded. Hardy-Weinberg equilibrium was confirmed for the genotypes of both codon 16 ($P = 0.62$) and codon 27 ($P = 0.98$).

Table 2 compares the symptoms and spirometric parameters used for classification of asthma. There was no association between the severity of the disease and the different alleles at the Arg16Gly or Gln27Glu loci. Except for the high incidence of asymptomatic males in the doubly heterozygous subgroup (Arg16Gly-Gln27Glu), there are no significant differences between the different *ADRB2* haplotypes with regard to the clinical and spirometric variables studied (Table 3).

Phase II

Table 4 shows the lack of association of the genotypes at loci 16 and 27 of the *ADRB2* gene with a series of demographic, clinical, spirometric and gene expression variables in 33 asthmatics (three out of 36 patient samples could not be processed) that initiated and successfully completed the anti-asthmatic treatment protocol. Similar results were obtained when the different haplotype pairs were considered (data not shown). Treatment with salbutamol (6 mg/day) and prednisolone (1 mg/kg/day) orally administered for 4 days, did not induce changes in spirometric parameters or mRNA levels of the *ADRB2* gene in the 33 patients grouped

according to their Arg16Gly and Gln27Glu genotypes (Table 5) or haplotypes (data not shown). Population stratification was not found when we analyzed the degree of genetic admixture of the patients, grouped according to their Arg16Gly and Gln27Glu genotypes. The ancestry component for the subset of asthmatic children that underwent the second phase of the study was: African 10%, European 48%, Asian 20%, and Native American 22%.

DISCUSSION

The *ADRB2* gene is highly polymorphic and allele prevalence changes significantly among different ethnic groups. Allele and genotype frequencies found in the schoolchildren included in this study (Table 1) are similar to those we previously reported in a Colombian mestizo population.²² Several investigations exploring the relationship of the Arg16Gly and Gln27Glu polymorphisms to different phenotypes of asthma have yielded discrepant results, which are accentuated among different ethnic groups.^{10–12} For example, Choudhry et al.⁸ found that ethnic-specific pharmacogenetic differences exist between Arg16Gly genotypes, asthma severity and bronchodilator response in Puerto Ricans and Mexicans with asthma, two Hispanic populations similar to Colombian mestizo (an admixture between Amerindian, Hispanic, and African descent). The fact that no genotypic differences at loci 16 and 27 in the *ADRB2* gene between asthmatic and non-asthmatic individuals were found (Table 1) indicates that neither polymorphism is a marker of susceptibility to asthma in the population represented in this study. Although the controversy about the contribution of the Arg16Gly and Gln27Glu polymorphisms in the *ADRB2* gene and the risk of asthma persists, there is increasing evidence that the *ADRB2* gene is included within the susceptibility loci to bronchial hyperresponsiveness and asthma,^{23,24} although these results have not been confirmed by others.^{25,26} Due to the high degree of admixture found among individuals with asthma (African 10%, European 48%, Asian 20%, and Native American 22%), the existence of significant haplotypes or mutations in gene regions other than the ones that have been genotyped by our group and others that did not find associations cannot be ruled out.

Furthermore, when we looked for a possible association between the allelic (Table 2) and haplotypic (Table 3) variants with asthma severity, we did not find any relationship to the main symptoms and spirometric findings that define severity of the disease. However, this result does not necessarily mean that the genotypes evaluated in this study are not important and a different benchmark should be used. Although some studies have found that Gly16 allele is a marker of asthma

TABLE 1—Allelic, Genotypic, and Haplotypic Distribution of the Arg16Gly and Gln27Glu Polymorphisms of the ADRB2 Gene Between Asthmatic Schoolchildren and Controls

	Asthmatic (n = 109)		Non-asthmatic (n = 137)		<i>P</i> -value
	n	Frequency (95% CI)	n	Frequency (95% CI)	
<i>ADRB2</i> allele					
Arg16	99	0.45 (0.39–0.52)	138	0.50 (0.44–0.56)	0.32
Gly16	119	0.55 (0.48–0.61)	136	0.50 (0.44–0.55)	
Gln27	181	0.83 (0.78–0.88)	235	0.86 (0.79–0.91)	
Glu27	37	0.17 (0.12–0.22)	39	0.14 (0.10–0.18)	0.48
<i>ADRB2</i> Genotype					
Arg16Arg	30	0.27 (0.22–0.33)	48	0.35 (0.27–0.43)	0.44
Arg16Gly	39	0.36 (0.29–0.42)	42	0.31 (0.23–0.38)	
Gly16Gly	40	0.37 (0.30–0.43)	47	0.34 (0.26–0.42)	
Gln27Gln	76	0.70 (0.64–0.76)	103	0.75 (0.68–0.82)	
Gln27Glu	29	0.27 (0.21–0.33)	29	0.21 (0.14–0.28)	
Glu27Glu	4	0.04 (0.01–0.06)	5	0.04 (0.01–0.07)	0.6
<i>ADRB2</i> Haplotype					
Arg16Arg-Gln27Gln	28	0.26 (0.18–0.34)	47	0.28 (0.22–0.33)	0.83
Arg16Gly-Gln27Gln	25	0.23 (0.15–0.31)	28	0.28 (0.22–0.33)	
Gly16Gly-Gln27Gln	23	0.21 (0.13–0.29)	28	0.26 (0.18–0.34)	
Arg16Gly-Gln27Glu	14	0.13 (0.07–0.19)	14	0.26 (0.18–0.34)	
Gly16Gly-Gln27Glu	13	0.12 (0.06–0.18)	14	0.26 (0.18–0.34)	
Gly16Gly-Glu27Glu	4	0.04 (0.02–0.07)	5	0.26 (0.18–0.34)	
Arg16Arg-Gln27Glu	2	0.02 (0.01–0.04)	1	0.28 (0.22–0.33)	

Associations for the differences between asthmatics and non-asthmatics were tested by means of the Chi-square test. CI, confidence intervals.

severity,^{27,28} our results match with most studies which found no association between this marker and disease severity. However, due to the small population studied, we were not able to detect any association with asthma or related phenotypes.

The Arg16Gly allele influence on drug response to short-acting beta2-agonistas (SABA) has been subject of intense research, for example, Israel et al.^{29,30} found that the Gly16 homozygous patients benefit most from

long-term treatment with SABA, that Arg16 homozygous patients. Opposite results have been reported by other authors who found that Gly16 allele was associated with a decreased response to acute exposure to SABA therapy.^{8,31,32}

The gene expression analysis using qRT-PCR suggests that genetic variability at sites 16 and 27 of the β_2 -adrenoreceptor is not associated with differences in the expression of the *ADRB2* gene, which is known

TABLE 2—Baseline Characteristics of 109 Asthmatic Individuals by Genotype

	Arg16Arg (n = 30)	Arg16Gly (n = 39)	Gly16Gly (n = 40)	<i>P</i> -value	Gln27Gln (n = 76)	Gln27Glu (n = 29)	Glu27Glu (n = 4)	<i>P</i> -value
Age (year)	10.9 ± 3.3	11.5 ± 3.2	11.6 ± 3.0	0.6	11.1 ± 3.0	11.6 ± 3.4	14 ± 3.0	0.09
Gender (M/F)	18/12	29/10	31/9	0.24	48/28	26/3	4/0	0.012
Cough (yes/no)	15/15	15/24	18/22	0.63	31/45	16/13	1/3	0.3
Exacerbations/year								
None	19	19	16	0.46	41	12	1	0.3
1-3	7	14	18		25	13	1	
4-12	3	6	5		9	3	2	
>12	1	0	1		1	1	0	
Nocturnal asthma								
Never	22	25	24	0.59	52	18	1	0.5
<1 week	7	10	10		17	8	2	
≥1 week	1	4	6		7	3	1	
Spirometry								
FEV ₁ (%)	97.6 ± 12	97 ± 18	92.7 ± 17	0.35	95.7 ± 17	95.7 ± 15	91.8 ± 18	0.64
FEF _{25–75} (%)	79.9 ± 23	88.6 ± 25	79.7 ± 21	0.16	81.4 ± 23	87.2 ± 22	80 ± 32	0.8

Quantitative variables are expressed as mean ± SD. Chi-square test was used for categorical variables, whereas ANOVA was used for numerical variables. M, male; F, female; FEV₁, forced expiratory volume in 1 sec; FEF, forced expiratory flow.

TABLE 3—Baseline Characteristics of 103 Asthmatic Individuals According to the More Prevalent Haplotypes

	Arg16Arg-Gln27Gln (n = 28)	Arg16Gly-Gln27Gln (n = 25)	Gly16Gly-Gln27Gln (n = 23)	Arg16Gly-Gln27Glu (n = 14)	Gly16Gly-Gln27Glu (n = 13)	P-value
Age (year)	10.8 ± 3.3	11.3 ± 2.9	11.3 ± 3.1	11.9 ± 3.8	11.2 ± 2.9	0.87
Gender (M/F)	17/11	15/10	16/7	14/0	11/2	0.05
Cough (yes/no)	13/15	5/20	13/10	10/4	4/9	0.02
Exacerbations/year						
None	18	16	7	3	8	0.06
1-3	6	6	13	8	4	
4-12	3	3	3	3	0	
>12	1	0	0	0	1	
Nocturnal asthma						
Never	21	18	13	7	10	0.65
<1 week	6	5	6	5	2	
≥1 week	1	2	4	2	1	
Spirometry						
FEV ₁ (%)	96.5 ± 11.7	95.7 ± 19.8	94.8 ± 18.8	99.3 ± 14.3	89.1 ± 12.4	0.56
FEF ₂₅₋₇₅ (%)	79.7 ± 23.3	82.9 ± 24.7	81.9 ± 23	98.8 ± 22.7	75.5 ± 15	0.07

Quantitative variables are expressed as mean ± SD. Chi-square test was used for categorical variables, whereas ANOVA was used for numerical variables. M, male; F, female; FEV₁, forced expiratory volume in 1 sec; FEF, forced expiratory flow.

to be one of the genes trans-activated by glucocorticoids. This suggests that the Arg16Gly and Gln27Glu alleles of the *ADRB2* gene are not involved in the therapeutic response of asthmatic patients to glucocorticoids and that such agents could overcome the downregulation disorders induced by these allelic variants. Although this result is in agreement with recent studies showing that the *ADRB2* genotype does not influence the therapeutic response to the combination of β_2 agonists and inhaled glucocorticoids in patients with asthma,³³ other authors have shown opposite results.³⁴⁻³⁷

These findings should be viewed with caution for several reasons: first, the gene expression level of messenger RNA was measured in a relatively small number of patients (n = 33) and the lack of differences could

be due to insufficient power. However, we used two internal controls for messenger RNA quantification in order to improve both the statistical power of the test as well as the reliability based on normalization of data; second, other authors have shown that the pharmacogenetic findings with respect to *ADRB2* and lung function occur after longer periods of time²⁹ but in this study, the drugs were prescribed only for a few days to individuals with controlled mild to moderate asthma, circumstances that do not allow evaluation of long-term results or examination during acute exacerbations, in order to detect glucocorticoid induced upregulation of the β_2 adrenergic receptor and its anti-inflammatory effects on the airways; also, since all asthmatic patients were compensated at the time of incorporation into the

TABLE 4—Baseline Characteristics of 33 Asthmatic Individuals Treated With Salbutamol Plus Prednisolone According to *ADRB2* Genotype

	Arg16Arg (n = 8)	Arg16Gly (n = 15)	Gly16Gly (n = 10)	P-value	Gln27Gln (n = 21)	Gln27Glu (n = 10)	Glu27Glu (n = 2)	P-value
Age (year)	11.4 ± 2.7	11 ± 2.6	12 ± 2.4	0.43	11.1 ± 2.5	11.8 ± 2.9	12.5 ± 0.4	0.57
Gender (M/F)	5/3	7/8	8/2	0.25	12/9	6/4	2/0	0.49
BMI (kg/m ²)	18 ± 3.6	19 ± 2.7	19 ± 3.2	0.61	18 ± 2.7	20 ± 3.1	19 ± 3	0.07
Asthma severity								
Mild-moderate/severe	7/1	14/1	10/0	0.45	19/2	10/0	2/0	0.5
Prednisolone (mg/day)	42 ± 15	39 ± 1.2	44 ± 11	0.51	39.5 ± 13	44 ± 11	43 ± 11	0.64
FVC (%)	110 ± 11	113 ± 12	110 ± 25	0.25	112 ± 16	110 ± 19	108 ± 0.0	0.75
FEV ₁ (%)	96 ± 13	96 ± 17	94 ± 14	0.92	94 ± 15	98 ± 15	108 ± 7	0.31
FEV ₁ /FVC (%)	77 ± 8	74 ± 8	77 ± 10	0.66	74 ± 9.0	79 ± 7	85 ± 5	0.09
FEF ₂₅₋₇₅ (%)	71 ± 20	69 ± 23	74 ± 20	0.92	65 ± 20	79 ± 21	94 ± 20	0.07
cDNA (ΔC_T)	4.8 ± 2.1	1.7 ± 1.0	4 ± 1.3	0.38	3.2 ± 1.1	3.6 ± 1.3	0.65 ± 0.08	0.98

Chi-square test was used for categorical variables, whereas Kruskal-Wallis was used for numerical variables. ΔC_T values are expressed as mean ± SEM, whereas the other quantitative variables are expressed as mean ± SD. BMI, body mass index; FVC, forced vital capacity; FEV₁, forced expiratory volume in 1 sec; FEF, forced expiratory flow; cDNA, complementary DNA; ΔC_T , change in threshold cycle.

TABLE 5—Spirometric and Gene Expression Parameters Before and After Treatment With Salbutamol Plus Prednisolone in 33 Asthmatic Individuals Stratified According to ADRB2 Genotype

	Arg16Arg (n = 8)	Arg16Gly (n = 15)	Gly16Gly (n = 10)	Gln27Gln (n = 21)	Gln27Glu (n = 10)	Glu27Glu (n = 2)
FVC (%)						
Basal	110 ± 11	113 ± 12	110 ± 25	112 ± 16	110 ± 19	108 ± 0
Post	106 ± 15	113 ± 19	95 ± 11	108 ± 19	103 ± 13	103 ± 6
P-value	0.33	0.84	0.32	0.43	0.29	0.18
FEV ₁ (%)						
Basal	96 ± 13	96 ± 17	94 ± 14	94 ± 16	98 ± 15	108 ± 7
Post	98 ± 12	95 ± 18	88 ± 13	94 ± 18	95 ± 9	94 ± 16
P-value	0.29	0.8	0.67	0.67	0.58	0.18
FEV ₁ /FVC (%)						
Basal	77 ± 8	74 ± 8	77 ± 10	74 ± 9	79 ± 7	85 ± 5
Post	82 ± 7	73 ± 8.3	80 ± 8	76 ± 9	81 ± 7	76 ± 9
P-value	0.06	0.7	0.53	0.2	0.77	0.18
FEF _{25–75} (%)						
Basal	71 ± 20	69 ± 23	74 ± 20	65 ± 21	79 ± 21	93 ± 5
Post	78 ± 15	63 ± 19	71 ± 23	66 ± 19	79 ± 19	66 ± 23
P-value	0.14	0.5	0.58	0.38	0.91	0.18
cDNA (ΔC_T)						
Basal	4.8 ± 2.1	1.7 ± 1.0	4 ± 1.3	3.2 ± 1.1	3.6 ± 1.3	0.7 ± 0.1
Post	3.3 ± 1.9	2 ± 1.2	3.6 ± 1.5	2.4 ± 1.0	3.9 ± 1.6	0.7 ± 0.6
P-value	0.12	0.49	0.42	0.12	0.58	1

Comparisons were performed using the Wilcoxon rank test. ΔC_T values are expressed as mean $\pm$ SEM, whereas the other quantitative variables are expressed as mean $\pm$ SD. FVC, forced vital capacity; FEV₁, forced expiratory volume in 1 sec; FEF, forced expiratory flow; cDNA, complementary DNA; ΔC_T , change in threshold cycle.

study, the diagnosis of asthma was not confirmed inducing bronchospasm with methacholine and demonstrating reversibility of bronchospasm using bronchodilators. We based the diagnosis of asthma only in clinical history and spirometric evaluation of patients; third, we recognize that a step-wise experimental design is optimal to evaluate the effects of corticosteroids on beta agonist response between the Arg16Gly and Gln27Glu genotypes because the corticosteroid may mask the effects of the β_2 agonist, but we had to use both drugs simultaneously. Finally, it is necessary to keep in mind that this study included only Colombian mestizo volunteers and that these results may not be applicable to other ethnic groups.

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