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Influence of β_2-adrenergic receptor polymorphisms on asthma exacerbation in children with severe asthma regularly receiving salmeterol

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ABSTRACT

Background: New evidence suggests that different β_2-adrenergic receptor (β2AR) polymorphisms may influence asthma control in patients receiving long-acting β_2-agonists (LABAs) as regular therapy.
Objectives: To determine the influence of β2AR polymorphisms on asthma exacerbations in children with severe asthma from Argentina receiving inhaled corticosteroid (ICS) and LABAs regularly.
Methods: Ninety-seven children with severe asthma were genotyped for polymorphisms of β2AR at codons 16 and 27. The number of severe exacerbations, the time of first asthma exacerbation, and the number of hospitalizations during 12 months were assessed. Changes on pulmonary function from the beginning to the end of the study were also evaluated.
Results: The number of overall asthma exacerbations and the proportion of children with these events were similar among β2AR genotypes at position 16 (Arg/Arg, Arg/Gly, and Gly/Gly) and at position 27 (Gln/Gln, Gln/Glu, and Glu/Glu). The time to first asthma exacerbation was similar among individuals carrying different β2AR polymorphisms. No β2AR genotype association was found in relation to the number of hospitalizations. Longitudinal analysis of forced expiratory volume in 1 second from baseline to the end of the study also showed no differences among β2AR genotypes at position 16 or 27. No association was observed among the 3 most common haplotypes (Arg/Arg-Gln/Gln, Gly/Gly-Gln/Gln, and Gly/Gly-Glu/Glu) and the number of participants with asthmatic crisis or with the overall number of exacerbations.
Conclusion: β2AR polymorphisms were not associated with an increased risk of having asthma exacerbations or lung function decline in a population of Argentinian children with severe asthma receiving ICS and LABAs regularly.

Introduction

Asthma is one of the most common chronic diseases in children. It is a complex disease influenced by multiple environmental and genetic factors. Different specific genetic determinants have been investigated. One of the most studied candidate genes is the β_2-adrenergic receptor (*β2AR*) gene located on chromosome 5q31-q32, which encodes β2AR, a G protein that is expressed in airway smooth muscle.

Several β2AR polymorphisms have been described in this gene. The 2 most common genetic variants are characterized by substitution of glycine (Gly16) for arginine (Arg16) at position 16 and glutamic acid (Glu27) for glutamine (Gln27) at position 27 of the extracellular domain of the receptor.[1] These polymorphisms have been known to alter β2AR function in the respiratory system because they induce bronchial relaxation. Several studies have reported that homozygous Arg16 patients either have a reduced bronchodilator effect or experience a decline in lung function. In addition, patients carrying this variant may present an increased risk of exacerbations when they receive short-acting β_2-agonists (SABAs) as regular therapy.[2–4] Similar results have been found among homozygous Arg16 individuals who were regularly treated with long-acting β_2-agonists (LABAs).[5–7]

These observations raise the possibility that the effects of long-term treatment with inhaled β_2-agonist drugs on asthma control may be determined, at least in part, by specific β2AR genotypes. The clinical implications of these findings could be significant, especially if we consider the effect of the use of LABAs administered with inhaled corticosteroids (ICSs) on asthma control in patients with persistent asthma. This combination is an effective and well-tolerated maintenance therapy for children and adults with persistent moderate and severe asthma.[8]

Asthma exacerbations account for the largest proportion of children with severe asthma. Exacerbations and asthma-related hospital admissions represent well-validated measures of asthma

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control in children and in adults.[9] Because treatment with LABAs has been associated with the number of asthma exacerbations, we have evaluated whether the presence of a particular β2AR polymorphism can increase the likelihood of asthma exacerbations in children with severe asthma under treatment with LABAs.

Methods

A population-based, prospective cohort study was conducted during a 3-year period (2009-2011). Of 280 genotyped children for β2AR polymorphisms belonging to a genetic asthma cohort at Hospital de Pediatría (Dr. Juan P. Garrahan), Buenos Aires, Argentina, every patient with current physician-diagnosed severe asthma was enrolled (n = 97).[10,11] Severe asthma was defined following the Global Initiative for Asthma guidelines.[8]

Patients of both sexes eligible for enrollment were 6 years or older with asthma of 12 months' duration or longer. At the moment of diagnosis they presented with continuous asthma symptoms, daily limitation to exercise, frequent exacerbations and night symptoms, and daily use of a bronchodilator. Patients also had a low forced expiratory volume in 1 second (FEV_1) of 60% or lower. They had a history of taking medium-high doses of ICS (fluticasone propionate, 250 μg or equivalent, given twice daily) for 12 weeks or longer and were following a stable asthma regimen (daily dose unchanged) for 2 weeks or more before the screening.

The influence of β2AR polymorphisms on asthma control was assessed by quantifying the overall number of exacerbations, the number of severe exacerbations, the time of first asthma exacerbation, and hospitalizations during the 12-month study period. Changes in pulmonary function from the beginning to the end of the study were also considered as a parameter to evaluate asthma control. Secondary outcomes considered were the use of as-needed medication for symptom relief and symptom-free days during 12 months.

During the study period, children were instructed to take an ICS (fluticasone propionate, 250 μg) plus an LABA (salmeterol, 25 μg) twice daily, using a metered-dose inhaler and a pediatric chamber. Medication was delivered by single inhaler device. Albuterol was used as needed for symptom relief. Inhaled treatment was provided by the hospital pharmacy as part of the severe asthma clinic program. Adherence to treatment was assessed by the total number of doses taken determined from the inhaler dose counter.

During the study, patients were seen in the severe asthma clinic setting. Clinic visits were scheduled monthly. Information on any asthma-related exacerbations, short courses of albuterol and oral steroids received, and asthma-related hospital admissions during the 12-month study were recorded. A physician who did not know the genetic variants of the β2AR collected the data.

The severity of asthma exacerbations was defined according to a consensus statement recently published by the American Thoracic Society/European Respiratory Society.[12] A severe exacerbation was considered an event requiring the use of oral corticosteroids for at least 3 days or an emergency department (ED) admission or hospitalization. A moderate exacerbation was an event with symptoms that lasted 2 or more days but not severe enough to require oral steroid or hospitalization. The patient could need ED care but did not require oral corticosteroid. Patients were treated according to their personalized action plans, as usually indicated. They were provided with albuterol and had scheduled clinic visits and 24-hour access to a physician consultation.

A pulmonary function test was performed at every visit before the morning dose of inhaled medication and was measured by means of spirometry. Studies were performed with the standardization of the American Thoracic Society.[13] The parameters considered were vital forced capacity, FEV_1, and forced middle maximum flow. Spirometries performed at the moment of enrollment and on the last day of the study were considered for the analysis.

Some atopic conditions other than asthma were also registered at the beginning of the study. In all patients, IgE levels (expressed as the number of fold increase over normal values according to age) and numbers of blood eosinophils were evaluated.

Molecular Methods

All children were genotyped for polymorphisms of the *β2AR* gene. Genomic DNA was extracted from peripheral blood leukocytes by standard procedures. The polymorphisms of *β2AR* at codons 16 and 27 were analyzed by polymerase chain reaction/restriction fragment length polymorphism as was previously described in the literature.[14] Briefly, a 168-bp PCR product, including both polymorphisms, was digested using *Nco*I restriction enzyme to identify Arg16/Gly16 alleles and *Bbv*I to identify polymorphisms at position 27 (Glu/Gln).

An informed consent, approved by the Ethics Committee of Hospital de Pediatría (Dr. Juan P. Garrahan), was signed by the patient's parent or legal guardian. No pharmaceutical company had input into a trial design, management, or interpretation of the information.

Statistical Analysis

Data were analyzed using a Stata version 10.0 software package (Stata Corp, College Station, Texas). Standard descriptive statistics were used to describe the baseline characteristics of the study population using means and SDs for continuous variables and number and percentages for ordinal variables.

The number of asthma exacerbations was analyzed by genotype group. This comparison was made by use of analysis of variance models on the assumption that outcomes were approximately normally distributed. This analysis also used age, sex, IgE values, number of blood eosinophils, and pulmonary function in the model. The percentage of patients with asthma exacerbations was analyzed using the χ^2 test.

Time to first exacerbation was described by Kaplan-Meier plots. The comparison of time to first exacerbation among genotypes was obtained from a log-rank test. Haplotype analyses were performed using similar models. A difference was considered statistically significant if the $P < .05$.

Results

A total of 97 Argentinean children with a diagnosis of severe asthma were genotyped for β2AR variants. At position 16, the frequency of the Arg/Arg variant was 21% (n = 20); Arg/Gly, 39% (n = 38); and Gly/Gly, 40% (n = 39). The prevalence of genotypes at position 27 was as follows: Gln/Gln, 62% (n = 60); Gln/Glu, 32% (n = 31); and Glu/Glu, 6% (n = 6). Polymorphisms were found to be in the Hardy-Weinberg equilibrium. The number of patients with each potential variant combination at positions 16 and 27 is shown in Table 1. Allele frequencies were 0.40 for Arg16 and 0.60 for Gly16. The frequencies for Gln27 and Glu27 were 0.78 and 0.22, respectively. This allele frequency was similar to that observed in our series of patients with mild and moderate asthma previously studied.[10,11]

Table 1
Association of β_2-adrenergic receptor genetic variants at positions 16 and 27

Genotype 16	Genotype 27			Total
	Gln-Gln	Gln-Glu	Glu-Glu	
Arg-Arg	20	0	0	**20**
Arg-Gly	23	14	1	**38**
Gly-Gly	17	17	5	**39**
Total	**60**	**31**	**6**	**97**

T frequency.

Table 2
Baseline characteristics of the 97 study patients by β_2-adrenergic receptor genotype

Characteristic	Genotype 16			Genotype 27		
	Arg/Arg (n = 20)	Arg/Gly (n = 38)	Gly/Gly (n = 39)	Gln/Gln (n = 60)	Gln/Glu (n = 31)	Glu/Glu (n = 6)
Male sex, No. (%)	6 (30)	13 (34)	18 (46)	23 (38)	12 (39)	2 (33)
Age, y[a]	12.1 (2.59)	13.2 (2.71)	13 (2.7)	12.9 (1)	12.6 (2)	15.1 (1)
IgE[b]	10.4 (2.8)	8.9 (1.2)	9.7 (2.5)	7.1 (1.1)	13 (3.2)	19.2 (10)
Blood eosinophils[a]	539 (155)	575 (106)	688 (95)	864 (232)	596 (236)	495 (105)
Pulmonary function[c]						
FVC	101 (94-108)	99 (95-104)	100 (96-104)	102 (99-106)	98 (94-102)	92 (81-102)
FMMF	80 (68-93)	71 (62-80)	77 (64-89)	77 (69-85)	71 (60-82)	81 (52-110)

Abbreviations: FEV_1, forced expiratory volume in 1 second; FMMF, forced middle maximum flow; FVC, forced vital capacity.
[a]Data are expressed as mean (SD).
[b]IgE values are expressed as fold increased over normal values.
[c]Pulmonary function values are in percentage of predicted values. Data are expressed as mean (95% confidence interval).

No significant differences were found related to sex, age, IgE levels, and number of blood eosinophils across different β2AR genotypes. Pulmonary function at baseline was also similar among variants at position 16 and 27 (Table 2).

The proportion of participants who had severe asthma exacerbation was similar across β2AR genotypes. During the study, 17 patients (85%) carrying the Arg/Arg variant had at least one exacerbation, as well as 28 patients (77%) having Arg/Gly and 39 (89%) of those with the Gly/Gly genotype ($P = .90$). For the variants at position 27, the number of individuals who presented with asthma exacerbations was 48 (80%) for Gln/Gln, 28 (90%) for those with Gln/Glu, and 4 (66%) for children with Glu/Glu ($P = .40$).

The rate of asthma exacerbations was presented as exacerbations per participant per year (Table 3).[12] The number of overall asthma exacerbations did not differ among genotypes at position 16 or 27 (Table 3). Variants at position 16 or 27 did not show any effect on the rate of asthma exacerbations measured as above, either when each position was considered in isolation or as a covariate with the other position genotypes ($P = .80$ and $P = .70$, respectively). A similar trend was seen in the rate of severe and moderate asthma exacerbations (Table 3).

Considering the time to first asthma exacerbation for the analysis, no statistically significant differences were observed among individuals carrying different β2AR polymorphisms at position 16 or 27 (Arg16Gly, $P = .07$; Gln27Glu, $P = .70$).

Asthma-related hospital admissions were uncommon (n = 4). The number of exacerbations that led to hospital admission did not differ among genotypes groups at position 16 ($P = .60$) or 27 ($P = .50$).

Concerning pulmonary function, longitudinal analysis of forced vital capacity, FEV_1, and forced middle maximum flow from baseline to the end of the study also revealed no differences among patients carrying different genotypes (data not shown).

In addition, no differences were noted among patients having different β2AR genetic variants in other secondary outcome measures, including use of as-needed medication for symptom relief (Arg16Gly, $P = .30$; Gln27Glu, $P = .40$) and symptom-free days (Arg16Gly, $P = .60$; Gln27Glu, $P = .50$).

Because haplotypes might be more informative than individual genotypes, the homozygous haplotypes Arg16Arg/Gln27Gln (n = 20), Gly16Gly/Gln27Gln (n = 14), and Gly16Gly/Glu27Glu (n = 6) were evaluated. No association was observed among haplotypes and the number of participants with asthma exacerbations ($P = .40$) or the overall number of exacerbations ($P = .50$). Furthermore, pulmonary function measurements were not affected by haplotypes either at the beginning or at the end of the study.

Discussion

Asthma is a disease with a heterogeneous clinical expression. This feature is even more marked in patients with severe asthma.[15] The management of the latter group is often problematic. Although heterogeneity in drug responses may indeed be due to inherent differences in the underlying disease, an alternative option to explain this variability might be the influence of genetics on drug response.[16]

The present study was conducted to investigate the role of β2AR polymorphisms on an important clinical outcome, such as asthma exacerbations. The findings showed no pharmacogenetic associations between β2AR genetic variants and the frequency of asthma exacerbations in Argentinean children with severe asthma receiving ICSs and LABAs regularly.

A particular concern of this study was to test the hypothesis regarding asthma worsening in children associated with severe asthma undergoing salmeterol therapy on a regular basis. Published data suggest that this drug used alone increases the risk of severe asthma events.[17,18] Furthermore, salmeterol shows greater heterogeneity of response and also seems to be less effective in children than in adults with asthma.[19] The US Food and Drug Administration issued recommendations that LABAs be used only in combination with ICSs and for the shortest duration possible.[20]

It has been proposed that LABAs' effects might be related to β2AR genetic variants. Different β2AR polymorphisms appear to be associated with variability in the response to β_2-agonists, influencing asthma control. Prospective clinical trials have reported that the homozygous presence of Arg16 variant reverses the benefits from the regular use of SABAs in asthmatic patients compared with the

Table 3
Number of asthma exacerbations by β_2-adrenergic receptor polymorphisms[a]

Outcome	Genotype 16				Genotype 27			
	Arg/Arg (n = 20)	Arg/Gly (n = 38)	Gly/Gly (n = 39)	*P* value	Gln/Gln (n = 60)	Gln/Glu (n = 31)	Glu/Glu (n = 6)	*P* value
Overall No. of asthma exacerbations	40	88	80		117	79	12	
Asthma exacerbations[b]	2.2 (1.1-3.3)	2.4 (1.7-3.2)	2.3 (1.6-2.8)	.20	2.2 (1.6-2.7)	2.7 (1.9-3.4)	1.7 (0.6-2.7)	.40
Mild asthma exacerbations[b]	1 (0.5-1.4)	1.2 (0.7-1.6)	1.1 (0.7-1.4)	.10	1.03 (0.7-1.3)	1.2 (0.8-1.6)	1 (0.1-1.8)	.40
Severe asthma exacerbation[b]	2.2 (0.4-2)	1.2 (0.7-1.7)	1.2 (0.7-1.6)	.30	1.1 (0.7-1.5)	1.4 (0.9-2)	0.7 (0.1-1.4)	.80
Asthma-related hospital admissions	1	2	1	.60	2	2	0	.50

[a]Analysis of variance test was applied for differences among genotypes.
[b]The rate of asthma exacerbations is exacerbations per participant per year. Data are presented as mean (95% confidential interval).

homozygous Gly16 genotype.[3,4] Palmer and colleagues confirmed these findings in a population of children and young adults treated following the 4-step British Thoracic Society therapy recommendations.[7] The authors of the present study have shown, in a group of Argentinean asthmatic children, that variants at position 27 also might influence the response to SABAs as was described in previous studies.[10,21] In reference to the influence of β2AR polymorphisms on the response to LABAs, the information is somehow conflicting. Retrospective studies have suggested that they might have some influence, but other studies have not.[6,22] Available information suggests that patients taking salmeterol who were homozygous Arg16 may be at greater risk of developing an asthma exacerbation in the absence of controller medication.[7] Basu and colleagues[23] have described a significant increase in the risk of exacerbations in children and young adults with mild persistent asthma receiving ICSs with inhaled albuterol according to need per copy of the Arg16 allele. This increased risk was present in patients who were also taking regular LABAs. No increase in the risk of exacerbations was observed in patients with more severe asthma receiving ICSs, LABAs, and albuterol according to need per Arg16 allele copy.

In the present study conducted in children with severe asthma there was no indication that the Arg/Arg16 genotype was responsible for outcomes different from any other genotype for either asthma exacerbations or lung function. Our findings are consistent with data reported by Taylor and colleagues[2] and Bleecker and colleagues.[24] In the study by Taylor et al, there was a similar pattern in the frequency of major exacerbations across genotypes during treatment with salmeterol. Similarly, 2 studies by Bleecker et al demonstrated a sustained improvement in lung function and a reduction in asthma exacerbations regardless of β2AR genotypes. Recently, Bleecker et al[25] have not found any pharmacogenetic effect of β2AR polymorphisms in patients receiving LABA monotherapy or in combination with an ICS.

In agreement, the results of the current study suggest that the genotypic effects on asthma exacerbations are not present among children receiving high doses of an ICS plus LABAs. In this study, the treatment provided was consistent with national and international guideline recommendations for the management of moderate and severe persistent asthma.[8,26,27] Our series did not evaluate salmeterol as monotherapy, which is not recommended in current asthma guidelines. This condition could have contributed to the failure to detect a polymorphic effect of β2AR on our study outcomes. It is worth mentioning that children were also instructed to use inhaled albuterol as reliever medication for their asthma. Thus, in our study the use of β_2-agonist relievers seems to be unrelated to deleterious effects on asthma control in patients taking LABAs.

A possible explanation for these findings could be the protection against β_2-agonist–induced downregulation and desensitization of β2AR conferred by ICS. β2AR desensitization for a long period is associated with a decrease in receptor number and increased receptor degradation. Previous observations reported in adults with asthma carrying homozygous Arg16 genotype have shown that this genotype predisposes patients to bronchoprotective subsensitivity of LABAs.[28–30] Steroids have shown in vitro and in vivo studies to reverse functional desensitization of β2AR and increase receptor expression and density.[31–33] However, in humans, loss of bronchoprotection from regularly administered β_2-agonists seems to reverse only with high doses of ICS, and it is not clear whether this happens with long-term use of ICS at low or medium doses.[34,35] In the present study, β2AR genotypic effects on asthma control were not present among ICS and LABA users, and this might be due to reversed β2AR desensitization by high ICS doses.

Children participating in this study had well-preserved lung function with an overall mean FEV_1 of 87% by the end of the study. Also, in the longitudinal analysis, pulmonary function revealed no significant differences from baseline in any genetic variant of β2AR. It is worth mentioning that patients were enrolled in the study only if they had their asthma controlled and the pulmonary function was close to normal. The deleterious effect of salmeterol, therefore, seems to be unrelated to airway caliber. It has been reported that the adverse effects related to salmeterol were possibly more defined by bronchoprotective than by bronchodilator subsensitivity during a period of increased airway tone, rather than during the quiescent state.[28,36] This finding is also suggested by previous studies using bronchial challenge in adults with asthma, for whom the degree of bronchoprotection conferred by LABAs was less in patients who had the Arg16 genotype.[28] However, as in this cohort, where children did not perform bronchoprovocation challenges, this mechanism might only be suggested as a putative hypothesis.

The present study, although not powered to evaluate haplotypes effect, supports the findings observed with individual genotypes. There was no evidence that the clinical outcomes and pulmonary function were affected by common β2AR haplotypes, in concordance with published data.[24]

Some methodologic issues are worth mentioning. The prospective nature of this study with adequately long follow-up and no losses to follow-up was a major strength and provided a special setting to evaluate genetic effects on asthma exacerbations and lung function variation for a period of 12 months. The observational nature of this series does not completely exclude potential for confounding. Further studies with a larger number of children with severe asthma are needed to confirm an association between the presence of these polymorphisms and asthma exacerbations.

In summary, we explore the practical consequences on the management of severe asthma with ICSs and LABAs in a population of Argentinean children through an effect on asthma control for a period of several months. On the basis of the results, it might be not necessary to avoid LABA therapy in patients with severe asthma carrying homozygous Arg16 variant. β2AR polymorphisms were not associated with an increased risk of having asthma exacerbations or with lung function decline. Genotypic effects on asthma control were not present among ICS and LABA users, which might be due to reversed β2AR desensitization by high ICS dose.

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