

Association between β_2 -adrenoceptor polymorphisms and asthma diagnosis among Mexican adults

Alfredo A. Santillan, MD, MPH,^{a,b} Carlos A. Camargo, Jr, MD, DrPH,^c
Alicia Ramirez-Rivera, MD,^b Ivan Delgado-Enciso, MD,^a Augusto Rojas-Martinez, MD,^a
Felipe Cantu-Diaz, MD,^b and Hugo A. Barrera-Saldaña, PhD^a Monterrey, Mexico, and
Boston, Mass

Background: Recent studies demonstrate that genetic variations in the human β_2 -adrenergic receptor (β_2 AR) structure at codons 16 and 27 alter receptor function in vitro and are associated with asthma severity and airway hyperresponsiveness but have not been linked to asthma diagnosis. The nature of the relation in a more homogeneous population is uncertain. **Objective:** We determined frequencies of these polymorphisms to explore the association between β_2 AR haplotypes and asthma diagnosis and phenotype.

Methods: This is a population-based, case-control study that involves a total sample of 907 unrelated Mexican Mestizos. Genotyping at β_2 AR was identified by polymerase chain reaction–restriction fragment length polymorphism analysis. Multivariate logistic regression analysis was used to estimate the odds ratio (OR) of the association between β_2 AR haplotype status and asthma diagnosis.

Results: A significant inverse association was found between subjects with Glu27 allele (OR, 0.5; 95% CI, 0.4 to 0.7) and Gly16-Glu27 alleles (OR, 0.5; 95% CI, 0.3 to 0.8) and asthma. Sex differences in this association were explored, given the complex relation between sex and asthma. Among men, a positive association was present between the “Gly16 allele without Glu27” (OR, 2.9; 95% CI, 1.26 to 6.8) and asthma. In contrast, a lower risk of asthma was found among women Gly16-Glu27 alleles (OR, 0.3; 95% CI, 0.2 to 0.6). Nocturnal asthma was associated with the Gly16 allele (OR, 1.8; 95% CI, 1.3 to 2.6). **Conclusions:** Variation in the β_2 AR gene is associated in the pathogenesis of asthma and acts as a disease modifier in nocturnal asthma. (*J Allergy Clin Immunol* 2003;112:1095-1100.)

Key words: Alleles, asthma, β_2 -adrenoceptor polymorphism, case-control studies, genetics, genotype, phenotype

Abbreviations used

β_2 AR: β_2 -Adrenergic receptor
BMI: Body mass index

Genetic variations in the human β_2 -adrenergic receptor (β_2 AR) structure have been the subject of much experimental and clinical research, given its potential role in the pathogenesis and treatment of asthma. The intronless gene encoding this G-protein-coupled receptor has been cloned and sequenced, and 13 different point mutations in the promoter and coding sequence have subsequently been described.¹⁻³ Four polymorphisms of the coding region demonstrate changes in the amino acid sequence; however, only substitutions of glycine for arginine at codon 16 (Arg16→Gly) and glutamic acid for glutamine at codon 27 (Gln27→Glu) of the β_2 AR gene have been widely studied in both subjects with and without asthma, since they occur in high allelic frequencies.⁴

Previous analyses of genetic risk of asthma among adults have not, to our knowledge, linked β_2 AR gene polymorphisms to asthma diagnosis.^{2,5-11} The nature of the relation of these polymorphisms and asthma in a homogeneous Mexican Mestizo population with respect to ethnic origin¹² and geography is uncertain. Therefore, we sought to determine the allele frequencies of the Arg16→Gly and the Gln27→Glu β_2 AR gene polymorphisms in 907 Mexican Mestizos. In addition, since few prior studies of genetic risk of asthma have analyzed Gly16 and Glu27 polymorphisms together,¹³ multilocus genotypes were introduced in an effort to better characterize the role of potential interactions between these two polymorphisms of the coding region in the risk of developing asthma.³ Next, given the complex relation between sex and asthma,¹⁴ we explored sex differences in the relation between these polymorphic variants and asthma diagnosis. Finally, we assessed the relation of these polymorphisms and asthma phenotype.

METHODS

Participants

This is a population-based, case-control study that involves a total sample of 907 unrelated Mexican Mestizos living in Monterrey, Mexico. A Mexican Mestizo is a person born in the country, having a Spanish-derived last name, with family antecedents of

From ^aDepartamento de Bioquímica, Facultad de Medicina, Universidad Autónoma de Nuevo León (UANL), Monterrey, México; ^bLaboratorio de Fisiología Pulmonar, Hospital de Enfermedades Cardiovasculares y del Tórax, Centro Médico Nacional del Norte, Instituto Mexicano del Seguro Social (IMSS), Monterrey, México; and ^cChanning Laboratory, Department of Medicine, Brigham and Women's Hospital, and the Department of Emergency Medicine, Massachusetts General Hospital, Harvard Medical School, Boston, Mass.

Received for publication March 15, 2003; revised September 3, 2003; accepted for publication September 12, 2003.

Reprint requests: Dr Carlos A. Camargo, Jr, Massachusetts General Hospital, 55 Fruit St, Clinics Bldg 397, Boston, MA, 02114-2969.

Supported by the Consejo Nacional de Ciencia y Tecnología grant 35392-M, Mexico, and by National Institutes of Health grant HL-03533, Bethesda, Md.

© 2003 American Academy of Allergy, Asthma and Immunology

0091-6749/2003 \$30.00 + 0

doi:10.1016/j.jaci.2003.09.029

Mexican ancestors at least back to the third generation.¹² Ethical approval was granted by the Mexican Institute of Social Security and Universidad Autonoma de Nuevo Leon, and written consent was obtained from all participants.

Outpatient cases were recruited from the Pulmonary Physiology Laboratory at the Mexican Institute of Social Security. Asthma diagnosis was based on the following criteria: (1) current physician diagnosis of asthma, (2) symptoms (wheezing), (3) use of antiasthma medications, and (4) spirometry (β -agonist reversibility). Subjects with concomitant physician diagnosis of chronic bronchitis and emphysema were excluded from the study. A total sample of 303 outpatients with a diagnosis of asthma agreed to participate in the study. On the other hand, a population-based, random sample of 660 healthy control subjects was ascertained from the member's insurance list of the Mexican Institute of Social Security. Of the identified control subjects, 56 refused to donate blood for the genotyping analysis; therefore 604 control subjects were included. The control group consisted of healthy subjects (98% denied history of any chronic disease) who met the following criteria: (1) no prior or current physician diagnosis of asthma; (2) no history of wheezing, chronic phlegm, or shortness of breath; and (3) no use of antiasthma medications.

A structured face-to-face interview and examination was conducted for all cases and control subjects by trained interviewers who used a structured questionnaire that assessed demographic and phenotypic characteristics. All interviews were conducted at the Mexican Institute of Social Security. Cases and control subjects were classified with respect to physical activity status as physically active if they reported at least a 30-minute walk per day, and/or lifting or carrying heavy objects at work daily, and/or participating in sports or physical exercise more than 2 hours per week. Cases were asked for history of nocturnal asthma, defined as a history of awakening caused by asthma at least once per week for 12 consecutive months.¹⁵ Furthermore, baseline pulmonary function and reversibility studies were evaluated in the case group, with values expressed as percentage of predicted normal values.¹⁶

Genotyping

Genotyping at β_2 AR-16 and β_2 AR-27 was identified by polymerase chain reaction–restriction fragment length polymorphism analysis, using the primers and protocol detailed by Large et al.¹⁷ Cases and control subjects were genotyped together, and individuals who performed β_2 AR genotyping were blinded to case-control status. In 10% of subjects, samples were genotyped by two investigators independently, and they obtained identical results ($\kappa = 1.0$).

Statistical analysis

Statistical analyses were performed with Stata 7.0 (StataCorp, College Station, Tex). Data were compiled according to the genotype, and the derived allelic frequencies were estimated on the basis of the observed numbers of the specific alleles from genotype data in each group. The genotype frequencies for each polymorphism were tested for deviation from the Hardy-Weinberg equilibrium by χ^2 goodness-of-fit analysis, with one degree of freedom used. Differences in the frequencies of the β_2 AR-2 polymorphisms by race were tested by comparison of the distribution of genotypes and alleles with $R \times C$ contingency table analysis and Fisher exact test as appropriate. Linkage disequilibrium was based on analysis of a 3×3 contingency table. Since unique interactions of multiple single nucleotide polymorphisms within a haplotype ultimately affect biological and therapeutic phenotype and individual single nucleotide polymorphisms may have poor predictive power as pharmacogenetic loci,³ analyses were performed across 3 multilocus genotypes, as described by Barr et al: Arg16 reference (Arg16Arg-Gln27Gln and

Arg16Arg-Gln27Glu), “Gly16 allele without Glu27” or Gly16 (Arg16Gly-Gln27Gln and Gly16Gly-Gln27Gln), and “Gly16 allele with Glu27” or Gly16-Glu27 alleles (Arg16Gly-Gln27Glu, Arg16Gly-Glu27Glu, Gly16Gly-Gln27Glu, and Gly16Gly-Glu27Glu).¹³ Associations were tested by means of the χ^2 test, *t* test, ANOVA, and Wilcoxon rank-sum test, as appropriate. Multivariate logistic regression analysis was used to estimate the odds ratio (OR) of the association between β_2 AR genotype status and asthma diagnosis, while controlling for age, sex, education, smoking, body mass index (BMI), and physical activity. Also, multivariate logistic regression analysis was used to look at the association between β_2 AR genotype status and nocturnal asthma, while controlling for demonstrated predictors of nocturnal asthma (see Results section). Multiplicative interaction terms were tested in the logistic regression models. A value of 2-tailed $P < .05$ was considered statistically significant. OR are presented with 95% confidence intervals (CI).

RESULTS

Table I shows the general characteristics of the study population. Cases differed from control subjects in several ways. Adults with asthma were older, more likely to be female, and were less likely to have graduated from high school. Although cases and control subjects had similar levels of having ever smoked (36% and 40%, respectively), adults with asthma were more likely to be former smokers, whereas control subjects were more likely to be current smokers. Subjects with asthma had higher BMI and were less likely to be physically active.

All individuals were genotyped for β_2 AR-16 and β_2 AR-27 gene polymorphisms, and all known genetic variations were found. As expected, the two sequence variants were in strong linkage disequilibrium ($P < .001$). β_2 AR-27 gene polymorphisms were in Hardy-Weinberg equilibrium in the general population. However, only among cases, an increased number of Gln27Gln homozygotes were present ($P = .007$). For the β_2 AR-16 gene polymorphisms, there were no significant deviations from Hardy-Weinberg equilibrium.

Asthma diagnosis

Table II shows β_2 AR allele frequencies in asthma and control groups. The frequency of β_2 AR-27 alleles differed significantly between cases and control subjects ($P = .001$). The Glu27 allele was highly associated with a decrease prevalence of asthma diagnosis (OR, 0.5; 95% CI, 0.4 to 0.7). Stratifying the analysis by sex did not materially change these results. On the other hand, the allelic frequency of the Glu27 polymorphism among the control group was 20%, significantly lower ($P < .001$) among our Mexican population than in white subjects, in whom the prevalence of the Glu27 allele is more frequent (47%).⁴ In contrast, there were no marked interethnic differences ($P = .27$) in the frequency of the Glu27 allele and previous reports among black (21%) and Hispanic (26%) populations.^{18,19} Finally, the allelic frequency of Gly16 among control subjects did not differ from these populations ($P = .34$).^{4,18,19}

Multilocus genotype frequencies between cases and control subjects are shown in Table III. The most common

TABLE I. Demographic and clinical characteristics of the study population

Characteristics	Cases (n = 303)	Control subjects (n = 604)
Age (y), mean $\pm$ SD*	42 $\pm$ 14	35 $\pm$ 12
Female (%)*	74	62
High school graduate (%)*	33	44
Smoking status (%)*		
Never-smokers	64	60
Former smokers	27	12
Current smokers	9	28
Median pack-years		
Among former smokers	1.0	0.5
Among current smokers	2.0	1.2
BMI (kg/m ²), mean $\pm$ SD*	30 $\pm$ 6	28 $\pm$ 5
Weight (kg), mean $\pm$ SD	73 $\pm$ 16	71 $\pm$ 16
Physically active (%)*	69	83

* $P < .01$.

combination for both cases and control subjects was Arg16Gly-Gln27Gln, 43% and 33%, respectively. In agreement with previous reports,^{13,17,19} there was no participant homozygous for both Arg16 and Glu27. Also, when compared with white individuals, Mexican Mestizos had a significantly higher frequency of the combined Gly16 and Gln27 (15% vs 5%) and a marked lower frequency of the combined Gly16 and Glu27 (3% vs 15%).¹⁹ Table IV presents adjusted ORs of the association between β_2 AR-16 and β_2 AR-27 multilocus genotype groups and asthma diagnosis stratified by sex. Adjustment for potential confounders had essentially no effect on the estimates by 10% or more.²⁰ Among all participants, with subjects with Arg16 used as the reference group, asthma was not associated with “Gly16 allele without Glu27” (OR, 1.1; 95% CI, 0.7 to 1.6), but a significant inverse association was found among subjects with Gly16-Glu27 alleles (OR, 0.5; 95% CI, 0.3 to 0.7). Stratifying the analysis by sex, we found a strong, positive, independent association between “Gly16 allele without Glu27” and asthma in men (OR, 2.9; 95% CI, 1.2 to 6.8) but not in women (OR, 0.7; 95% CI, 0.4 to 1.1). Furthermore, a strong and independent inverse association between Gly16-Glu27 alleles and asthma was found in women (OR, 0.3; 95% CI, 0.2 to 0.6) but not in men (OR, 0.8; 95% CI, 0.3 to 2.1). The interaction was statistically significant ($P = .003$), with “Gly16 without Glu27” increasing risk in men but not in women. On the other hand, Gly16-Glu27 alleles appeared equally protective for both men and women (P for interaction term = .09).

Asthma phenotype

Table V presents phenotypic characteristics of asthma cases across β_2 AR multilocus genotype groups. Among subjects with asthma, “Gly16 allele without Glu27” and Gly16-Glu27 alleles were significantly associated with nocturnal asthma but not with several demographic, clinical, and severity characteristics of asthma. Subjects with nocturnal asthma (n = 90) reported a significantly higher

TABLE II. β_2 -adrenoceptor gene allele frequencies between cases and control subjects*

Allele	Cases (n = 303)	Control subjects (n = 604)	OR (95% CI)
Arg16	275 (45%)	520 (43%)	0.9 (0.7-1.1) [†]
Gly16	331 (55%)	688 (57%)	
Gln27	535 (88%)	972 (80%)	0.5 (0.4-0.7) [‡]
Glu27	71 (12%)	236 (20%)	

*Counts represent actual number of alleles and not subjects.

[†] $P = .34$.

[‡] $P < .001$.

TABLE III. β_2 -adrenoceptor multilocus genotype frequencies between cases and control subjects

Genotype	Cases (n = 303)	Control subjects (n = 604)	P value
Arg16Arg	56 (18%)	101 (17%)	.61
Arg16Gly	163 (53%)	318 (53%)	
Gly16Gly	84 (28%)	185 (31%)	
Gln27Gln	241 (80%)	385 (64%)	<.001
Gln27Glu	53 (17%)	202 (33%)	
Glu27Glu	9 (3%)	17 (3%)	
Arg16Arg-Gln27Gln	56 (19%)	96 (16%)	<.001*
Arg16Gly-Gln27Gln	131 (42%)	198 (33%)	
Arg16Gly-Gln27Glu	31 (10%)	118 (20%)	
Gly16Gly-Gln27Gln	54 (18%)	91 (15%)	
Gly16Gly-Gln27Glu	22 (7%)	79 (13%)	
Gly16Gly-Glu27Glu	8 (2%)	15 (3%)	
Arg16Gly-Glu27Glu	1 (<1%)	2 (<1%)	
Arg16Arg-Gln27Glu	0 (0%)	5 (1%)	
Arg16Arg-Glu27Glu	0 (0%)	0 (0%)	

* $P < .001$ after Bonferroni correction for multiple comparisons.

history of hay fever ($P = .03$) and presented a significantly lower baseline FEV₁ ($P = .04$) compared with subjects with asthma with nonnocturnal symptoms. Also, adults with nocturnal asthma were more likely to report a history of ever having been hospitalized for asthma ($P < .01$), history of emergency department visit in the last year ($P < .01$), use of inhaled steroids during the past 4 weeks ($P < .01$), and use of inhaled β -agonists during the past 4 weeks ($P < .01$). We found that the Gly16 allele was highly associated with an increased prevalence of nocturnal asthma, with an OR of 1.8 (95% CI, 1.3 to 2.6). Furthermore, in the multivariate analysis, nocturnal asthma was strongly associated with “Gly16 allele without Glu27” (OR, 4.9; 95% CI, 1.7 to 13.9) and with Gly16-Glu27 alleles (OR, 4.2; 95% CI, 1.3 to 13.7). Stratifying the analysis by sex did not materially change these results (data not shown).

DISCUSSION

The current study reports an association between human β_2 AR gene polymorphisms at codons 16 and 27 and asthma diagnosis among a total sample of 907 unrelated adults. We found that the allelic frequency of the Glu27 polymorphism of the β_2 AR gene was less preva-

TABLE IV. β_2 -Adrenoceptor gene polymorphism frequencies among cases versus control subjects stratified by sex

Genotype groups [†]	Total population			Men			Women		
	Cases (n = 303)	Control subjects (n = 604)	OR (95% CI)	Cases (n = 80)	Control subjects (n = 227)	OR (95% CI)	Cases (n = 223)	Control subjects (n = 377)	OR (95% CI)
Arg16 (%)	18	17	1.0	10	19	1.0	22	15	1.0
Gly16 (%)	61	48	1.1 (0.7-1.6)	75	48	2.9 (1.2-6.8)	56	48	0.7 (0.4-1.1)
Gly16-Glu27 (%)	20	35	0.5 (0.3-0.7)	15	33	0.8 (0.3-2.1)	22	37	0.3 (0.2-0.6)

*Odds ratios for asthma diagnosis are adjusted for age, sex, education, smoking status, BMI (kg/m²), and physical activity.

[†]In the genotype groups, subjects with genotypes Arg16Arg-Gln27Gln and Arg16Arg-Gln27Glu are the reference group (Arg16) and are compared with subjects with genotypes Arg16Gly-Gln27Gln and Gly16Gly-Gln27Gln (Gly16) and with subjects with genotypes Arg16Gly-Gln27Glu, Arg16Gly-Glu27Glu, Gly16Gly-Gln27Glu, and Gly16Gly-Glu27Glu (Gly16-Glu27).

TABLE V. Phenotypic characteristics of asthma cases across β_2 -adrenoceptor genotype groups^{*}

Phenotypic characteristics	Arg16	Gly16	Gly16-Glu27
Mean age (y), mean $\pm$ SD	36 $\pm$ 13	38 $\pm$ 13	37 $\pm$ 13
Female (%)	68	65	68
High school graduate (%)	34	40	44
Never-smokers (%)	66	61	59
No exercise (%)	71	76	76
Mean age of asthma diagnosis (y), mean $\pm$ SD	32 $\pm$ 16	32 $\pm$ 16	34 $\pm$ 16
Maternal history of asthma (%)	15	10	18
Paternal history of asthma (%)	6	8	8
History of hay fever (%)	70	68	66
Upper respiratory infection as trigger of attacks (%)	73	76	69
Mean FEV ₁ (% predicted), mean $\pm$ SD	82 $\pm$ 28	87 $\pm$ 24	84 $\pm$ 21
Mean FVC (% predicted), mean $\pm$ SD	92 $\pm$ 26	97 $\pm$ 20	94 $\pm$ 20
Ever hospitalized for asthma (%)	30	30	27
Emergency department visit in last year (%)	34	46	44
Ever taken steroid medicine for asthma (%)	30	35	35
Inhaled steroids during past 4 weeks (%)	50	52	55
Inhaled β -agonist during past 4 weeks (%)	64	68	73
History of nocturnal asthma (%) [†]	11	35	32

*Subjects with genotypes Arg16Arg-Gln27Gln and Arg16Arg-Gln27Glu are the reference group (Arg16) and are compared with subjects with genotypes Arg16Gly-Gln27Gln and Gly16Gly-Gln27Gln (Gly16) and with subjects with genotypes Arg16Gly-Gln27Glu, Arg16Gly-Glu27Glu, Gly16Gly-Gln27Glu, and Gly16Gly-Glu27Glu (Gly16-Glu27).

[†]P < .01 after Bonferroni correction for multiple comparisons.

lent among Mexican Mestizos than in white populations. This could explain the significant inverse association between the Glu27 allele and asthma diagnosis in men and women found in our study population.

The results of our study are consistent with expectations one can infer from in vitro studies that assessed the phenotype of β_2 AR gene polymorphisms. Site-directed mutagenesis studies have demonstrated that Gly16 variant has an enhanced agonist-promoted downregulation compared with the wild-type receptor.^{21,22} On the contrary, the Glu27 polymorphism was associated with an impaired agonist downregulation.^{21,22} It was shown that β_2 AR-27 polymorphism affects the agonist-promoted downregulation in that the Gln27 polymorphism confers increased downregulation when compared with the Glu27 receptor form. Similar results have been reported in primary cultures of human airway smooth muscle cells.²³

As a result, it is possible that the enhanced agonist-promoted β_2 AR downregulation associated with the Gln27 polymorphism correlates with the increased risk of asthma. Moreover, the association of the "Gly16 allele

without Glu27" with asthma diagnosis in our population could be explained by the fact that the haplotype Gly16-Gln27 has the greatest potential for downregulation.²² On the other hand, the result that a protective association was present between the receptor form Glu27 and asthma is consistent with its resistance to downregulation. Individuals with the Gln27 variant may downregulate their β_2 AR in vivo in response to circulating catecholamines. Subjects harboring the Glu27 form, which is resistant to downregulation, would therefore express higher levels of β_2 AR in the airways compared with the Gln27 form. Thus, individuals with the Glu27 variant of the receptor may be less susceptible to inflammation and bronchoconstriction and hence have less reactive airways than individuals with the Gln27 form.⁹ Moreover, Dishy et al²⁴ reported that the Glu27 polymorphism is associated with increased agonist-mediated responsiveness.

The in vitro and in vivo effects of several polymorphic combinations of the β_2 AR gene have been shown to be different from those previously obtained with individual polymorphisms.³ Drysdale et al identified different

effects on the β_2 AR gene and protein expression in vitro, consistent with the in vivo response to a β_2 AR agonist, based on haplotype pairs. Individuals with asthma with haplotype Arg16Arg-Gln27Gln had a depressed responsiveness in contrast to individuals with haplotype Gly16Gly-Glu27Glu, who had an increased responsiveness. In the current study, men with the “Gly16 allele without Glu27” were at increased risk of asthma diagnosis, whereas men and women harboring the Gly16-Glu27 alleles were at lower risk of asthma diagnosis.

Our findings of an association among men with the “Gly16 allele without Glu27” and asthma diagnosis are also consistent with previous clinical data. Hall et al found that the Gln27 allele was associated with enhanced bronchial hyperresponsiveness and elevated IgE levels among asthmatic persons. Furthermore, sex differences in our study are consistent with a previous analysis of β_2 AR haplotypes reported by D’Amato et al¹¹ in a population-based study of men that showed that Gly16-Gln27 was strongly associated with persistent bronchial hyperresponsiveness. Finally, although Ulbrecht et al reported an underrepresentation of Gly16-Gln27-Thr164 haplotype carriers within the group of individuals with bronchial hyperresponsiveness, only a significant protective effect of the Gly16-Gln27-Thr164 haplotype on bronchial hyperresponsiveness was present among women and not in men.²⁵

The protective effect of the Glu27 polymorphism is also consistent with several clinical studies. Hopes et al found that this form of the receptor was associated with a decreased prevalence of self-reported asthma in a random population of children from the United Kingdom.²⁶ Finally, this polymorphism has not been associated with increased levels of IgE and bronchial hyperactivity among subjects with asthma.^{8,9} Thus, our results confirm previous studies that hypothesized that subjects harboring the Glu27 form of the receptor could be protected from the development of asthma.^{9,10}

With regard to the relation between β_2 AR gene polymorphisms at codons 16 and 27 and asthma phenotype, we found a strong association between the Gly16 variant and history of nocturnal asthma. This observation is consistent with Turki et al,¹⁵ who reported that the Gly16 form of the receptor was strongly associated with nocturnal asthma. In addition, we performed analyses across genotype groups and found that the combination of the Gly16 variant with both Gln27 and Glu27 polymorphisms were more common in subjects with a history of nocturnal asthma. As previously noted, the mechanism whereby the Gly16 polymorphism might lead to the development of nocturnal asthma is related to its enhanced agonist-mediated β_2 AR downregulation.

The current study has some potential limitations. First, it could be possible that several potential confounding variables such as BMI, physical activity, and mainly, smoking, could be altering our results, since they may have gene-environment interactions with β_2 AR gene polymorphisms.^{17,27,28} However, the multivariate analyses showed that the associations of β_2 AR gene polymor-

phisms at codons 16 and 27 with asthma were independent of these factors. Also, there was no clear evidence in this sample for an interaction among BMI ($P = .38$), physical activity ($P = .89$), and smoking ($P = .62$) with genotype groups. Second, the case definition of nocturnal asthma was based on history and not defined by spirometry. Nevertheless, since we found that subjects with a history of nocturnal asthma had a higher prevalence of multiple markers of asthma severity, those participants who reported nocturnal symptoms probably do exhibit the phenotype of nocturnal asthma. Finally, it cannot be excluded that other polymorphisms or complex haplotypes within the promoter and coding region of the β_2 AR gene or in adjacent genes might contribute to the genetic inheritance of asthma while being in linkage with those studied here.³ In this regard, polymorphisms of the 5' leader cistron of the β_2 AR, which are known to be important for controlling the level of expression of the β_2 AR in cells,^{29,30} deserve further investigation of linkage analyses with β_2 AR gene polymorphisms at codon 16 and 27.

The current study reports an association between β_2 AR-16 and β_2 AR-27 gene polymorphisms with asthma diagnosis and nocturnal symptoms among Mexican Mestizos. These results provide evidence that variation in the β_2 AR gene is associated with asthma pathogenesis and may act as a disease modifier in nocturnal asthma. The results of our study are consistent with expectations one can infer from in vitro and in vivo studies that evaluated the phenotype of β_2 AR gene polymorphisms. Furthermore, these results give emphasis to the necessity of accounting for ethnic background in performing β_2 AR genotyping. Interethnic differences in the β_2 AR structure could explain differences in the incidence and prevalence of asthma, disease susceptibility, asthma severity, and treatment response. Further investigation is required to assess the sex difference that could be of major importance in β_2 AR function and its role in the pathogenesis of asthma.

The authors are most grateful to Dr Edwin Silverman for his thoughtful comments on the manuscript.

REFERENCES

1. Kobilka BK, Dixon RAF, Frielle T, Dohlman HG, Bolanowski MA, Sigal IS, et al. cDNA for the human β_2 -adrenergic receptor: a protein with multiple membrane-spanning domains and encoded by a gene whose chromosomal location is shared with that of the receptor for platelet-derived growth factor. *Proc Natl Acad Sci U S A* 1987;84:46-50.
2. Reihnsaus E, Innis M, MacIntyre N, Liggett SB. Mutations in the gene encoding for the β_2 -adrenergic receptor in normal and asthmatic subjects. *Am J Resp Cell Mol Biol* 1993;8:334-9.
3. Drysdale CM, McGraw DW, Stack CB, Stephens JC, Judson RS, Nandabalan K, et al. Complex promoter and coding region β_2 -adrenergic receptor haplotypes alter receptor expression and predict in vivo responsiveness. *Proc Natl Acad Sci U S A* 2000;97:10483-8.
4. Liggett SB. The pharmacogenetics of β_2 -adrenergic receptors: relevance to asthma. *J Allergy Clin Immunol* 2000;105:S487-92.
5. Dewar JC, Wheatley AP, Venn A, Morrison JFJ, Britton J, Hall IP. β_2 -Adrenoceptor polymorphisms are in linkage disequilibrium, but are not associated with asthma in an adult population. *Clin Exp Allergy* 1998;28:442-8.
6. Summerhill E, Leavitt SA, Gidley H, Parry R, Solway J. β_2 -Adrenergic receptor Arg16/Arg16 genotype is associated with reduced lung function,

- but not with asthma, in the Hutterites. *Am J Respir Crit Care Med* 2000;162:599-602.
7. Emala CW, McQuitty CK, Eleff SM, Hopkins-Price P, Lawyer C, et al. Asthma, allergy, and airway hyperresponsiveness are not linked to the beta(2)-adrenoceptor gene. *Chest* 2002; 121:722-31.
 8. Dewar JC, Wilkinson J, Wheatley A, Thomas NS, Doull I, Morton N, et al. The glutamine 27 β_2 -adrenoceptor polymorphism is associated with elevated immunoglobulin E levels in asthmatic families. *J Allergy Clin Immunol* 1997;100:261-5.
 9. Hall IP, Wheatley A, Wilding P, Liggett SB. Association of the Glu27 β_2 -adrenoceptor polymorphism with lower airway reactivity in asthmatic subjects. *Lancet* 1995;345:1213-4.
 10. Ramsay CE, Hayden CM, Tiller KJ, Burton PR, Goldblatt J, LeSouef PN. Polymorphisms in the β_2 -adrenoceptor gene are associated with decreased airway responsiveness. *Clin Exp Allergy* 1999;29:1195-203.
 11. D'Amato M, Vitiani LR, Petrelli G, Ferrigno L, di Pietro A, Trezza R, et al. Association of persistent bronchial hyperresponsiveness with β_2 -adrenoceptor (ADRB2) haplotypes. *Am J Respir Crit Care Med* 1998;158:1968-73.
 12. Gorodezky C, Alaez C, Vazquez-Garcia MN, de la Rosa G, Infante E, Balladares S, et al. The genetic structure of Mexican Mestizos of different locations: tracking back their origins through MHC genes, blood group systems, and microsatellites. *Hum Immunol* 2001;62:979-91.
 13. Barr RG, Cooper DM, Speizer FE, Drazen JM, Camargo CA Jr. Beta(2)-adrenoceptor polymorphism and body mass index are associated with adult-onset asthma in sedentary but not active women. *Chest* 2001;120:1474-9.
 14. Singh AK, Cydulka RK, Stahmer SA, Woodruff PG, Camargo CA Jr. Sex differences among adults presenting to the emergency department with acute asthma: Multicenter Asthma Research Collaboration Investigators. *Arch Intern Med* 1999;159:1237-43.
 15. Turki J, Park J, Green S, Martin R, Liggett SB. Genetic polymorphisms of the β_2 -adrenergic receptor in nocturnal and non-nocturnal asthma: evidence that Gly16 correlates with the nocturnal phenotype. *J Clin Invest* 1995;95:1635-41.
 16. American Thoracic Society. Standardization of spirometry: 1994 update. *Am J Respir Crit Care Med* 1995;152:1107-36.
 17. Large V, Hellstrom L, Reynisdottir S, Lonnqvist F, Eriksson P, Lannfelt L, et al. Human beta-2 adrenoceptor gene polymorphisms are highly frequent in obesity and associate with altered adipocyte beta-2 adrenoceptor function. *J Clin Invest* 1997;100:3005-13.
 18. Martinez FD, Graves PE, Baldini M, Solomon S, Erickson S. Association between genetic polymorphisms of the β_2 -adrenoceptor and response to albuterol in children with and without history of wheezing. *J Clin Invest* 1997;100:3184-8.
 19. Xie HG, Stein CM, Kim RB, Xiao ZS, He N, Zhou HH, et al. Frequency of functionally important beta-2 adrenoceptor polymorphisms varies markedly among African-American, Caucasian and Chinese individuals. *Pharmacogenetics* 1999;9:511-6.
 20. Greenland S. Model and variable selection in epidemiologic analysis. *Am J Public Health* 1989;79:340-9.
 21. Green SA, Cole G, Jacinto M, Innis M, Liggett SB. A polymorphism of the human β_2 -adrenergic receptor within the fourth transmembrane domain alters ligand binding and functional properties of the receptor. *J Biol Chem* 1993;268:23116-21.
 22. Green SA, Turki J, Innis M, Liggett SB. Amino-terminal polymorphisms of the human β_2 -adrenergic receptor impart distinct agonist-promoted regulatory properties. *Biochem* 1994;33:9414-9.
 23. Green A, Turki J, Bejarano P, Hall IP, Liggett SB. Influence of β_2 -adrenergic receptor genotypes on signal transduction in human airway smooth muscle cells. *Am J Resp Cell Mol Biol* 1995;13:25-33.
 24. Dishy V, Sofowora GG, Xie HG, Kim RB, Byrne DW, Stein CM, et al. The effect of common polymorphisms of the beta2-adrenergic receptor on agonist-mediated vascular desensitization. *N Engl J Med* 2001;345:1030-5.
 25. Ulbrecht M, Hergeth MT, Wjst M, Heinrich J, Bickeboller H, Wichmann H-E, et al. Association of β_2 -adrenoreceptor variants with bronchial hyperresponsiveness. *Am J Respir Crit Care Med* 2000;161:469-74.
 26. Hopes E, McDougall C, Christie G, Dewar H, Wheatley A, Hall IP, et al. Association of glutamine 27 polymorphism of β_2 adrenoceptor with reported childhood asthma: population based study. *BMJ* 1998;316:664.
 27. Meirhaeghe A, Helbecque N, Cotel D, Amouyel P. β_2 -Adrenoceptor gene polymorphism, body weight, and physical activity. *Lancet* 1999;353:896.
 28. Wang Z, Chen C, Niu T, Wu D, Yang J, Wang B, et al. Association of asthma with beta(2)-adrenergic receptor gene polymorphism and cigarette smoking. *Am J Respir Crit Care Med* 2001;163:1404-9.
 29. Scott MGH, Swan C, Wheatley AP, Hall IP. Identification of novel polymorphisms within the promoter region of the human β_2 -adrenergic receptor gene. *Br J Pharmacol* 1999;126:841-4.
 30. McGraw DW, Forbes SL, Kramer LA, Liggett SB. Polymorphisms of the 5' leader cistron of the human beta2-adrenergic receptor regulate receptor expression. *J Clin Invest* 1998;102:1927-32.