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Effects of In Utero and Childhood Tobacco Smoke Exposure and β2-Adrenergic Receptor Genotype on Childhood Asthma and Wheezing

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Abstract

Objective—Associations between single-nucleotide polymorphisms in the β2-adrenergic receptor gene and asthma and wheeze have been inconsistent. Recent studies indicated that tobacco smoke affects β2-adrenergic receptor gene expression and associations of β2-adrenergic receptor gene variants with asthma in adults. We aimed to investigate the joint effects of in utero and childhood secondhand tobacco smoke exposure and 2 well-characterized functional single-nucleotide polymorphisms (*Arg*16*Gly* and *Glu*27*Gln*) of β2-adrenergic receptor gene on asthma and wheezing in 3128 non-Hispanic and Hispanic white children of the Children's Health Study.

Methods—We fitted logistic regression models to estimate odds ratios and 95% confidence intervals for the independent and joint effects of these single-nucleotide polymorphisms and in utero and secondhand tobacco smoke exposure on asthma and wheeze outcomes.

Results—Exposures to in utero maternal smoking and secondhand tobacco smoke were associated with wheezing. Children who were homozygous for the *Arg*16 allele and were exposed to maternal smoking in utero were at a threefold increased risk for lifetime wheeze compared with children who were unexposed and had at least 1 *Gly*16 allele. We found similar joint effects of secondhand tobacco smoke and *Arg*16*Gly* with wheezing. The risk for lifetime, current, and nocturnal wheeze increased with the number of smokers at home among *Arg*16 homozygous children. The results were consistent in 2 cohorts of children recruited in 1993 and 1996. Diplotype-based analyses were consistent with the single-nucleotide polymorphism–specific results. No associations were found for *Glu*27*Gln*.

Conclusions—Both in utero and childhood exposure to tobacco smoke were associated with an increased risk for wheeze in children, and the risks were greater for children with the *Arg*16*Arg* genotype or 2 copies of the *Arg*16–*Gln*27 diplotype. Exposures to smoking need to be taken into account when evaluating the effects of β2-adrenergic receptor gene variants on respiratory health outcomes.

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What's Known on This Subject: *ADRB2* is a candidate gene for asthma and wheeze; however, no consistent associations have been found between the functional *ADRB2* variants and asthma and wheeze in epidemiologic studies.

What This Study Adds: Smoking exposures in utero and during early childhood modify the associations between *Arg*16*Gly* genotypes and wheeze outcomes in children. Environmental exposures that could modulate gene expression should be examined in epidemiologic studies that determine genetic determinants of disease risk.

Keywords

β-2 adrenergic receptor; prenatal exposure; secondhand-smoke exposure; asthma; wheeze

Wheezing is a common respiratory symptom associated with reversible airway obstruction. The airway obstruction may be enhanced by irritant exposures, particularly in individuals with airway hyperresponsiveness (AHR) and/or airway inflammation. In the airway, catecholamines or *β*2-agonists activate the *β*2-adrenergic receptors (*β*2AR), which results in bronchodilation and relief of wheezing symptoms. Because variants in the *β*2-adrenergic receptor gene (*ADRB2*) that encodes for this receptor modulate receptor activity that may affect airway tone, we hypothesized that functional variants in this gene affect the occurrence of wheezing.

In *ADRB2*, *Arg*16*Gly* and *Glu*27*Gln* are the 2 most common nonsynonymous single-nucleotide polymorphisms (SNPs). In an in vitro study, Green et al[1] found that the *Gly*16 allele was more susceptible to agonist-promoted downregulation, and the *Glu*27 allele was resistant to agonist-mediated receptor downregulation; however, subsequent ex vivo and in vivo studies provided inconsistent findings.[2] The majority of the epidemiologic studies have investigated the associations of these 2 SNPs and asthma/wheeze and found inconsistent results. In addition, meta-analyses that pooled data from these studies failed to show any significant associations with asthma; however, these authors could not rule out effects of these SNPs on asthma symptoms.[3–6]

The inconsistencies across studies may have resulted from not taking into account environmental factors that could modulate the receptor activity. Tobacco smoke is 1 such factor that is associated with an increase in AHR and oxidant stress–mediated airway inflammation, biological mechanisms that could underlie the increased risk for wheeze from tobacco smoke as observed in many epidemiologic studies. To date, 1 study has reported the joint effects of functional variants in *ADRB2* and personal active smoking on asthma. Among Chinese adults, Wang et al[7] found that individuals who were homozygous for the *Arg*16 allele were at an increased risk for asthma only in smokers. Whether exposure to maternal smoking in utero or secondhand smoke (SHS) in childhood modifies the effect of these functional *ADRB2* genetic variants on wheezing is yet to be determined.

To assess the joint effects of tobacco smoke exposure and functional ADRB2 variants, we asked 3 research questions: (1) Are *ADRB2 Arg*16*Gly* and *Glu*27*Gln* variants associated with asthma/wheezing? (2) Are exposures to maternal smoking in utero and SHS in childhood associated with asthma/wheezing? (3) Do tobacco smoke exposures show stronger associations with asthma/wheezing in children with *ADRB2* variants? To address these hypotheses, we examined respiratory health and genetic data from the Children's Health Study, a population-based study of school-aged children in 12 southern California communities.

Methods

Study Design and Participants

Study children participated in the Children's Health Study, which has been described previously.[8,9] Briefly, school children were enrolled in the study during 1993–1996 from 12 communities of southern California. The mean age at study entry was 11.3 years (SD: 2.3 years). To provide an ethnic-specific analysis, we restricted our analysis to non-Hispanic (n = 2150) and Hispanic white children (n = 978), because of insufficient representation of other races. Data on children's asthma status, age at asthma diagnosis, wheezing history, sociodemographic factors, and exposures associated with respiratory health were collected

from self-administered questionnaires that were completed by parents/guardians at study entry. The University of Southern California institutional review board approved the study protocols.

Outcome Assessment

Lifetime asthma was defined by parental report of physician-diagnosed asthma at study entry. Lifetime wheeze was defined as parent-reported history of child's chest ever sounding wheezy or whistling, including times when the child had a cold. Current wheeze was defined as any wheeze in the previous 12 months. Nocturnal wheeze was defined as waking up at night as a result of wheeze in the previous 12 months.

Environmental Exposures of Interest

A child's exposure to maternal smoking in utero was based on smoking by the biological mother during pregnancy. Secondhand exposure to smoking was based on exposure to the number of smokers in the home (0, 1, and ≥2) and was collected on the basis of the smoking status of each participant's mother, father, and other adult household members.

Genotyping

Buccal cells were collected as a source of genomic DNA from consenting study participants. DNA from participants' buccal cells was extracted using a DNA-isolation kit (Puregene, Minneapolis, MN). Samples were analyzed in ABI PRISM 7700 Sequence Detector (Applied BioSystems, Foster City, CA) by using Applied BioSystems Sequence Detection Systems 1.9.1 software to discriminate allelic genotypes. Each reaction was prepared by TaqMan polymerase chain reaction (PCR), which contained QuantiTec probe PCR Master Mix (Qiagen, Valencia, CA), a pair of forward and reverse primers (designed by ABI Primer Express software [Applied Biosystems]), a minor groove binder (MGB) probe for each allele, and genomic DNA as a template. All primer and probe sequences are listed in Table 1. PCR was conducted on a prepared sample plate in the following manner: 95°C for 15 minutes (AmpliTaq Gold DNA polymerase activation), followed by 40 cycles of 92°C for 15 seconds (denature) and 60°C for 60 seconds (anneal/extend). Assays were repeated on 10% of the samples for quality control. The results from TaqMan PCR were validated by using PCR/restriction fragment length polymorphism methods and automatic sequencing (BigDye version 3.1, 377XL DNA sequencer [Applied Biosystems]). For minimization of error between plates, interplate control samples were also placed. In the presence of intraplate or interplate genotype discrepancy, the entire plate was regenotyped. When the genotype discrepancy could not be resolved, we then labeled the result as “unavailable” and removed the participant from data analyses.

Diplotype Estimation

We estimated the *ADRB2* diplotype frequencies in non-Hispanic and Hispanic white children separately by using the SAS macro code written by Daniel Stram (available at www-rcf.usc.edu/~stram/tagSNPs.html). This haplotype estimation technique provides the maximum likelihood estimates of the haplotype frequencies assuming Hardy-Weinberg equilibrium.[10]

Statistical Analysis

Recessive genetic models were used for both *Arg*16*Gly* and *Glu*27*Gln* SNPs on the basis of previous studies.[11,12] Because the effects of these SNPs and smoking exposures on asthma/wheeze were similar within each ethnic group (data not shown; available on request), we conducted pooled analyses. Logistic regression models were fitted to compute odds ratios (ORs) and 95% confidence intervals (95% CIs). In the final models, we included factors on which children who participated in this study differed from the nonparticipants from the original cohort (age, gender, annual family income, parent/guardian's education, health

insurance coverage, and in utero and SHS exposures) and study design variable (community of residence). The models were adjusted for ethnicity to address the potential for population stratification. Wheeze-related outcomes were further adjusted for asthma status. Models that were used to assess the effect of the number of smokers at home and in utero exposure to maternal smoking were mutually adjusted. We used likelihood ratio tests to determine whether in utero exposure to maternal smoking and the number of household smokers modified the relationship of the *ADRB2* polymorphisms and diplotypes with asthma-and wheeze-related outcomes. Two types of diplotype-based analyses were conducted: 1 in which the most common diplotype served as the reference category (log additive model) and the other in which the diplotype-specific ORs were calculated using 0 copies of each diplotype as the reference (codominant, dominant models). A likelihood ratio test was performed to test the global association of *ADRB2* diplotypes with susceptibility to childhood asthma and wheeze outcomes by using the diplotype information. All tests were 2-sided at a 5% significance level. SAS 9.1 (SAS Institute, Inc, Cary, NC) was used for all analyses.

Results

At study enrollment, the majority of children were between 8 and 10 years of age (Table 2). Lifetime wheeze and asthma prevalences at study entry were 35% and 15%, respectively. These prevalences were similar in non-Hispanic and Hispanic white children. Non-Hispanic white children were more likely to have parents with at least some college education and annual family income higher than $50 000. Approximately 17.4% children were exposed to maternal smoking in utero, and 28.3% children were exposed to at least 1 smoker at home. Nearly 29% of children who had exposure to maternal smoking in utero were not reported to have exposure to SHS, whereas nearly 20% of children who had exposure to SHS were not exposed to maternal smoking in utero.

In each ethnic group, both SNPs were in Hardy-Weinberg equilibrium. The minor allele frequencies (MAFs) of *Arg*16*Gly* and *Glu*27*Gln* were 0.37 and 0.38, respectively (Table 3). There was a strong linkage disequilibrium between Arg16Gly and *Glu*27*Gln* ($D' = 0.99$). We found 3 diplotypes (*Gly*16–*Gln*27, *Gly*16–*Glu*27, and *Arg*16–*Gln*27), and no participants carried the *Arg*16–*Glu*27 diplotype.

Exposure to maternal smoking in utero and SHS in early childhood was associated with wheeze and asthma outcomes (Table 4). Exposure to ≥2 smokers at home was associated with 40% increased risk for wheeze and asthma. Neither variant in *ADRB2* was associated with asthma and wheeze. Exposure prevalences for maternal smoking, SHS, and MAFs varied by ethnicity (Tables 2 and 3); however, the relationships of these exposures and SNPs with asthma/wheeze did not vary by ethnicity (data not shown). Therefore, we conducted a pooled analysis adjusting for ethnicity.

We found that the associations between *Arg*16*Gly* and wheeze outcomes varied among children who were exposed to maternal smoking (in utero exposure) and SHS during childhood (Tables 5 and 6). Children who were homozygous for the *Arg*16 allele and were exposed to maternal smoking in utero were at an increased risk for lifetime wheeze (OR: 3.1 [95% CI: 1.8–5.4]), current wheeze (OR: 3.0 [95% CI: 1.5–6.0]), and nocturnal wheeze (OR: 4.0 [95% CI: 1.3–11.9]) after adjustment for SHS and other potential confounders (Table 5). These associations did not substantially differ by ethnic groups (data not shown).

After adjustment for in utero exposure to maternal smoking, children who were homozygous for the *Arg*16 allele and were exposed to ≥2 smokers at home during childhood were at an increased risk for lifetime wheeze (OR: 4.2 [95% CI: 1.8–10.1]), current wheeze (OR: 3.0 [95% CI: 1.0–9.6]), and nocturnal wheeze (OR: 5.9 [95% CI: 1.2–30.2]; Table 6). There was a

statistically significant increasing trend for risk for wheeze outcomes with the number of smokers at home among *Arg*16 homozygotes.

Because there was overlap between exposures to in utero and SHS, we conducted sensitivity analyses of children who were not exposed to maternal smoking in utero and of children who were not exposed to SHS to determine whether the associations represented independent effects. Of children who were not exposed to SHS ($n = 2223$), those with *Arg*/*Arg* genotype and exposure to maternal smoking were at 3.8-fold (95% CI: 1.3–10.9) increased risk for lifetime wheeze compared with children with no maternal smoking exposure and at least 1 *Gly*16 allele (data not shown). Among children who were not exposed to maternal smoking in utero ($n = 2519$), children who were homozygous for the *Arg*16 allele and were exposed to ≥2 smokers had fivefold (95% CI: 1.3–20.4) increased risk for lifetime wheeze compared with children who were not exposed to SHS and had at least 1 *Gly*16 allele (data not shown).

Although we found strong associations with wheeze outcomes, we found little evidence for variation in the effects of maternal smoking during pregnancy and number of household smokers on the association between the *Arg*16*Gly* polymorphism and lifetime asthma (Tables 5 and 6). We also did not find a strong association for early persistent or late-onset asthma phenotypes (data not shown). There was little evidence that the *Glu*27*Gln* polymorphism contributed to wheeze or asthma irrespective of maternal smoking during pregnancy or SHS (data not shown).

In diplotype-based analyses, children with 2 copies of the *Arg*16–*Gln*27 diplotype (ie, *Arg*16 homozygous) had an increased risk for wheeze-related end points among those who were exposed to in utero maternal smoking or SHS compared with those who had no copies of the *Arg*16–*Gln*27 diplotype and were unexposed (data not shown). Children who carried the *Gly*16–*Glu*27 and *Gly*16–*Gln*27 diplotypes were not at an increased risk irrespective of smoking exposures. These associations further were consistent with the recessive genetic modeling for the SNP-based analyses. Child's ethnicity and gender and parental asthma did not modify any of the genetic associations.

To assess consistency of our findings in different populations, we compared the association in 2 cohorts of children. In our sample, 2 fourth-grade cohorts (average 10 years of age in both groups) were recruited in 1993 ($N = 1114$) and 1996 ($N = 1191$), which provided us with the opportunity to examine the associations in 2 comparable populations with similar smoking exposure prevalences and MAFs. The results showed similar magnitudes of associations between 2 fourth-grade student groups (ie, no significant heterogeneities across cohorts) and are also consistent with the results found for the overall sample (data not shown).

Discussion

We found that the *ADRB2 Arg*16*Gly* genotype contributed to the occurrence of wheeze among children who were exposed to tobacco smoke. Across a spectrum of wheeze outcomes, associations of in utero exposure to maternal smoking and childhood exposure to SHS were larger in children with the homozygous *Arg*16 genotype. Among the genetically susceptible children, a strong dosage-response relationship was found for wheeze-related outcomes, and the risks increased with the number of household smokers. These associations were consistent in 2 cohorts of children who were recruited 3 years apart. Our findings indicate that there are important long-term effects of in utero and postnatal exposure to tobacco smoke in a genetically susceptible group of children.

In *ADRB2*, 2 other functional SNPs (*Arg*19*Cys* in the 5′ promoter region and *Thr*164*Ile*) have been studied in addition to the 2 SNPs that we studied (*Arg*16*Gly* and *Glu*27*Gln*). The promoter *Arg*19*Cys* SNP is in strong linkage disequilibrium with the coding SNPs *Arg*16*Gly* and

*Glu*27*Gln*, and 3 common haplotypes (*Cys*19-*Gly*16-*Gln*27, *Arg*19-*Gly*16-*Glu*27, and *Cys*19-*Arg*16-*Gln*27) exist.[13] Because the MAF for *Ile*164 is very low (~1%) and the alleles at the *Arg*19*Cys* locus could be predicted from the diplotypes estimated from *Arg*16*Gly* and *Glu*27*Gln* genotypes, it was not necessary to genotype *Arg*19*C*ys and *Thr*164*Ile* SNPs.

Previous in vitro and in vivo studies provided conflicting data on the functional significance of these SNPs. These inconsistencies may have resulted from conducting experiments on different cell lines (eg, airway smooth muscle, respiratory epithelium, lymphocyte, mast cell, macrophage) with pleiotropic effects of the SNPs on these cells.[13–16] Because these SNPs are tightly linked, some researchers have also argued that assessment of individual SNP effects without considering haplotypes in previous studies may have resulted in inconsistent associations between these SNPs and agonist-promoted β2AR desensitization. Given the haplotype distributions, the *Arg*16 allele is carried in the *Cys*19-*Arg*16-*Gln*27 haplotype (*Arg*16-*Gln*27 diplotype in this study). Emerging data from in vivo studies suggest that *Arg*16 homozygosity is associated with enhanced desensitization of the β2AR,[13] reduced β2AR density,[17] and reduced lung function and lung fluid clearance.[16] Our data also suggested a recessive genetic model for the joint effects of smoking and *Arg*16*Gly* on wheeze outcomes.

The MAFs of these 2 tightly linked SNPs vary across racial/ethnic groups, and there are geographic differences and temporal trends in childhood smoke exposure. These heterogeneities in exposure prevalence and genotype distributions across populations and differential impact of these SNPs on wheeze outcomes by smoking exposures could explain the inconsistent findings in previous studies. Similar to some of the previous studies, we also did not find statistically significant associations between these 2 functional *ADRB2* polymorphisms and wheeze/asthma[6,18]; however, tobacco smoke exposures modified the effect of the *Arg*16 allele on wheeze outcomes. Smoking is a strong risk factor for respiratory health outcomes (asthma, wheeze, lung function, and AHR).[19] The adverse effects of smoking could be mediated directly by an increase in AHR and allergic sensitization and by impairment of lung function.[20–22] Tobacco smoke may also reduce β2AR density and ligand binding.[23] Besides these direct adverse effects of smoking on these respiratory outcomes and β2AR activity, a growing body of evidence also indicates that tobacco smoke exposures could modulate the effects of the *Arg*16*Gly* genotype on asthma,[7] lung function,[24] and AHR.[25] Additional research is warranted to examine the joint effects of *Arg*16*Gly* and tobacco smoke exposures on β2AR expression.

Although the underlying reason for the seeming discrepancy in results observed for wheeze and asthma remains unclear, our results are consistent with previous reports. Holloway et al[26] found that *ADRB2* variants were associated with asthma severity; however, that study was based on a small sample. That finding was not replicated in a larger study and a meta-analysis by Hall et al,[6] which included the data from the study by Holloway et al.[26] Given the existing evidence, we can speculate that the effect of *Arg*16 allele is mediated by increasing AHR,[25] which was further accentuated by smoking's leading to wheezing. It could be that these wheezing episodes are attributable to airway hyperreactivity and results in a clinical pattern that supports an asthma diagnosis. This hypothesis needs to be evaluated in a larger prospective study with direct measures of AHR.

We acknowledge that there were some overlaps among the wheeze phenotypes; however, we presented them to show consistency in our results and to be consistent with our previous articles. In addition, heterogeneity in reporting respiratory symptoms has been a concern in epidemiologic studies; therefore, we decided to present data on a few of the symptoms that were examined previously in other articles on this topic.

We considered the potential effects of limitations in this study. Demographic factors, socioeconomic factors, exposure to maternal smoking during pregnancy, SHS after birth, and prevalence of asthma showed significant differences between participants and nonparticipants (data not shown). To address any potential for selection bias from these modest differences, we adjusted for these factors in the final models and found very little change (<10%) in the risk estimates. Furthermore, restricting our analyses to children with health insurance yielded similar results (data not shown). Several studies found that maternal recall of smoking during pregnancy is reliable even 10 to 30 years later.[27–30] Parental report of SHS has been found to be reliable in some[31–33] and was found to be underreported in children with asthma[34]; therefore, although recall bias is possible for in utero and SHS exposures, this may have attenuated the risk estimates because children with asthma were more likely to be in the unexposed group. This potential bias could account for the lack of significant modification of the association between *Arg*16*Gly* and asthma by smoking exposures. Because genotyping for the *ADRB2* SNPs is not used in diagnosing asthma, diagnostic bias with respect to the studied SNPs is not likely.

Bias from population stratification is a concern in association studies involving multiethnic populations. To address this potential bias, we excluded children who were black or Asian or belonged to a mixed ethnic background because of insufficient sample sizes to conduct ethnic-specific analyses. We acknowledge that substructure within each ethnic group could lead to population stratification; however, this is unlikely given the homogeneity across non-Hispanic and Hispanic white groups. Furthermore, for examining joint effects of exposures and the genetic variants, such intraethnic substructure would have to be related to both factors, which is unlikely. Because the associations between the SNPs and exposures showed similar results by ethnicity and the joint effects of *Arg*16*Gly* and maternal smoking during pregnancy showed similar magnitude of associations by ethnicity, our analytic approach of conducting pooled analyses adjusting for ethnicity is unlikely to introduce a bias that could account for the findings.

The modifying role of smoking on the association between ADRB2 *Arg*16*Gly* and asthma/wheeze was reported previously for adults,[7] and existing data suggest that both smoking and these functional SNPs modulate β2AR density and activity[13,23]; therefore, our analyses were based on specified a priori hypotheses. In this setting, we do not consider it appropriate to disregard previous information on the biology of β2AR expression and to adjust the *P* values for testing a set of selected a priori hypotheses. Some of the results were based on small sample sizes, and we conducted multiple tests on the basis of a priori hypotheses; therefore, these results should be interpreted with some caution.

Conclusions

Children who have 2 copies of the *Arg*16 allele or *Arg*16–*Gln27* diplotype marked by this variant allele are at highest risk for developing wheezing when exposed to maternal smoking in utero or to SHS at home in childhood. Because of the high prevalence of childhood wheeze, intervention strategies are needed to reduce smoke exposures to children in general and, in particular, to those who are genetically susceptible to the adverse effects of smoking.

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Abbreviations

AHR
airway hyperresponsiveness

***β*2AR**
*β*2-adrenergic receptor

ADRB2
*β*2-adrenergic receptor gene

SNP
single-nucleotide polymorphism

SHS
secondhand smoke

PCR
polymerase chain reaction

OR
odds ratio

CI
confidence interva

MAF
minor allele frequency

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TABLE 1

Primers and Probes for Genotyping *ADRB2* SNPs at Codons 16 and 27

***ADRB2* SNPs**	**Primer/Probe Sequence**
*Arg*16*Gly* (rs1042713)	
Forward primer	5′-GGCAGCGCCTTCTTGCT-3′
Reverse primer	5′ AGGACGATGAGAGACATGACGAT-3′
16A probe	5′-(6FAM)-ACCCAATAGAAGCC-3′
16G probe	5′-(VIC)-ACCCAATGGAAGCC-3′
*Glu*27*Gln* (rs1042714)	
Forward primer	5′-GGCAGCGCCTTCTTGCT-3′
Reverse primer	5′-AGGACGATGAGAGACATGACGAT-3′
27G probe	5′-(6FAM)-TCACGCAGGAAAG-3′
27C probe	5′-(VIC)-TCACGCAGCAAAG-3′

6FAM and VIC are reporter dyes.

TABLE 2
Selected Demographic Characteristics and Respiratory Outcomes

Characteristics/Outcome	Non-Hispanic White (*N* = 2150), *n* (%)	Hispanic White (*N* = 978), *n* (%)	Combined Population (*N* = 3128), *n* (%)
Female	1113 (51.8)	558 (57.1)	1671 (53.4)
Age, y			
8–10	1144 (53.2)	570 (58.3)	1714 (54.8)
11–12	403 (18.7)	190 (19.4)	593 (19.0)
>12	603 (28.1)	218 (22.3)	821 (26.3)
Parent/guardian's education			
Did not finish high school	119 (5.6)	240 (26.2)	359 (11.8)
Completed high school	384 (18.1)	200 (21.8)	584 (19.2)
Some college or technical school	1033 (48.7)	361 (39.4)	1394 (45.9)
Completed college	255 (12.0)	57 (6.2)	312 (10.3)
Some graduate training after college	329 (15.5)	59 (6.4)	388 (12.8)
Income, US $			
<7500	55 (2.9)	75 (9.4)	130 (4.8)
7500–14 999	125 (6.6)	109 (13.7)	234 (8.7)
15 000–29 999	221 (11.7)	187 (23.5)	408 (15.2)
30 000–49 999	511 (27.1)	211 (26.5)	722 (26.9)
50 000–99 999	816 (43.2)	191 (24.0)	1007 (37.5)
≥100 000	159 (8.4)	24 (3.0)	183 (6.8)
Health insurance	1903 (90.1)	728 (76.6)	2631 (85.9)
Parental asthma history	449 (22.2)	158 (17.4)	607 (20.7)
Maternal smoking during pregnancy	423 (20.2)	106 (11.2)	529 (17.4)
No. of smokers inside home			
0	1471 (69.0)	752 (77.6)	2223 (71.7)
1	418 (19.6)	172 (17.8)	590 (19.0)
≥2	243 (11.4)	45 (4.6)	288 (9.3)
Respiratory outcome prevalences			
Lifetime wheeze	772 (37.5)	279 (30.2)	1051 (35.2)
Current wheeze	148 (10.3)	76 (10.6)	591 (23.5)
Nocturnal wheeze	772 (37.5)	279 (30.2)	224 (10.4)
Lifetime asthma	331 (15.6)	136 (14.3)	467 (15.2)

Percentages are reported among nonmissing observations.

TABLE 3

Distribution of *ADRB2* SNPs and Diplotype Frequencies in the Combined Population

Genotypes/Diplotypes	Non-Hispanic White	Hispanic White	Combined Population
*Arg*16*Gly*, *n* (%)			
Gly/*Gly*	912 (42.4)	326 (33.3)	1238 (39.6)
Gly/*Arg*	963 (44.8)	484 (49.5)	1447 (46.3)
Arg/*Arg*	275 (12.8)	168 (17.2)	443 (14.2)
*Arg*16 allele frequency	0.35	0.42	0.37
*Glu*27*Gln*, *n* (%)			
Gln/*Gln*	694 (32.3)	566 (57.9)	1260 (40.3)
Gln/*Glu*	1015 (47.2)	360 (36.8)	1375 (44.0)
Glu/*Glu*	441 (20.5)	52 (5.3)	493 (15.8)
*Glu*27 allele frequency	0.44	0.24	0.38
*Gly*16*Arg*–*Glu*27*Gln* diplotypes, frequency			
Gly-*Gln*	0.21	0.34	0.25
Gly-*Glu*	0.44	0.24	0.38
Arg-*Gln*	0.35	0.42	0.37

Numbers do not always add up because of missing values. Percentages are reported among nonmissing observations.

TABLE 4

Associations Among *ADRB2* SNPs, Maternal Smoking During Pregnancy, and Number of Smokers and Respiratory Outcomes

Parameter	Lifetime Wheeze, OR (95% CI)	Current Wheeze, OR (95% CI)	Nocturnal Wheeze, OR (95% CI)	Lifetime Asthma, OR (95% CI)
Maternal smoking during pregnancy				
No	1.0 (reference)	1.0 (reference)	1.0 (reference)	1.0 (reference)
Yes	1.7 (1.3–2.2)	1.7 (1.2–2.4)	1.4 (0.7–2.6)	1.1 (0.8–1.5)
No. of smokers				
0	1.0 (reference)	1.0 (reference)	1.0 (reference)	1.0 (reference)
1	1.1 (0.9–1.4)	0.9 (0.6–1.3)	1.0 (0.6–1.8)	1.1 (0.9–1.5)
≥2	1.4 (1.0–2.0)	1.4 (0.9–2.2)	1.4 (0.6–3.2)	1.4 (1.0–2.0)
P for trend[a]	.06	.40	.49	.09
*Arg*16*Gly*				
Gly/*Gly* or *Gly*/*Arg*	1.0 (reference)	1.0 (reference)	1.0 (reference)	1.0 (reference)
Arg/*Arg*	1.2 (0.9–1.5)	1.1 (0.8–1.6)	1.0 (0.6–1.9)	1.2 (0.9–1.6)
*Glu*27*Gln*				
Gln/*Gln* or *Gln*/*Glu*	1.0 (reference)	1.0 (reference)	1.0 (reference)	1.0 (reference)
Glu/*Glu*	0.9 (0.7–1.1)	0.8 (0.6–1.2)	0.7 (0.4–1.2)	1.0 (0.7–1.3)

Models are adjusted for age, gender, ethnicity, parent/guardian's education, total annual family income, health insurance, and community. For wheezing-related outcomes, asthma status is also adjusted. Maternal smoking and number of smokers are mutually adjusted.

[a] *P* for trend was obtained from a 1 degree of freedom (*df*) trend test that was used to evaluate possible exposure–response relationships across categories of the SHS variables within each genotype category.

TABLE 5

Joint Association of *Arg*16*Gly* and Maternal Smoking During Pregnancy on Respiratory Outcomes

Parameter	Maternal Smoking During Pregnancy			
	No		Yes	
	Case/Control[a]	OR (95% CI)	Case/Control[a]	OR (95% CI)
Lifetime wheeze				
Gly/*Gly* or *Gly*/*Arg*	681/1402	1.0 (reference)	196/240	1.5 (1.1–2.0)
Arg/*Arg*	114/219	1.0 (0.7–1.4)	41/31	3.1 (1.8–5.4)
Interaction *P*[b]				.03
Current wheeze				
Gly/*Gly* or *Gly*/*Arg*	384/1400	1.0 (reference)	106/240	1.5 (1.0–2.2)
Arg/*Arg*	69/218	0.9 (0.6–1.4)	20/31	3.0 (1.5–6.0)
Interaction *P*[b]				.08
Nocturnal wheeze				
Gly/*Gly* or *Gly*/*Arg*	152/1400	1.0 (reference)	36/240	1.1 (0.5–2.1)
Arg/*Arg*	25/219	0.8 (0.4–1.6)	9/31	4.0 (1.3–11.9)
Interaction *P*[b]				.03
Lifetime asthma				
Gly/*Gly* or *Gly*/*Arg*	314/1826	1.0 (reference)	77/370	1.1 (0.8–1.5)
Arg/*Arg*	58/289	1.3 (0.9–1.7)	13/62	1.3 (0.7–2.6)
Interaction *P*[b]				.90

Models are adjusted for age, gender, ethnicity, parent/guardian's education, total annual family income, health insurance, community, and number of household smokers. For wheeze-related outcomes, models were adjusted for asthma status.

[a] Cases are defined as participants with respiratory outcome, whereas control subjects had no respiratory outcome.

[b] *P* values for *Arg*16*Gly* by maternal smoking during pregnancy were obtained from likelihood ratio tests from nonstratified models with appropriate interaction terms and were based on 1*df*.

TABLE 6

Joint Association of *Arg*16*Gly* and Numbers of Household Smokers on Respiratory Outcomes

Outcomes/Gene	No. of Household Smokers						*P* for Trend[b]
	0		1		≥2		
	Case/Control[a]	OR (95% CI)	Case/Control[a]	OR (95% CI)	Case/Control[a]	OR (95% CI)	
Lifetime wheeze							
Gly/*Gly* or *Gly*/*Arg*	598/1232	1.0 (reference)	179/298	1.0 (0.8–1.4)	109/132	1.2 (0.8–1.7)	.3700
Arg/*Arg*	97/199	0.9 (0.7–1.3)	36/47	1.5 (0.9–2.6)	23/9	4.2 (1.8–10.1)	.0007
Interaction *P*[c]							.0100
Current wheeze							
Gly/*Gly* or *Gly*/*Arg*	344/1230	1.0 (reference)	87/298	0.8 (0.5–1.1)	66/132	1.2 (0.8–2.0)	.8800
Arg/*Arg*	60/198	0.8 (0.5–1.3)	20/47	1.5 (0.8–2.9)	10/9	3.0 (1.0–9.6)	.0100
Interaction *P*[c]							.0500
Nocturnal wheeze							
Gly/*Gly* or *Gly*/*Arg*	132/1230	1.0 (reference)	38/298	0.8 (0.5–1.6)	18/132	1.1 (0.4–2.6)	.9100
Arg/*Arg*	23/199	0.7 (0.3–1.4)	7/47	2.0 (0.6–6.4)	4/9	5.9 (1.2–30.2)	.0080
Interaction *P*[c]							.0500
Lifetime asthma							
Gly/*Gly* or *Gly*/*Arg*	271/1601	1.0 (reference)	78/420	1.1 (0.8–1.5)	44/201	1.4 (0.9–2.0)	.1300
Arg/*Arg*	48/260	1.3 (0.9–1.8)	14/69	1.3 (0.7–2.4)	9/30	2.1 (1.0–4.7)	.3200
Interaction *P*[c]							.8400

Models are adjusted for age, gender, ethnicity, parent/guardian's education, annual family income, health insurance, community, and maternal smoking during pregnancy. For wheeze outcomes, asthma status is further adjusted.

[a] Cases are defined as participants with respiratory outcome, whereas control subjects do not have the particular respiratory outcome.

[b] *P* for trend was obtained from a 1-*df* trend test that was used to evaluate possible exposure–response relationships across categories of the SHS variables within each genotype category.

[c] *P* values for *Arg*16*Gly* by number of smokers at home were obtained from likelihood ratio tests from nonstratified models with appropriate interaction terms and were based on 2 *df*.