

Original article

Associations of β 2-adrenergic receptor genotypes and haplotypes with wheezing illness in Taiwanese schoolchildren

Background: Increasing attention was focused on the β 2-adrenergic receptor gene (ADRB2), whose genetic variability has been implicated as a risk factor for asthma-related phenotypes. However, only a few studies reported the associations by utilizing haplotypic approaches. We therefore examined the relationship of childhood wheezing illness with polymorphisms at codons 16 and 27, and evaluated the influence of polymorphisms individually and in combination as haplotypes.

Methods: We conducted a genetic case-control study comprising 215 wheezing children and 183 nonwheezing controls, all of whom were selected from 2524 fourth- to ninth-grade schoolchildren in southern Taiwan.

Results: All participants were homozygous at the ADRB2 Thr164 locus. After controlling for possible confounders, ADRB2 Glu27 allele was significantly associated with wheezing illness in all genetic models, but the risks on Arg16Gly genotypes were inconclusive. Estimated frequencies for the three main haplotypes were Arg16/Gln27 57.2%, Gly16/Gln27 35.3%, Gly16/Glu27 7.4% in wheezing children, and Arg16/Gln27 56.3%, Gly16/Gln27 32.2%, Gly16/Glu27 10.4% in controls. The protective effect of Gly16/Glu27 haplotype remained relative to all other ADRB2 haplotypes [adjusted relative risk (aRR) = 0.58; 95% confidence interval (CI) 0.35–0.97]. As compared with children without Gly16/Glu27 haplotype, those with Gly16/Glu27 haplotype had a significantly lower risk for wheezing illness (aRR = 0.56; 95% CI 0.33–0.99). The copy numbers of Gly16/Glu27 haplotype also showed a clear dose-response relationship on the decreased risks. No significant association was found with the prevalence of wheezing illness for other haplotypes.

Conclusion: We concluded that ADRB2 Glu27 allele and Gly16/Glu27 haplotype were significantly protective factors for wheezing illness in Taiwanese schoolchildren.

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Wheezing illness is the most common chronic childhood disease in developed nations (1). Current studies indicate that many regions of the human genome containing susceptibility genes are associated with various wheezing/asthma phenotypes (2, 3). 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. It has long been hypothesized that defects in this receptor might represent a pathogenic role in asthma (4).

β 2-adrenergic receptor gene (ADRB2), an intronless gene on chromosome 5q31-q32 encoding 413 amino acids, is expressed on the surface of airway smooth muscle cells and plays a key role in tuning airways reactivity (5, 6). Several functional variants in this gene have been investigated as potential asthma-susceptibility loci or as disease modifiers (7, 8). Previous studies indicated the two most common nonsynonymous single-

nucleotide polymorphisms (SNPs) occurred at codons 16 and 27 (7, 9).

The ADRB2 genotypes have been inconsistently associated with intermediate or asthma-associated phenotypes, such as response to medications (10–12), lung function (13, 14), airway hyper-responsiveness (15, 16), and asthma severity (17). In a previous *in vitro* study, Green et al. found that the Gly16 allele was more susceptible to agonist-promoted receptor downregulation, and the Glu27 allele was resistant to agonist-mediated downregulation (18); however, subsequent *ex vivo* and *in vivo* studies provided inconsistent findings (19). Moreover, meta-analyses that pooled data from epidemiologic studies failed to show conclusive associations with wheezing/asthma phenotypes (20). For the reason that there may be interactions between multiple SNPs within a haplotype, analyses based on haplotypes may be more informative than SNPs in association

studies (21). In an *in vivo* study, Drysdale et al. reported that the interaction of multiple SNPs within a haplotype of the ADRB2 gene was correlated with individual responses to β 2-stimulants, whereas no correlation was found with individual SNPs (22). Haplotypes may characterize the linkage disequilibrium pattern of a region more precisely, so that associations with interesting variants can be identified (21).

To date, only a few population-based studies utilizing haplotype approaches were reported to evaluate the risks for asthma-associated phenotypes (23–26). In order to investigate the association between ADRB2 genetic variants and childhood wheezing illness, we recruited schoolchildren for genotype determination from our previous Taiwanese population (27). Information would be provided on the distribution of the haplotypic combinations of ADRB2 polymorphisms. We also examined the association of wheezing illness with polymorphisms at codons 16 and 27 and evaluated the influence of these SNPs individually, and in combination as haplotypes.

Methods

Study design

In 2001, we conducted a national, cross-sectional, school-based survey for respiratory diseases and symptoms in middle- and elementary-school children. The study protocol has been described previously (27). Briefly, the standard ISAAC-Chinese version questionnaire was taken home by students and answered by parents. Some information concerning basic demography, residential environmental factors, and history of family atopic diseases was also collected from the questionnaire. Stratified sampling by grade was applied in each school, and classroom incentives but not individual incentives were used to encourage participation. In total, we investigated 35 036 children from 22 elementary and 22 middle schools, and the overall response rate was 92.8%. In June 2001, we conducted this study focusing on the 2853 fourth- to ninth-grade schoolchildren who completed the questionnaire survey and resided in three southern Taiwan communities. A parent of each participating child provided written informed consent. The study protocol was approved by the Institutional Review Board at our university hospital, and it complied with the principles outlined in the Helsinki Declaration (28).

Questionnaire and subjects selection

The definition of ever wheezing was determined by a positive response to the question, 'Has your child ever had wheeze or whistling in the chest at any time in the past when he/she did not have a cold or the flu?' Nonwheezing controls were defined as those reporting not ever having dyspnoea with wheezing (from the parental questionnaire), no nocturnal dyspnoea associated with wheezing (from the video questionnaire), and without physician-diagnosed asthma. After excluding questionnaires with unanswered questions, we found only 1.5% and 1.0% of subjects to have *in utero* environmental tobacco smoke (ETS) and active smoking habits in our population. Because of sample size limitation for stratification analysis, we excluded subjects with any of these two kinds of tobacco smoke exposure at the study entry. Based on criteria established from questionnaire information and parental informed

Table 1. Demographic and selected characteristics of the study population among fourth- to ninth-grade school children in Taiwan, 2001

Categories	Participants genotyped		All eligible participants	
	Wheezing subjects (n = 215)	Controls (n = 183)	Wheezing subjects (n = 441)	Controls (n = 2083)
Age (years)	12.0 $\pm$ 1.6	12.1 $\pm$ 1.8	11.8 $\pm$ 1.7	12.1 $\pm$ 1.8
Gender				
Boys	119 (55.4)	88 (48.1)	255 (57.8)	942 (45.2)
Girls	96 (44.7)	95 (51.9)	186 (42.2)	1141 (54.8)
Parental atopy*				
Yes	91 (42.3)	44 (24.0)	189 (42.9)	520 (25.0)
No	124 (57.7)	139 (76.0)	252 (57.1)	1563 (75.0)
Parental education level (years)				
<10	50 (23.3)	59 (32.2)	127 (28.8)	747 (35.9)
10–12	87 (40.5)	73 (39.9)	169 (38.3)	853 (41.0)
$\geq$ 13	78 (36.3)	51 (27.9)	145 (32.9)	483 (23.2)
ETS exposure at home				
Yes	106 (49.3)	92 (50.3)	241 (54.6)	1174 (56.4)
No	109 (50.7)	91 (49.7)	200 (45.4)	909 (43.6)

ETS, environmental tobacco smoke.

Results were shown as mean $\pm$ SD or n (%). We excluded samples with *in utero* ETS or active smoking habits in this table.

*Defined as presence of paternal or maternal asthma, allergic rhinitis or atopic eczema.

consent, we randomly selected 50% of the children with ever wheezing and 10% of the nonwheezing controls for oral mucosa sampling, with the response rate of 93.7%. All of the selected subjects were of the same ethnic origin. Table 1 provides the demographic characteristics of the study population.

DNA collection and genotyping

Cotton swabs containing oral mucosa were collected and were immediately maintained at -80°C throughout the transfer and storage. Genomic DNA was isolated using the phenol/chloroform extraction method previously described with some modifications (29). Briefly, the cotton swabs were directly immersed in 300 μl cell lysis buffer [50 mM Tris-HCl, 1 mM ethylenediaminetetraacetic acid (EDTA), 0.1 M NaCl, pH 8.0] containing 2% sodium dodecyl sulfate (SDS) and 20 $\mu\text{g}/\text{ml}$ proteinase K in a 1.5-ml microcentrifuge tube. After incubation overnight at 55°C , the swabs were discarded, and the DNA in the supernatants was purified by phenol/chloroform extraction and then precipitated with ethanol.

Three ADRB2 functional SNPs were selected for genotyping in this study: rs1047213 (Arg16Gly), rs1042714 (Gln27Glu) and rs180088 (Thr164Ile). The SNP genotypes were determined by polymerase chain reaction and restriction fragment length polymorphism methods (16, 30). All assays were performed by workers unaware of the clinical status of individual subjects, and genotype assignments were based on two consistent experimental results. About 15% of randomly selected samples were directly sequenced, and all of them were concordant with the initial genotyping results.

Statistical analysis

Unconditional logistic regression models were used to estimate the association of the ADRB2 genotypes with wheezing illness in schoolchildren. For the SNP analyses, dominant, co-dominant genetic, and additive models were utilized. Crude and adjusted

odds ratios (ORs) and relative risks (RRs) with 95% confidence intervals (CIs) were shown. A global test of significance testing the effect of haplotypes was conducted by including the three haplotype variables in the model as compared with a model with only covariates, using the likelihood ratio test.

Haplotype frequencies of unphased ADRB2 SNPs for wheezing children and non wheezing controls were estimated using TagSNPs (31). The estimated number of copies of each haplotype was used as a proxy for the true haplotype, a single imputation procedure that provides unbiased estimates and appropriate confidence intervals (31). Determining haplotypes is helpful for genetic association study because genetic inheritance operates through the transmission of chromosomal segments. Experimental methods for haplotype determination exist, but they are currently time-consuming and expensive. TagSNPs is a statistical method for inferring haplotypes although it is only a mathematical estimation. Log additive haplotype analyses were conducted and likelihood ratio tests were performed to test the global association of ADRB2 gene with wheezing illness using haplotype information. All tests were assumed to be a two-sided alternative hypothesis and a 0.05 significance level, with corrections for the number of haplotypes observed. All analyses were conducted using SAS software version 9.1 (SAS Institute, Cary, NC, USA).

Results

Our study finally comprised 215 wheezing subjects and 183 nonwheezing controls from three communities in southern Taiwan. Table 1 presents the demographic and household ETS exposure data. Children with wheezing illness were slightly younger, included a higher proportion of boys than did the controls, tended to have parents with higher education levels, and were more likely to have parental atopic histories. However, the distribution of household ETS exposure was not significantly different between cases and controls. Participants who had genotyping data had higher parental education level and lower proportion of household ETS exposure than did those without genotyping data (Table 1), and all of the other factors were almost identical between children with and without genotyping.

We collected oral mucosa samples from 398 school-children and our DNA extraction rate was 100%. All participants were homozygous at the ADRB2 Thr164 locus. The relationship of two ADRB2 functional SNPs and wheezing illness is presented in Table 2. Among each group, the frequencies of the ADRB2 genotypes were consistent with the Hardy–Weinberg equilibrium. Children with wheezing illness were prone to have a lower percentage of the ADRB2 Glu27 allele than controls. Using an additive genetic coding and adjusting for possible confounders, ADRB2 Glu27 allele was significantly associated with wheezing illness (aOR 0.55; 95% CI 0.34–0.90) (Table 2). Co-dominant models also revealed that children with any Glu27 allele were significantly associated with wheezing illness (aOR 0.57; 95% CI 0.33–0.99). In our series, the risks of wheezing illness on Arg16Gly genotypes were inconclusive. Among the 215 wheezing children, 72 subjects had been diagnosed

Table 2. The relationship of two ADRB2 SNPs on wheezing illness among schoolchildren

ADRB2 genotypes	Controls, <i>n</i> (%)	Wheezing subjects		
		<i>n</i> (%)	cOR (95% CI)	aOR* (95% CI)
<i>Arg16Gly</i>				
Dominant model				
Arg/Arg	66 (36.1)	73 (34.0)	1.00	1.00
Arg/Gly or Gly/Gly	117 (63.9)	142 (66.0)	1.10 (0.73–1.66)	1.05 (0.68–1.62)
Co-dominant model				
Arg/Arg	66 (36.1)	73 (34.0)	1.00	1.00
Arg/Gly	78 (42.6)	100 (46.5)	1.16 (0.74–1.81)	1.13 (0.71–1.80)
Gly/Gly	39 (21.3)	42 (19.5)	0.97 (0.56–1.69)	0.90 (0.51–1.59)
Additive model				
MAF	42.6%	42.8%	1.01 (0.77–1.32)	0.97 (0.73–1.28)
<i>Gln27Glu</i>				
Dominant model				
Gln/Gln	146 (79.8)	184 (85.6)	1.00	1.00
Gln/Glu or Glu/Glu	37 (20.2)	31 (14.4)	0.66 (0.39–1.10)	0.57 (0.33–0.99)
Co-dominant model				
Gln/Gln	146 (79.8)	184 (85.6)	1.00	1.00
Gln/Glu	32 (17.5)	30 (14.0)	0.74 (0.43–1.28)	0.65 (0.37–1.14)
Glu/Glu	5 (2.7)	1 (0.5)	0.16 (0.02–1.37)	0.11 (0.01–1.00)
Additive model				
MAF	11.5%	7.4%	0.64 (0.40–1.02)	0.55 (0.34–0.90)

cOR, crude odds ratio; aOR, adjusted odds ratio; CI, confidence interval; MAF, minor allele frequency.

Hardy–Weinberg equilibrium tests showed insignificance ($P > 0.05$) in each case and control group.

*Models are adjusted for age, gender, community of residence, parental atopic history, parental education level and ETS exposure at home.

with asthma by physicians. The reduced sample size for asthma diminished the capacity of this study to detect small effects. Restricting the analyses to children without asthma did not substantially alter the above findings (data not shown).

Based on the available genotype information, only three haplotypes were estimated to have a frequency $> 2\%$. Arg16/Glu27 haplotype was omitted for statistical analyses because of very low frequency. Examining the association between ADRB2 haplotypes and childhood wheezing illness showed a significant association after controlling for confounders (global P -value = 0.04) (Table 3). When examining the ADRB2 haplotype as one block, we found the respective estimated frequencies for the three main haplotypes were Arg16/Gln27 = 57.2%, Gly16/Gln27 = 35.3%, Gly16/Glu27 = 7.4% in wheezing children, and Arg16/Gln27 = 56.3%, Gly16/Gln27 = 32.2%, Gly16/Glu27 = 10.4% in non-wheezing controls. As compared with Gly16/Glu27 haplotype, subjects with Arg16/Gln27 and Gly16/Gln27 haplotypes were at increased risks of wheezing illness (aRR 1.77, 95% CI 1.07–2.92; and aRR 1.89, 95% CI 1.12–3.22, respectively). The protective effect of Gly16/

Table 3. The association between haplotypes of two ADRB2 functional SNPs and wheezing illness among schoolchildren

ADRB2 haplotypes	Frequency		cRR (95% CI)	aRR* (95% CI)
	Controls	Wheezing subjects		
Arg16/Gln27	0.563	0.572	1.52 (0.94–2.45)	1.77 (1.07–2.92)
Gly16/Gln27	0.322	0.353	1.65 (1.00–2.75)	1.89 (1.12–3.22)
Gly16/Glu27	0.104	0.074	1.00	1.00
Arg16/Glu27	0.011	0.000		
Global <i>P</i> -value			0.05	0.04
Gly16/Glu27 vs other haplotypes			0.67 (0.41–1.10)	0.58 (0.35–0.97)

cRR, crude relative risk; aRR, adjusted relative risk; CI, confidence interval.

All relative risks are calculated based on additive genetic models.

*Models are adjusted for age, gender, community of residence, parental atopic history, parental education level and ETS exposure at home.

Glu27 haplotype remained relative to all other ADRB2 haplotypes (aRR 0.58; 95% CI 0.35–0.97) (Table 3). The decreased risk conferred by Gly16/Glu27 haplotype was independent of age, gender, community of residence, parental atopic history, parental education level, and ETS exposure at home.

In Table 4, the association between ADRB2 haplotypes and wheezing illness was assessed. As compared with children without Gly16/Glu27 haplotype, those with one or two copies of Gly16/Glu27 haplotype had a significantly lower risk for wheezing illness (aRR 0.56; 95% CI 0.33–0.99). The copy numbers of Gly16/Glu27 haplotype also showed a clear dose-response relationship on the decreased risks, with aRR 0.61 (95% CI 0.34–1.08) for those with one copy of Gly16/Glu27 haplotype, and aRR 0.24 (95% CI 0.02–2.46) for those with two copies (Table 4). No significant association was found with the prevalence of wheezing illness for other haplotypes.

Table 4. Relative risks (95% CI) of ADRB2 haplotype copy numbers on wheezing illness among schoolchildren

ADRB2 haplotypes	0 Copies	1 Copy	2 Copies	≥1 Copy
Univariate models				
Arg16/Gln27	1.00	1.28 (0.75–2.16)	1.10 (0.64–1.90)	1.19 (0.73–1.94)
Gly16/Gln27	1.00	1.24 (0.81–1.91)	1.25 (0.68–2.32)	1.25 (0.84–1.85)
Gly16/Glu27	1.00	0.72 (0.41–1.25)	0.27 (0.03–2.59)	0.66 (0.40–1.10)
Mutually adjusted models*				
Arg16/Gln27	1.00	1.38 (0.80–2.39)	1.21 (0.68–2.13)	1.30 (0.79–2.16)
Gly16/Gln27	1.00	1.23 (0.79–1.93)	1.28 (0.67–2.42)	1.24 (0.82–1.88)
Gly16/Glu27	1.00	0.61 (0.34–1.08)	0.24 (0.02–2.46)	0.56 (0.33–0.99)

CI, confidence interval.

*Models are adjusted for age, gender, community of residence, parental atopic history, parental education level and ETS exposure at home.

Discussion

In this study, we tested for an association between ADRB2 genotypes, haplotypes, and the risk of childhood wheezing illness. After controlling for age, gender, community of residence, parental atopic history, parental education level, and ETS exposure at home, children who carried Glu27 allele were found to have a significantly decreased risk of wheezing illness. The risk of wheezing illness was not associated with Arg16Gly genotype in our population. In haplotype analyses using Gly16/Glu27 haplotype as reference group, Arg16/Gln27 and Gly16/Gln27 haplotypes showed significantly increased risks for childhood wheezing illness. As compared with children without Gly16/Glu27 haplotype, those with Gly16/Glu27 haplotype had a significantly lower risk after controlling for potential confounders. Our study showed an instance with the use of haplotype information compared with analyses using individual SNP information.

Age, gender, ethnic factors, active smoking habits, *in utero* exposure to maternal smoking, household ETS exposure, parental atopic history, and parental education level were believed to contribute to childhood wheezing illness (23, 27, 32, 33). We minimized interference from these confounders by recruiting lifelong non smokers without *in utero* exposure to maternal smoking at study entry, and adjusting potential confounders by regression models. As all of the recruited children had the same ethnic origin, population stratification was therefore very unlikely to have influenced the findings. In this study, wheezing was noted to be associated with parental atopic history and parental education level (Table 1). These risk factors were controlled by regression model in our further analyses.

It has been known that activated ADRB2 gene has an important role in regulation of bronchial smooth muscle tone (5, 6). Several coding region variants occur at a high frequency, which would be relevant to a population as a whole, and show physiologic effects (34). Arg16Gly, Gln27Glu, and Thr164Ile all seem to be functionally relevant based on data generated with recombinant cell systems or cells studied *ex vivo* from individuals with known genotype (18, 35). In ADRB2, another functional SNP (Arg19Cys in the 5' promoter region) has been studied in addition to the three SNPs we studied. A recent report showed that the Arg19Cys SNP was in strong linkage disequilibrium with the coding SNPs Arg16Gly and Gln27Glu, and three common haplotypes (Gly16/Cys19/Gln27, Gly16/Arg19/Glu27, and Arg16/Cys19/Gln27) existed (36). Because the alleles at the Arg19Cys locus could be predicted from the haplotypes estimated from Arg16Gly and Gln27Glu genotypes (37), it was not necessary to genotype Arg19Cys in our study.

Although we did not find a significant risk for Arg16Gly genotypes, our data suggested an obviously protective effect for Glu27 allele on childhood wheezing

illness (Table 2). There were emerging data from *in vivo* studies of ADRB2, which suggest that Glu27 homozygosity is associated with reduced desensitization of the β 2AR (36, 38). The effect of ADRB2 polymorphisms on the functional properties of the corresponding β 2AR variants has been studied in β 2AR-transfected Chinese hamster fibroblasts and in human bronchial smooth muscle cell lines (35). It was shown that amino-terminal polymorphisms 16 and 27 affect the agonist-promoted receptor downregulation in that Gly16 confers increased downregulation when compared with Arg16, as does Gln27 when compared with Glu27 (18, 35). Our results are consistent with previous reports on Gln27Glu genotypes. Ramsay et al. assessed airway hyper-responsiveness and found subjects with the Glu27 allele were less responsive than those with the Gln27 allele (15). Hall et al. reported that asthmatic subjects with the Glu27 allele had significantly less airway hyper-responsiveness but did not find any association with the Arg16Gly SNP (39). Hopes et al. also found that Glu27 allele was associated with a reduced risk for childhood asthma in general population (40); 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. (20), which included the data from the study by Hopes et al.

Results from our laboratory showed that there are more of ADRB2 Arg16 allele and Gln27 allele in Taiwanese population than in Caucasians. The frequency of Arg16 allele in Western populations ranged from 35% to 42% throughout Italy, America and England (20, 23, 24, 40). Nearly 57% subjects had a Gln27 allele in Caucasian population (20, 23, 24, 40). In contrast, we found that the frequency of Gln27 allele was around 88.5% (controls) to 92.6% (wheezing subjects), and Arg16 allele was over 55% in Taiwanese population. These allele frequencies are similar to those reported in Chinese population: Arg16 = 56.4% and Gly16 = 43.6%, Gln27 = 91.5% and Glu27 = 8.5% (41). The higher frequency in Taiwanese population would make the genetic effects of risky alleles carriers for acquiring wheezing illness lower than that of Caucasian populations.

Because of strong linkage disequilibrium, the SNPs in ADRB2 gene result in only a limited number of haplotypes. The three haplotypes (Arg16/Gln27, Gly16/Glu27 and Gly16/Gln27), which are found in our data (Table 3), correspond to haplotypes prevalent in other population-based studies (23–25). In the system of β 2AR-transfected Chinese hamster fibroblasts, the Arg16/Glu27 haplotype was found to be strongly resistant to agonist-induced downregulation (35). Accordingly, it suggested that a virtual absence of this haplotype could be the result of a negative selection acting on such a poorly 'flexible' signaling protein.

Some haplotypes of the ADRB2 gene have been suggested to play an important role in modifying clinical characteristics of asthma phenotypes (21). Although the

exact relationships have not been well studied, some researchers have argued that assessment of individual SNP effects without considering haplotypes may have resulted in inconsistent associations because the SNPs in ADRB2 gene are tightly linked. As compared with Gly16/Glu27 haplotype, our data showed that children with Gly16/Gln27 haplotype were associated with wheezing illness, proposing this variant as an independent risk factor for the development of wheezing (Table 3). D'Amato et al. conducted analyses in an unselected Italian cohort and found that the Gly16/Gln27 haplotype was associated with persistent airways hyper-responsiveness over a period of 8 months (24). In US, a positive association of the Grg16/Gln27 haplotype with airway hyper-responsiveness has also been reported among non smoking adults (16). We also found that one or two copies of the Gly16/Glu27 haplotype were significantly related to a lower risk for childhood wheezing illness (Table 4). The copy numbers of Gly16/Glu27 haplotype also showed a clear dose-response relationship on the decreased risks. The protective effect of Gly16/Glu27 haplotype remained relative to all other ADRB2 haplotypes (Table 3). Although functional properties of the Gly16/Glu27 haplotype need exploration in greater detail, our findings contribute to the understanding of the complex relationship between ADRB2 gene and respiratory function.

The number of subjects involved in our analyses was not sufficiently large for marked differences between haplotypes we identified. As the effect estimates between the haplotypes include a twofold difference, it is possible that true differences may exist. Although there remains the possibility that our positive results may have been because of chance alone, the fact that the results from the individual genotype and haplotype analyses were consistent suggests that this may not be the case. We investigated the haplotypes from only two polymorphisms (codons 16 and 27) because most data suggest that haplotypes that are tagged by these two polymorphisms are the most common and might drive significant effects. In the promoter region (ADRB2 upstream peptide) of ADRB2 gene, eight SNPs were also identified and suggested to have the potential to alter human ADRB2 gene expression (42). Extensive haplotypes including the SNPs in the promoter region need to be further investigated.

Our study has some limitations. We did not have genotyping data from all subjects, which made selection bias possible. However, participants with genotyping data included in this analysis did not differ from those without genotyping data on many demographic factors, but did show small differences in the proportion of parental education levels (Table 1). False-positive associations may arise because of multiple comparisons, which have been corrected by reducing alpha levels in our analyses. Children from families with higher education level were less likely to have moved and to be lost to follow-up.

Because the differences in distribution were modest and are probably not associated with the genotypes, it is unlikely that selection of subjects biased the effect estimates in our results. The study subjects were recruited in an unselected population, unbiased observations of the association between genetic effects and outcomes were expected. Childhood wheezing illness was ascertained by parental-reported questionnaire, so misclassification of wheezing status may have arisen from imperfect parental recall of events. Because wheezing illness was defined without the knowledge of genotype, differential misclassification of wheezing by ADRB2 genetic polymorphisms is probably not a major source of bias that accounts for our results. Therefore, although there is likely to be non differential misclassification of wheezing status, such misclassification would not account for the genetic associations we observed.

In conclusion, we found that ADRB2 genotypes and haplotypes might be important determinants to the development of wheezing illness in schoolchildren, based on the functional biology of this gene. This stresses the

importance of the variation in functional properties of β_2 AR that may be used as markers of individuals susceptible to inhalational insults. Our data suggest that childhood wheezing illness is a complex genetic disease, and the use of haplotype information may be as powerful as SNP analysis for association studies and for instances in which only a few SNPs are genotyped. We believe that ADRB2 gene, along with its disease-modifying effects, does play an inducing role contributing to the genetic background that predisposes to asthma-associated phenotypes.

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