

ADRB2 polymorphisms in allergic asthma in Han Chinese children

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Background: Single-nucleotide polymorphisms (SNPs) in the human β_2 adrenergic receptor (*ADRB2*) gene have been linked to pathogenesis of and treatment responses in asthma. In China, where asthma is common, little information exists regarding *ADRB2* variants and their effects on asthma. The current study sought to investigate the relationship between variations in *ADRB2* and childhood asthma.

Methods: Blood samples were collected from 298 pediatric asthma cases treated in our hospital from July 2013 to September 2014; 304 samples were collected from healthy children for a control group. Six loci (at base positions -47, 79, -2387, 46, 523, and 491) of *ADRB2* were genotyped using the TaqMan probe assay; SHEsis was used to determine haplotypes. Allele frequencies and genotype distributions were compared between groups using chi square and *t* tests.

Results: No significant differences in genotype or allele frequencies were found between the groups for the polymorphic loci -2387 bp (T>C), 46 bp (G>A), and 523 bp (C>A) ($p > 0.05$). However, genotype frequencies for the

ADRB2 SNPs at -47 bp (C>T) and 79 bp (G>C) differed significantly between asthma and control groups ($p < 0.05$). Further, linkage disequilibrium was found between these 2 SNPs ($D' = 0.990$, $r^2 = 0.962$), as well as between SNPs at 46 and 523 bp ($D' = 0.985$, $r^2 = 0.607$). Finally, of the 4 haplotypes analyzed in these samples (haplotypes III, IV, IX, and XI), the frequency of haplotype III was significantly lower in the asthma group than the control group ($p < 0.05$; odds ratio [OR] = 0.72; 95% confidence interval [CI], 0.48 to 0.97).

Conclusion: Variations of *ADRB2* at base positions -47 (C>T) and 79 (G>C), as well as haplotype III, may contribute to susceptibility to childhood asthma. © 2015 ARS-AAOA, LLC.

Key Words:

asthma; *ADRB2* gene; polymorphism; genotype; haplotype

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Asthma is the most common chronic allergic disease of the respiratory tract in childhood, and has become a global public health problem in recent years.¹ Asthma, characterized by bronchial hyperresponsiveness and airway inflammation, is a polygenic disease produced by the combined action of genetic, immune, and environmental factors.²

The β_2 adrenergic receptor (β_2AR) is a G-protein coupled receptor found in respiratory epithelium, airway

smooth muscle, and lymphocytes. The gene encoding this receptor, *ADRB2*, was mapped to chromosome 5q31-33, which was regarded as harboring an atopy, asthma-susceptibility, or bronchial hyperresponsiveness locus.³ Importantly, β_2 -agonists are the mainstay of therapy for acute bronchospasm associated with asthma, but interindividual differences in response to these medications have been observed. These differences could be associated with genetic variation at the receptor level.⁴

Indeed, single-nucleotide polymorphisms (SNPs) in *ADRB2* have been correlated with various aspects of asthma, from severity and susceptibility to responses to drug treatment.⁵⁻⁷ These variations likely change the expression and functions of β_2AR .⁸⁻¹⁰ Currently, several loci in the *ADRB2* coding region have been identified as SNP sites; of these, SNPs at base positions 46G>A, 79G>C, and 491C>T can cause amino acid alterations.¹¹ Similarly, a meta-analysis published in 2014 showed that 46G>A, 79G>C, 491C>T, and -47C>T were reproducibly associated with a risk of asthma across ethnic groups in the general population.¹²

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In China, where the prevalence of asthma in children is 0.5% to 3.3%, 13 SNPs in *ADRB2* have been studied in children with asthma.¹³ A meta-analysis of 2 common polymorphisms in *ADRB2*, 46G>A and 79G>C, was performed for adult and pediatric populations separately, and concluded that the C79G genotype has a decreased risk for asthma in children.¹⁴ Here, genotyping of 6 loci, base positions –2387T>C, –47C>T (rs1042711), 46G>A (rs1042713), 79G>C (rs1042714), 491C>T (rs1800888), and 523C>A (rs1042718), in the coding and regulatory regions of *ADRB2*, was performed in children with asthma to determine any potential correlation between polymorphisms/haplotypes in this gene and asthma in children.

Subjects and methods

Subjects

This prospective case-control study comprised an asthma group and a healthy control group. Participants in the asthma group were treated in our hospital between July 2013 and September 2014. A total of 298 hospitalized patients with asthma were selected by random digit dialing (RDD); of them, 117 were males, and the mean group age was 5.6 ± 10.3 years (range, 9 months to 16 years). According to the Breathing Study Group of the Pediatrics Branch of Chinese Medical Sciences, the diagnostic criteria for asthma in infants were as follows: (1) a history of more than 3 asthma attacks and age less than 3 years; (2) at onset, both lungs had expiratory wheezing by auscultation, with extended expiratory phase; (3) the infant exhibited an allergic constitution, with symptoms such as allergic eczema or allergic rhinitis; (4) a history of allergy, including asthma, in the parents; and (5) other diseases were excluded as the cause of breathing symptoms. Individuals meeting criteria 1, 2, and 5 were diagnosed with asthma.¹⁵ Individuals meeting criteria 2 and 5 and with a history of more than 2 asthma attacks were diagnosed with asthma.¹⁵ The diagnostic criteria for asthma in children were as follows: (1) repeated sharp breaths and age over 3 years; (2) at the onset, both lungs had expiratory wheezing by auscultation, with extended expiratory phase; (3) treatment with a bronchial dilation agent produced improvements; and (4) other diseases associated with sharp breaths, chest tightness, and cough were ruled out. The patients who met all 4 of the criteria were diagnosed with asthma.¹⁵ Other diseases, including heart, liver, kidney, and other respiratory tract diseases were ruled out. Children in the control group were given a physical examination in our hospital. Of the 304 control individuals, 139 were males, and the mean group age was 5.2 ± 9.8 years (range, 2 to 14 years). No participants in the control group had a history of asthma, respiratory tract diseases, parasitic infections, or allergic diseases. All participants were Han people from Nanjing, China, who had no mutual blood relations. There were no statistically significant differences in age or sex between the asthma group and the control group ($p > 0.05$). This

study was approved by the hospital Ethics Committee, and informed consent was given by all participants' parents.

Genomic DNA extraction

For each participant, 4 mL of venous blood was collected. DNA was extracted according to the instructions in the Human Blood Genomic DNA Extraction Kit (Generay Biotech, Shanghai, China). Extracted DNA was stored at -80°C until use.

SNP genotyping

The TaqMan probe method was used to detect the genotypes at 6 loci in the coding and regulatory regions of *ADRB2*.¹⁶ Two probes (HEX and FAM) and primers were designed and synthesized by BioTeke Corporation (Beijing, China). Polymerase chain reaction (PCR) was performed in $2 \times \text{HotTaq}^{\text{®}}$ PCR Reaction Mix (Merck, Germany). The 10- μL reactions comprised 0.45 μL of upstream primer and 0.45 μL of downstream primer, 0.25 μL each of HEX and FAM, 5 μL of $2 \times \text{HotTaq}$ PCR Reaction Mix, 2.6 μL of double-distilled water (ddH₂O), and 1 μL of DNA template. Reactions were performed in the ABI7500 (Adicon Clinical Laboratories, Shanghai, China) under the following thermal cycling settings: pre-read for 1 minute; 40 cycles of 95°C for 10 minutes, then 95°C for 15 seconds, and 60°C for 1 minute; and a final read for 1 minute. Genotyping was performed with ABI SDS1.4 software (Applied Biosystems).

Statistical analysis

Double data entry was performed using EpiData version 3.1 (EpiData Association, Copenhagen, Denmark) to create a data bank, and logic checks were performed. SAS 9.2 (SAS Institute, Inc., Cary, NC) was used to analyze the data. Statistical methods included the *t* test and chi square test, Hardy-Weinberg equilibrium test for the sample representative of the population, as well as the SHEsis online platform (<http://analysis2.bio-x.cn/myAnalysis.php>) to calculate linkage disequilibrium among various loci and compare the haplotype frequencies between the 2 groups. Odds ratios (ORs) and 95% confidence intervals (CIs) were used to denote the strength of correlation between the SNPs and the risk of occurrence of asthma. A value of $p < 0.05$ was considered to indicate a statistically significant difference.

Results

Genotype and allele distributions for SNP loci of *ADRB2* in children with asthma and healthy children

All genotype frequencies at the 6 SNP loci of *ADRB2* in both groups met Hardy-Weinberg equilibrium ($p > 0.05$). At locus 491 in both groups, only the wild-type TT genotype was detected; therefore, this locus was not incorporated into the statistical analysis.

TABLE 1. Allele and genotype distributions for *ADRB2* polymorphic loci in children with and without asthma

	Asthma group (n = 298)	Control group (n = 304)		
SNP	n (%)	n (%)	p	OR (95% CI)
-47				
Genotype			0.069 ^{a,b}	
TT	247 (82.89)	231 (75.99)		
CT	49 (16.44)	67 (22.04)	0.043	0.69 (0.37–0.96)
CC	2 (0.67)	6 (1.97)	0.167 ^b	0.37 (0.09–2.30)
CT+ CC	51 (17.11)	73 (24.01)	0.036	0.65 (0.51–0.98)
Allele			0.023	0.69 (0.41–0.95)
T	543 (91.11)	529 (87.01)		
C	53 (8.89)	79 (12.99)		
79				
Genotype			0.069 ^{a,b}	
CC	247 (82.89)	231 (75.99)		
CG	49 (16.44)	67 (22.04)	0.043	0.69 (0.37–0.96)
GG	2 (0.67)	6 (1.97)	0.167 ^b	0.37 (0.09–2.30)
CG+ GG	51 (17.11)	73 (24.01)	0.036	0.65 (0.51–0.98)
Allele			0.023	0.69 (0.41–0.95)
C	543 (91.11)	529 (87.01)		
G	53 (8.89)	79 (12.99)		
-2387				
Genotype			0.584 ^a	
TT	152 (51.01)	144 (47.37)		
CT	122 (40.94)	130 (42.76)	0.493	0.87 (0.58–1.26)
CC	24 (8.05)	30 (9.87)	0.351	0.81 (0.41–1.43)
CT+ CC	146 (48.99)	160 (52.63)	0.372	0.85 (0.59–1.21)
Allele			0.302	0.87 (0.67–1.30)
T	426 (71.48)	418 (68.75)		
C	170 (28.52)	190 (31.25)		
46				
Genotype			0.312 ^a	
AA	104 (34.90)	90 (29.61)		
AG	145 (48.66)	154 (50.66)	0.267	0.79 (0.48–1.13)
GG	49 (16.44)	60 (19.74)	0.148	0.70 (0.41–1.16)
AG+ GG	194 (65.10)	214 (70.40)	0.165	0.75 (0.51–1.09)
Allele			0.132	0.83 (0.59–1.07)
A	353 (59.23)	334 (54.93)		
G	243 (40.77)	274 (45.07)		
523				

(Continued)

TABLE 1. Continued

	Asthma group (n = 298)	Control group (n = 304)		
SNP	n (%)	n (%)	p	OR (95% CI)
Genotype			0.688 ^a	
CC	145 (48.66)	140 (46.05)		
CA	123 (41.28)	136 (44.74)	0.430	0.86 (0.59–1.23)
AA	30 (10.07)	28 (9.21)	0.906	0.96 (0.60–1.71)
CA+ AA	153 (51.35)	164 (53.95)	0.522	0.90 (0.63–0.19)
Allele			0.743	0.93 (0.69–1.20)
C	413 (69.30)	416 (68.42)		
A	183 (30.70)	192 (31.58)		

^aThe *p* values of 3 kinds of genotypes were compared in the frequency of asthma and control groups.

^bFisher's exact test.

CI = confidence interval; OR = odds ratio; SNP = single-nucleotide polymorphism.

At locus –47, there was a significant difference in genotype frequencies between groups when the CT genotype was assessed (CT+CC vs TT: $p < 0.05$, OR = 0.65, 95% CI 0.51 to 0.98; CT vs TT: $p < 0.05$, OR = 0.69, 95% CI 0.37 to 0.96) (Table 1). However, there was no difference in frequency of the CC genotype vs the TT genotype between groups ($p > 0.05$). Allele frequencies also differed at this locus, with the C allele less frequent in the asthma group than in the control group ($p < 0.05$; OR = 0.69; 95% CI, 0.41 to 0.95).

At locus 79 there was also a significant difference in genotype distributions between groups when the CG allele genotype was compared (CG+GG vs CC: $p < 0.05$, OR = 0.65, 95% CI 0.51 to 0.98; CG vs CC genotype, $p < 0.05$, OR = 0.69, 95% CI 0.37 to 0.96) (Table 1). However, there was no difference in frequency of the GG genotype vs the CC genotype ($p > 0.05$). Further, the frequency of the G allele at this locus was significantly lower in the asthma group than in the control group ($p < 0.05$, OR = 0.69, 95% CI 0.41 to 0.95).

Differences between the groups regarding their genotype and allele frequencies at loci –2387, 46, and 523 were not statistically significant (each *p* value >0.05).

Linkage disequilibrium for 5 SNPs of *ADRB2*

The SHEsis online platform was used to analyze linkage disequilibrium among the 5 significant SNPs of *ADRB2*, in which the extent of linkage disequilibrium between 2 loci was expressed by D' and r^2 . Linkage disequilibrium was detected between loci 79 and –47 ($D' = 0.990$, $r^2 = 0.962$) as well as loci 523 and 46 ($D' = 0.985$, $r^2 = 0.607$) (Table 2).

Haplotype distributions for the 5 SNP loci of *ADRB2*

SHEsis was also used to determine haplotypes for the 5 significant SNPs. A total of 16 haplotypes were found; 12

TABLE 2. Linkage disequilibrium for the 5 polymorphic loci of *ADRB2*^a

	SNP loci			
SNP loci	–47	46	79	523
–2387	0.892 (0.254)	0.213 (0.031)	0.935 (0.283)	0.992 (0.204)
–47	—	0.915 (0.174)	0.990 (0.962)	0.987 (0.073)
46	—	—	0.989 (0.210)	0.985 (0.607)
79	—	—	—	0.993 (0.062)

^aValues are D' (r^2).

SNP = single-nucleotide polymorphism.

had a frequency <0.03 and therefore were not incorporated into the statistical analysis. The 4 remaining haplotypes were haplotypes III, IV, IX, and XI. The frequency of haplotype III was significantly lower in the asthma group than in the control group ($p < 0.05$; OR = 0.72; 95% CI, 0.48 to 0.97). Differences between the groups in their frequencies of haplotypes IV, IX, and XI were not statistically significant ($p > 0.05$) (Table 3).

Discussion

Understanding the pathogenesis of asthma is a fundamental way by which the disease can be prevented and treated. Other studies have demonstrated that the risk of asthma increases with increasing body mass index (BMI) and with early-life and environmental exposure,¹⁷ and with second-hand smoke exposure.¹⁸ Importantly, however, the contribution of genetic factors in contributing to the development of asthma should not be overlooked.¹⁹

Because the β_2 ARs are key components of asthma pathology and β_2 -agonists are commonly used to treat the disease, the *ADRB2* gene has been widely studied for its

TABLE 3. Haplotype analysis for 5 polymorphic loci of *ADRB2* in children with and without asthma

Haplotype	SNP loci					Asthma group (n = 298)	Control group (n = 304)	p	OR (95% CI)
	–47	79	–2387	46	523	n (%)	n (%)		
I	C	G	C	A	C	0.00 (0.00)	0.15 (0.02)	—	—
II	C	G	C	G	A	0.00 (0.00)	0.23 (0.04)	—	—
III	C	G	C	G	C ^a	51 (8.56)	73 (12.01)	0.031	0.72 (0.48–0.97)
IV	T	C	C	A	C ^a	115.6 (19.40)	117 (19.24)	0.937	1.04 (0.81–1.46)
V	T	C	C	G	C	1 (0.17)	0.01 (0.00)	—	—
VI	C	C	T	G	C	0.00 (0.00)	0.6 (0.10)	—	—
VII	C	G	T	G	C	0.00 (0.00)	0.7 (0.12)	—	—
VIII	T	C	T	A	A	0.00 (0.00)	1.2 (0.20)	—	—
IX	T	C	T	A	C ^a	238 (39.93)	219.6 (36.12)	0.091	1.23 (0.85–1.53)
X	T	G	T	A	C	0.00 (0.00)	0.11 (0.02)	—	—
XI	T	C	T	G	A ^a	180 (30.20)	193 (31.74)	0.702	0.91 (0.69–1.24)
XII	T	C	T	G	C	8 (1.34)	9 (1.48)	—	—
XIII	T	G	T	G	A	0.00 (0.00)	0.6 (0.10)	—	—
XIV	C	C	C	A	C	0.6 (0.10)	0.00 (0.00)	—	—
XV	T	C	C	G	A	1.2 (0.20)	0.00 (0.00)	—	—
XVI	T	G	C	G	C	0.6 (0.10)	0.00 (0.00)	—	—

^aFrequency of haplotype >0.03.

CI = confidence interval; OR = odds ratio; SNP = single-nucleotide polymorphism.

roles in asthma. This study sought to determine the roles of genetic variation in *ADRB2* in childhood asthma among Han Chinese by analyzing 6 known SNP sites among children with asthma and controls.

Two SNP sites, at base positions 79 and –47, had different genotype and allele distributions between cases and controls. At locus 79, the G allele was associated with a reduced risk of asthma. At locus –47, the C allele was also associated with reduced risk of asthma. Further, loci 79 and –47 as well as loci 523 and 46 displayed linkage disequilibrium. The results were similar to the findings of Li et al.²⁰ and Al-Rubaish,²¹ in which the CG allele at locus 79 could reduce the risk of occurrence of asthma in children. However, in their study on a population in northern India, Birbian et al.²² found that the CG allele was not correlated with asthma.

An approach looking only at the correlation between SNPs of *ADRB2* and asthma is not sufficient; the action of SNPs alone could possibly be influenced by the action of other polymorphisms or haplotypes.⁸ In this study, 4 common haplotypes (haplotypes III, IV, IX, and XI) were identified for the 5 SNP sites. Haplotype III was associated with a reduced risk of asthma. The differences between the groups regarding the frequency of haplotype III and the frequencies of the other 3 haplotypes were because of loci 79

and –47: for haplotype III, locus 79 was the G allele and locus –47 was the C allele. Thus, the roles of the G allele and the C allele in the reduction of asthma risk might be due not to SNPs alone but to having haplotype III, which contains both alleles. Therefore, compared with the G allele alone or the C allele alone, haplotype III could better predict the risk of asthma. These results are similar to the findings of Saadi et al.²³ However, in their study on a Han population with asthma in southern China, Fu et al.²⁴ found that the Arg19Cys, Arg16Gly, and Glu27Gln polymorphisms were not correlated with the susceptibility to asthma.

One limitation of this study is that data on potential confounding factors, such as BMI, exposure to secondhand smoke, and sociodemographic risk factors for asthma, were not collected. Therefore, the results need to be interpreted cautiously, taking into consideration that other factors could have contributed to asthma risk in our population.

Conclusion

In summary, the polymorphisms and haplotype III at locus –47 and 79 of the *ADRB2* gene in Chinese children are correlated with susceptibility to asthma. In future studies, we will increase the sample size and assess the

polymorphisms in the 3'-untranslated region to analyze the role of a complete haplotype of the *ADRB2* gene, thereby

more fully reflecting the role of polymorphisms/haplotypes of *ADRB2* in the pathogenesis of asthma. 4

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