Int J Clin Exp Med 2014;7(10):3812-3817
www.ijcem.com /ISSN:1940-5901/IJCEM0002000

Original Article

Association between polymorphisms and haplotypes of the beta-2 adrenergic receptor gene and asthma in a Chinese Han population

Zi-Qi Liu, Yi Jiao, Chan-Juan Liu, Hao-Cheng Zhang, Bao-Rong Hu

Department of Pharmacy, The First Affiliated Hospital, Harbin Medical University, Harbin 150001, Heilongjiang Province, P. R. China

Received August 20, 2014; Accepted September 25, 2014; Epub October 15, 2014; Published October 30, 2014

Abstract: Conflicting data have been reported on the association of variants of the beta-2 adrenergic receptor (*ADRB2*) gene with asthma etiology. To provide a scientific basis for the prevention and treatment of asthma, the relationship between single nucleotide polymorphisms and haplotypes of *ADRB2* and asthma was examined in a Chinese Han population. Six loci (base positions -2387, -47, 46, 79, 491, and 523) of *ADRB2* were examined in a group of individuals with asthma (n = 429) and a control group of individuals who do not have the disease (n = 483). Polymorphisms at the -47 locus [odds ratio (*OR*) = 0.789, 95% confidence interval (CI): 0.398~0.961, $P < 0.05$) and the 79 locus (*OR* = 0.788, 95% CI: 0.394~0.948, $P < 0.05$) and haplotype III (CCGGC; *OR* = 0.705, 95% CI: 0.412~0.973, $P < 0.05$) were correlated with asthma susceptibility and may affect the incidence of asthma in a Chinese Han population by conferring a protective effect. By examining several polymorphisms and haplotypes, this study yields a more comprehensive examination of the role of the *ADRB2* gene in the pathogenesis of asthma.

Keywords: ADRB2, asthma, single nucleotide polymorphism, haplotypes

Introduction

Bronchial asthma (asthma), a non-specific chronic airway inflammatory disease, is one of the most common chronic respiratory diseases. Currently, the global prevalence of asthma is 1%-18%, with the number of asthma patients totaling around 300 million [1], and the incidence of this disease is on the rise. The overall prevalence of asthma in China is 1%, but in children is up to 3%, with both increasing [2]. As a disease with complex clinical symptoms, the pathogenesis of asthma is still undefined.

The etiology of asthma is affected by both genetic and environmental factors. It is considered a polygenic disease with significant familial aggregation propensity. In genetic studies of asthma, the beta-2 adrenergic receptor (*ADRB2*) gene has been shown to be an important factor linked to the disease. *ADRB2*, mapped to 5q31-32, is a non-intronic gene [3]. The expression of the gene product is affected by single nucleotide polymorphisms (SNPs) that alter the haplotype of *ADRB2* [4, 5] and are related to the onset of the disease, phenotype, nocturnal asthma, and medication reactivity [6, 7]. However, results from several studies examining the role of *ADRB2* in asthma have yielded inconsistent results. Previous research on *ADRB2* in the Han Chinese population has focused primarily on the coding region, with few studies examining other loci. In the current study, six loci (base positions -2387, -47, 46, 79, 491, and 523) within the regulatory and coding regions of *ADRB2* were examined to determine the genotypes and haplotypes present within the population and, ultimately, investigate the relationship between *ADRB2* haplotypes and asthma.

Materials and methods

Study participants

This is a case-control study comparing individuals with asthma with individuals who do not have the disease. In the asthma group there

Table 1. Allele and genotype distributions of five polymorphic loci of the ADBR2 gene in individuals with and without asthma

SNP loci	Asthma group [n (%)]	Control group [n (%)]	*P*	*OR* (95% *CI*)
-2387				
Genotype			0.416*	
TT	220 (51.28)	229 (47.41)		
CT	175 (40.79)	207 (42.86)	0.528	0.960 (0.589~1.275
CC	34 (7.93)	47 (9.73)	0.339	0.903 (0.376~1.562)
CC + CT	209 (48.72)	254 (53.59)	0.243	0.967 (0.551~1.273)
Allele				
T	615 (71.68)	665 (68.84)		
C	243 (28.32)	301 (31.16)		
-47				
Genotype			0.056*	
TT	357 (83.22)	373 (78.47)		
CT	70 (16.32)	104 (20.70)	0.039	0.784 (0.393~0.915)
CC	2 (0.47)	6 (0.83)	0.491	0.524 (0.096~2.967)
CC + CT	72 (16.79)	112 (21.53)	0.031	0.765 (0.375~0.962)
Allele			0.018	0.789 (0.398~0.961)
T	784 (91.38)	850 (87.99)		
C	74 (8.62)	116 (12.01)		
46				
Genotype			0.398*	
AA	150 (34.97)	153 (31.68)		
AG	209 (48.72)	237 (49.07)	0.915	0.912 (0.497~1.216)
GG	70 (16.32)	93 (19.25)	0.248	0.820 (0.439~1.169)
GG + AG	279 (65.04)	330 (68.32)	0.293	0.891 (0.513~1.154)
Allele			0.179	0.942 (0.598~1.167)
A	509 (59.32)	543 (56.21)		
G	349 (40.68)	423 (43.79)		
79				
Genotype			0.054*	
CC	356 (82.98)	370 (76.60)		
CG	70 (16.32)	107 (22.15)	0.026	0.775 (0.381~0.980)
GG	3 (0.70)	6 (1.24)	0.408	0.521 (0.091~1.911)
GG + CG	73 (17.02)	113 (23.39)	0.017	0.771 (0.378~0.942)
Allele			0.016	0.788 (0.394~0.948)
C	782 (91.14)	847 (87.68)		
G	76 (8.86)	119 (12.32)		
523				
Genotype			0.615*	
CC	209 (48.72)	223 (46.17)		
CA	178 (41.49)	216 (44.72)	0.326	0.899 (0.572~1.283)
AA	42 (9.79)	44 (9.11)	0.726	0.979 (0.512~1.731)
AA + CA	220 (51.28)	260 (53.83)	0.442	0.891 (0.589~1.284)
Allele			0.667	0.958 (0.667~1.273)
C	596 (69.46)	662 (68.53)		
A	262 (30.54)	304 (31.47)		

Note: *Three genotypes in the asthma and control groups were compared in the calculation of *P* values. n = total number of participants; OR = Odds Ratio; CI = confidence interval.

were 429 asthma participants selected from patients hospitalized from September 2012 to September 2013. The asthma group comprised 170 males and 259 females with an average

age of 45.15 ± 16.10 years. All of the participants in the asthma group met the diagnotic criteria for asthma described in the Global Initiative for Asthma, 2009 [8] and excluded the patients with heart, liver, kidney, chronic obstructive pulmonary, and other respiratory diseases. The asthma patients were divided into three groups according to the degree of asthma attack experienced: intermittent/mild group (203 patients), moderate group (80 patients), and severe group (146 patients). The control group comprised 483 participants selected from individuals who received physical examination at the hospital during the same time period and had been confirmed as healthy. This control group comprised 221 males and 262 females with an average age of 43.51 ± 12.10 years. All 483 participants in the control group exhibited no asthma, diabetes, hypertension, or other diseases. All participants in this study were Han people of China without any genetic connection. The gender and age distributions of the participants in the asthma group and control group were not significantly different ($P > 0.05$). The study was approved by the ethics committee of the hospital, and informed consent was obtained from all participants.

DNA extraction

A 4-mL sample of venous blood was collected from each participant, and a human blood genome extraction kit (Beijing Tiangen Biochemistry Co., Beijing, China) was used to extract DNA following the kit instructions. Extracted DNA was stored at -80°C.

SNP genotyping of target gene

Taqman probes were used for genotyping six loci in the regulatory and coding regions of *ADRB2*. Two probes were designed for each locus, labeled as FAM and HEX. All Taqman probes and primers were designed and synthesized by Takara Biotechnology (Dalian, China). The 2× HotTaq®PCR Reaction Mix was provided by Nanjing Bio-Technique Co. (Nanjing, China). PCR reactions were carried out on an ABI 7500 PCR system (Applied Biosystems, Foster, CA, USA) in 10-μL reactions containing: 5 μL 2× HotTaq®PCR Reaction Mix, 20 pmol of each upstream and downstream primer, (0.45 μL of the FAM primers and 0.25 μL of the HEX primers), 1 μL DNA, and 2.6 μL ddH_2O. The PCR program used was an initial pre-read of 1 min followed by 40 cycles of denaturation at 95°C for 10 min, annealing at 95°C for 15 s, and extension at 60°C for 1 min, and a final post-read of 1 min. Applied Biosciences SDS 1.4 PCR analysis software (Applied Biosystems, Foster, CA, USA) was used for genotype analysis.

Statistical methods

SAS9.2 software (SAS, Cary, NC, USA) and the SHEsis online platform (http://analysis.bio-x.cn/myAnalysis.php) were used for statistical analysis. The Hardy-Weinberg model was used to determine the genotype and allele frequencies of the samples. The averages between the two groups were compared using a *t*-test and the comparison of the genotype and allele frequencies at each locus between the two groups was performed with a chi-square test. The SHEsis online platform was used to compare linkage disequilibrium between the loci and haplotype frequencies between the two groups. $P < 0.05$ was regarded as statistically significant.

Results

Comparison of genotype frequency and allele frequency at ADRB2 SNP loci

The genotype frequency and allele frequencies of individuals who have asthma were compared with individuals who do not have the disease using six loci (base positions -2387, -47, 46, 79, 491, and 523) of *ADRB2*. Both the asthma group and the control group genotype frequencies were consistent with Hardy-Weinberg equilibrium ($P > 0.05$). The 491 locus in both groups was the wild-type homozygous TT genotype and no variant homozygous CC or heterozygous TC genotypes were detected. Therefore, the 491 locus was not included in the statistical analysis. The allele frequency of the C allele (encoding Arg19) at the -47 locus in the asthma group was significantly lower than in the control group ($OR = 0.789$, 95% CI: 0.398~0.961, $P < 0.05$; **Table 1**). Also at this locus, the CT genotype frequency was significantly lower in the asthma group compared to the control group ($OR = 0.784$, 95% CI: 0.393~0.915, $P < 0.05$; **Table 1**), while the frequency of the CC genotype was not significantly different between the two groups ($OR = 0.524$, 95% CI: 0.096~2.967, $P > 0.05$; **Table 1**). When both genotypes carrying the C allele were compiled in the CC+CT group

Table 2. Linkage disequilibrium of five polymorphic loci of ADRB2

SNP loci	SNP loci [D' (r^2)]			
	-47	46	79	523
-2387	0.968 (0.259)	0.169 (0.012)	0.968 (0.259)	0.981 (0.182)
-47	-	0.974 (0.148)	0.980 (0.967)	0.994 (0.052)
46	-	-	0.999 (0.155)	0.992 (0.589)
79	-	-	-	0.994 (0.052)

(Arg19/Arg + Arg19/Cys), the difference between the asthma and control groups was statistically significant, with the asthma group having a lower genotype frequency than the control group (*OR* = 0.765, 95% CI: 0.375~0.962, *P* < 0.05; **Table 1**). At the 79 locus the G allele (Glu27) frequency in the asthma group was significantly lower than that of the control group (*OR* = 0.788, 95% CI: 0.394~0.948, *P* < 0.05; **Table 1**). Also at the 79 locus, the frequency of the CG genotype was significantly lower in the asthma group compared to the controls (*OR* = 0.775, 95% CI: 0.381~0.980, *P* < 0.05; **Table 1**), while the frequency of the GG genotype was not significantly different between the two groups (*OR* = 0.521, 95% CI: 0.091~1.911, *P* > 0.05; **Table 1**). The GG+CG group (Glu27/Glu + Gln27/Glu), which includes both genotypes carrying the G allele, had a significantly lower frequency in the asthma group than the control group (*OR* = 0.771, 95% CI: 0.378~0.942, *P* < 0.05; **Table 1**). The genotype frequencies and allele frequencies at the -2387, 46, and 523 loci were not significantly different between the asthma group and the control group (*P* > 0.05), as shown in **Table 1**.

Linkage disequilibrium analysis in five SNP loci of the ADRB2

The SHEsis online platform was used to analyze the linkage disequilibrium of five SNP loci of *ADRB2*. Two parameters, D' and r^2, were used to indicate the degree of linkage disequilibrium between two loci. There was strong linkage disequilibrium between the -47 locus and 79 locus (D' = 0.980, r^2 = 0.967) and between the 46 locus and 523 locus (D' = 0.992, r^2 = 0.589), as shown in **Table 2**.

Comparison of the haplotype frequency distribution at five SNP loci of ADRB2

SHEsis online platform analysis of the 5 SNP loci in both the asthma and control groups found a total of 16 different haplotypes, including 12 haplotypes with frequency < 0.03 (**Table 3**) that were not included in the statistical analysis. The remaining four common haplotypes of the participants in this study were haplotype III (CCGGC), haplotype IV (CTACC), haplotype IX (TTACC), and haplotype XI (TTGCA). The frequency of haplotype III (CCGGC) in the asthma group was significantly lower compared to the control group (*OR* = 0.705, 95% CI: 0.412~0.973, *P* < 0.05; **Table 3**). In contrast, the frequencies of haplotypes IV (CTACC), IX (TTACC), and XI (TTGCA) were not significantly different between the two groups (both *P* > 0.05), as shown in **Table 3**.

Discussion

Genetic studies of asthma have prompted further investigation of the relationship between gene polymorphisms and asthma. Polymorphisms in the *ADRB2* gene have been investigated in asthma, nocturnal asthma, asthma severity, serum IgE levels, bronchial hyperreactivity, and other asthma-related phenotypes [5-7, 9]. Interestingly, results from these studies are inconsistent, revealing a need for further study of the link between *ADRB2* polymorphisms and asthma.

In this study, the relationship between polymorphisms and haplotypes of the *ADRB2* identified several variants and haplotypes associated with asthma. Of the six SNP loci (-2387, -47, 46, 79, 491, 523) investigated in *ADRB2*, two variants were significantly associated with the disease. The C allele at the -47 locus had a protective effect on the occurrence of asthma. Similar results were found for the G allele at the 79 locus. The results of this study are similar to that obtained by two other groups [10, 11]; however, some studies have reported that the polymorphisms at the 46 locus (corroborated by our findings) and 79 locus are not correlated with asthma in Chinese adults [12]. Further, other studies have reported that the polymorphisms at the 46 locus, 79 locus, and 491 locus are not correlated with onset risk of asthma [13]. Finally, numerous studies have shown that *ADRB2* polymorphisms are not related to the occurrence of asthma, yet one study found that a variant at the 46 locus can reduce the onset of asthma [14]. The results presented in this study suggest that these inconsistencies

Table 3. Haplotype analysis of five polymorphic loci of ADRB2

Haplotype	SNP loci					Asthma group [n (%)]	Control group [n (%)]	*P*	*OR* (95% CI)
	-2387	-47	46	79	523				
I	C	C	A	G	C	0.00 (0.00)	0.10 (0.01)	-	-
II	C	C	G	G	A	0.00 (0.00)	0.20 (0.02)	-	-
III	C	C	G	G	C	73.70 (8.59)	115.00 (11.90)	0.031	0.705 (0.412~0.973)
IV	C	T	A	C	C	165.70 (19.3)	184.50 (19.10)	0.948	1.012 (0.695~1.497)
V	C	T	G	C	C	1.70 (0.20)	0.05 (0.01)	-	-
VI	T	C	G	C	C	0.00 (0.00)	0.97 (0.10)	-	-
VII	T	C	G	G	C	0.00 (0.00)	0.97 (0.10)	-	-
VIII	T	T	A	C	A	0.00 (0.00)	1.93 (0.20)	-	-
IX	T	T	A	C	C	341.50 (39.80)	345.40 (35.76)	0.097	1.236 (0.882~1.579)
X	T	T	A	G	C	0.00 (0.00)	0.07 (0.01)	-	-
XI	T	T	G	C	A	260.10 (30.31)	302.40 (31.30)	0.752	0.961 (0.652~1.275)
XII	T	T	G	C	C	11.15 (1.30)	13.56 (1.40)	-	-
XIII	T	T	G	G	A	0.00 (0.00)	0.85 (0.09)	-	-
XIV	C	C	A	C	C	0.85 (0.10)	0.00 (0.00)	-	-
XV	C	T	G	C	A	2.45 (0.29)	0.00 (0.00)	-	-
XVI	C	T	G	G	C	0.85 (0.10)	0.00 (0.00)	-	-

are likely to be because the majority of the studies only focus on a single SNP without taking into account other SNPs. In support of this idea, Drysdale *et al.* [15] showed that the interaction of several SNPs in a haplotype can affect the physiological reaction and response to treatment, and that a single SNP is not sufficient to predict the genetics basis of drug response.

The inconsistencies in the results of these studies may also be linked to the different research and experimental methods used as well as differences in sample size, populations, and ethnic groups. Consistent with this idea, comparison of the distribution of allele frequency at the six loci of the *ADRB2* gene between Chinese Han people and Japanese, African-American, and Caucasian-American populations revealed the allele frequency of Chinese Han people is similar to that of Japanese and African-American populations [16], but significantly different from that of Caucasian-Americans [17]. This indicates that *ADRB2* gene polymorphisms differentially affect asthma in different ethnicities.

Linkage disequilibrium analysis of 5 of the SNP loci in *ARDB2* revealed strong linkage disequilibrium between the -47 locus and the 79 locus and between the 46 locus and the 523 locus. Further, of the four common haplotypes of participants in this study [haplotype III (CCGGC), haplotype IV (CTACC), haplotype IX (TTACC), and haplotype XI (TTG-CA)], haplotype III was less common in the asthma group than in the control group, suggesting this haplotype may reduce susceptibility to asthma, consistent with results obtained by Saadi *et al.* [18]. A study by Panebra *et al.* [5] showed that haplotype II-1, which includes the -2387C, 46G, 79G, and 523C alleles, is correlated with high expression of ADRB2 and is consistent with the higher ADRB2 expression level of the Drysdale haplotype 2 [15]. These findings support the results of this study.

In summary, this analysis of six SNP loci (-2387, -47, 46, 79, 491, and 523) in the regulatory and coding regions of *ADRB2* revealed that variants at the -47 locus and 79 locus and that haplotype III (CCGGC) are correlated with asthma susceptibility in a Chinese Han population. This was a more comprehensive study that investigated the role of the *ADRB2* gene in the pathogenesis of asthma by examining several polymorphisms and haplotypes, rather than just a single polymorphism.

Acknowledgements

This work was supported by Heilongjiang Provincial Project of Applied Technology Research and Development (No. GZ 13C 001).

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Bao-Rong Hu, Department of Pharmacy, The First Affiliated Hospital, Harbin Medical University, Nangang District, Youzheng Street, Harbin 150001, Heilongjiang Province, P. R. China. E-mail: hubrhmu@163.com

References

[1] Masoli M, Fabian D, Holt S and Beasley R. The global burden of asthma: Executive summary of the GINA Dissemination Committee report. Allergy 2004; 59: 469-478.

[2] Wong GW, Ko FW, Hui DS, Fok TF, Carr D, von Mutius E, Zhong NS, Chen YZ and Lai CK. Factors associated with difference in prevalence of asthma in children from three cities in China: multicentre epidemiological survey. BMJ 2004; 329: 486.

[3] Leineweber K and Brodde OE. β2-adrenoceptor polymorphisms: relation between in vitro and in vivo phenotypes. Life Sci 2004; 74: 2803-2814.

[4] Ferdinands JM, Mannino DM, Gwinn ML and Bray MS. ADRB2 Arg16Gly polymorphism, lung function, and mortality: results from the Atherosclerosis Risk in Communities study. PloS One 2007; 2: e289.

[5] Panebra A, Wang WC, Malone MM, Pittler DR, Weiss ST, Hawkins GA and Liggett SB. Common ADRB2 haplotypes derived from 26 polymorphic sites direct β2-adrenergic receptor expression and regulation phenotypes. PLoS One 2010; 5: e11819.

[6] Lee MY, Cheng SN, Chen SJ, Huang HL, Wang CC and Fan HC. Polymorphisms of the β2-adrenergic receptor correlated to nocturnal asthma and the response of terbutaline nebulizer. Pediatr Neonatol 2011; 52: 18-23.

[7] Asano K, Yamada-Yamasawa W, Kudoh H, Matsuzaki T, Nakajima T, Hakuno H, Hiraoka R, Fukunaga K, Oguma T, Sayama K, Yamaguchi K, Nagabukuro A, Harada Y, Ishizaka A. Association between β-adrenoceptor gene polymorphisms and relative response to β2-agonists and anticholinergic drugs in Japanese asthmatic patients. Respirology 2010; 15: 849-854.

[8] Kroegel C. Global Initiative for Asthma (GINA) guidelines: 15 years of application. Expert Rev Clin Immunol 2009; 5: 239-49.

[9] Giubergia V, Zelazko M, Roy A, Gravina LP, Gonzalez Pena H and Chertkoff L. β2-adrenergic polymorphisms and total serum IgE levels in children with asthma from Argentina. Ann Allergy Asthma Immunol 2009; 102: 308-313.

[10] Hizawa N. Beta-2 adrenergic receptor genetic polymorphisms and asthma. J Clin Pharm Ther 2009; 34: 631-643.

[11] Thakkinstian A, McEvoy M, Minelli C, Gibson P, Hancox B, Duffy D, Thompson J, Hall I, Kaufman J, Leung TF, Helms PJ, Hakonarson H, Halpi E, Navon R, Attia J. Systematic review and meta-analysis of the association between β2-adrenoceptor polymorphisms and asthma: a HuGE review. Am J Epidemiol 2005; 162: 201-211.

[12] Li X, Zhang Y, Zhang J, Xiao Y, Huang J, Tian C, He C, Deng Y, Yang Y, Fan H. Asthma susceptible genes in Chinese population: A meta analysis. Respir Res 2010; 11: 129.

[13] Thomsen M, Nordestgaard BG, Sethi AA, Tybjærg-Hansen A, Dahl M. β2-adrenergic receptor polymorphisms, asthma and COPD: two large population based studies. Eur Respir J 2012; 39: 558-566.

[14] Birbian N, Singh J, Jindal SK, Singla N. Association of β2-adrenergic receptor polymorphisms with asthma in a North Indian population. Lung 2012; 190: 497-504.

[15] Drysdale CM, McGraw DW, Stack CB, Stephens JC, Judson RS, Nandabalan K, Arnold K, Ruano G, Liggett SB. Complex promoter and coding region beta 2-adrenergic receptor haplotypes alter receptor expression and predict in vivo responsiveness. Proc Natl Acad Sci U S A 2000; 97: 10483-10488.

[16] Munakata M, Harada Y, Ishida T, Saito J, Nagabukuro A, Matsushita H, Koga N, Ohsaki M, Imagawa K, Shiratsuchi T. Molecular-based haplotype analysis of the beta 2-adrenergic receptor gene (ADRB2) in Japanese asthmatic and non-asthmatic participants. Allergol Int 2006; 55: 191-198.

[17] Hawkins GA, Tantisira K, Meyers DA, Ampleford EJ, Moore WC, Klanderman B. Sequence, haplotype, and association analysis of ADRbeta2 in a multiethnic asthma case-control study. Am J Respir Crit Care Med 2006; 174: 1101-1109.

[18] Saadi AV, Gupta H, Angural A, Dhanya SK, Mony S, Oberoi D, D'Souza SC, Sahoo RC, Hande MH, Gopinath PM, Satyamoorthy K. Single nucleotide polymorphisms of ADRB2 gene and their association with susceptibility for plasmodium falciparum malaria and asthma in an Indian population. Infect Genet Evol 2013; 20: 140-147.