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Beta-catenin promoter polymorphism is associated with asthma risk in Korean subjects ☆☆☆

Sungmin Bae ^a, Haeyong Lee ^{a,1}, Byoung Whui Choi ^b, Hyeong-Kyu Lee ^c, Sang-In Chung ^a, Wonyong Kim ^a, Kijeong Kim ^a, Seong Jun Seo ^d, Dong-Seok Kim ^e, Seok-min Kim ^f, Yoosik Yoon ^{a,*}

^a Department of Microbiology, Chung-Ang University College of Medicine, Seoul 156-756, Republic of Korea

^b Division of Pulmonary and Allergy Disease, Department of Internal Medicine, Chung-Ang University College of Medicine, Seoul, 156-756, Republic of Korea

^c Korea Research Institute of Bioscience and Biotechnology, Ochang, Chungbuk, 363-883, Republic of Korea

^d Department of Dermatology, Chung-Ang University College of Medicine, Seoul 156-756, Republic of Korea

^e Department of Biochemistry, Chung-Ang University College of Medicine, Seoul 156-756, Republic of Korea

^f Department of Mechanical Engineering, Chung-Ang University College of Engineering, Seoul 156-756, Republic of Korea

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ABSTRACT

Objectives: The effects of β-catenin promoter haplotypes on its mRNA expression levels and asthma risks were investigated in Korean subjects.

Design and methods: The genotype analyses were conducted by a Taqman method for 684 Korean subjects, 400 controls and 284 with asthma. Measurement of mRNA expression levels in peripheral blood nucleated cells were conducted on subjects whose buffy coat fractions were available (n = 185). Logistic regression analyses were conducted to test the associations of the β-catenin promoter haplotypes with asthma risks.

Results: Four SNPs, −10,288C>T (rs7630377), −6,426C>G (rs9859392), −4,361G>C (rs9870255), and −765G>A (rs3864004), were identified in the promoter region of the β-catenin gene, and three common haplotypes were constructed from them. Haplotype ht1[CCGG] was associated with decreased β-catenin mRNA expression levels and a lower asthma risk with an odds ratio of 0.53, while ht2[TGCA] was associated with increased mRNA expression levels and a higher asthma risk with an odds ratio of 2.34. Ht3[TCGG] had no significant effects on both.

Conclusions: Our findings show that β-catenin promoter polymorphism affects its mRNA expression levels, and also is significantly associated with the asthma risk of Korean subjects.

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Introduction

β-catenin, encoded by the *CTNNB1* gene, is a component of the cadherin cell-adhesion complex and is also a transcriptional co-activator [1]. It plays important roles in the regulation of differentiation, proliferation, development, and tissue regeneration [2,3]. β-catenin is also well known as a transcriptional co-activator located in the WNT/

β-catenin pathway. The activation of the WNT/β-catenin pathway induces the nuclear translocation of β-catenin followed by the transcription of its target genes including cyclin D1, PPAR δ and so on [4]. Recently, some studies have reported controversial roles of β-catenin in the immune responses induced by bacteria and lipopolysaccharide. Duan et al. reported that β-catenin negatively regulates the inflammatory response induced by *Salmonella typhimurium*, while Kim et al. reported that β-catenin positively regulates the NADPH oxidase expression and reactive oxygen species generation in macrophage cells stimulated by lipopolysaccharide through the toll-like receptor 4 pathway [5,6]. In our previous study, we compared the gene expression patterns of the peripheral blood nucleated cells from asthma patients and normal subjects using microarrays, and found that the expression levels of some genes in the WNT/β-catenin pathway were different between asthmatic and normal subjects. We also found that β-catenin negatively regulates cytokine production in lipopolysaccharide-stimulated macrophages [7]. Genetic association study of human subjects will be a very helpful approach for the elucidation of the roles of β-catenin in the immune response. Until now, however, few association studies of β-catenin have been conducted in relation to immune disorders of human subjects. In this study, the associations of the genetic

Abbreviations: CI, confidence interval; OR, odds ratio; SNP, single-nucleotide polymorphism.

☆ The protocol of this study was approved by the Institutional Review Board of Chung-Ang University Hospital and written informed consent was obtained from all subjects.

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* Corresponding author at: Department of Microbiology, Chung-Ang University College of Medicine, 221 Heukseok-dong, Dongjak-gu, Seoul, 156-756, Republic of Korea. Fax: +82 2 823 5423.

E-mail address: thanks@cau.ac.kr (Y. Yoon).

¹ Current address: Forensic DNA center, National Forensic Service, 331-1 Shinwol 7-Dong, Yangcheon-Gu, Seoul, 158-707, Republic of Korea.

polymorphisms of β -catenin with asthma risks were investigated in Korean subjects.

Subjects and methods

Subjects

The human subjects for this study were recruited from the Division of Pulmonary and Allergy Disease, Department of Internal Medicine, Chung-Anug University Hospital (Seoul, Korea). All subjects participating in this study were ethnically Korean. All patients with asthma currently had one or more asthma symptoms, based on the definition of asthma by the American Thoracic Society, upon physical examination [8]. Normal subjects were recruited from the general population after answering negatively to a screening questionnaire for respiratory symptoms. The protocol of this study was reviewed and approved by the Institutional Review Board, in accordance with the Declaration of Helsinki, and written informed consent was obtained from all subjects. A total of 684 subjects, 400 controls and 284 with asthma, were included. The general characteristics of the human subjects are summarized in Table 1.

Genotype analysis and haplotype construction

The genomic DNAs of each subject were extracted from whole blood using a QIAamp® DNA Blood Mini Kit (Qiagen, Hilden, Germany). The Haploview program (available at <http://www.broadinstitute.org/scientific-community/software>) was used to identify SNPs in the promoter region of the β -catenin gene among the Asian population (CHB + JPT, denoting Chinese and Japanese) from the HapMap data release 22 [9]. The genotype analyses of the identified SNPs were conducted by a Taqman method, as described previously [10], and PCR primer/probe sets for the genotype analyses were obtained from Applied Biosystems (Foster City, CA, USA) (Supplementary Data 1). The calculation of the linkage disequilibrium (LD) coefficient, $|D'|$, and r^2 , and the construction of haplotypes for each subject were conducted using the SNPAnalyzer 2.0 program (available at <http://snp.istech21.com>) [11].

Measurement of mRNA expression levels in peripheral blood cells

Measurement of mRNA levels in peripheral blood cells were conducted on a subset of 185 subjects whose buffy coat fractions were available. Even though we had frozen blood samples of all subjects, freshly collected unfrozen blood samples were needed to get buffy coat fractions. Some subjects ($n = 185$) agreed to collect fresh 10 mL samples of their peripheral blood, and their buffy coat fractions were available for mRNA expression analyses. The age and sex distributions of this subset of subjects were not significantly different from

those of whole study subjects ($P > 0.05$). Ten milliliters of their peripheral blood were centrifuged at 4000 rpm for 20 min to collect 0.4 mL of each buffy coat fraction, which were mainly composed of peripheral blood nucleated cells. The buffy coat fractions were mixed with 1.3 mL RNeasy lysis solution (Ambion, Austin, TX, USA) and stored at -70°C . Total RNA was extracted using a RiboPure-Blood Kit (Ambion). One microgram of RNA was converted into its complementary DNA (cDNA) using a cDNA Reverse Transcription Kit (Applied Biosystems). The Assay-on-Demand Gene Expression products (Applied Biosystems) were used to evaluate the expression levels of β -catenin (Hs00170025_m1) and 18 S rRNA (Hs99999901_s1). 18 S rRNA was used as an endogenous control. For each sample, the levels of β -catenin mRNA were normalized to those of 18 S rRNA, and are presented according to the comparative Ct method [12].

Statistical analyses

All data were expressed as the mean $\pm$ standard error (SE). Logistic regression analyses were conducted to test the associations of the β -catenin promoter haplotypes with asthma risk using the covariates of gender, age, and haplotype. β -catenin mRNA expression levels of the subjects were compared by ANCOVA, adjusted for age and gender. A p -value of <0.05 was considered statistically significant. All analyses were conducted using SPSS version 19.0 (SPSS, Chicago, IL, USA).

Results

Analysis of the promoter polymorphisms of β -catenin

In this study, genetic polymorphisms in the promoter region of β -catenin gene were searched to find genetic factors which can directly affect expression levels of β -catenin in humans. Presently, the 41,205–41,216 kb region of chromosome 3, which includes a greater than 10 kb region upstream of the human β -catenin gene, was searched to identify four SNPs. These identified SNPs included $-10,288\text{C}>\text{T}$ (rs7630377), $-6,426\text{C}>\text{G}$ (rs9859392), $-4,361\text{G}>\text{C}$ (rs9870255), and $-765\text{G}>\text{A}$ (rs3864004), as shown in Fig. 1A and Table 2. After genotyping the 684 Korean subjects, their minor allele frequencies in the Korean population were found to be 0.220–0.291, which were similar to those of Chinese and Japanese populations, according to the HapMap data (0.221–0.320). Their genotype distributions were in Hardy–Weinberg equilibrium ($P > 0.05$) (Table 2). Next, these SNPs were analyzed for linkage disequilibrium (LD) and haplotype construction [13]. The LD coefficients between the four SNPs are shown in Fig. 1B. Three of these, $-6,426\text{C}>\text{G}$, $-4,361\text{G}>\text{C}$, and $-765\text{G}>\text{A}$, were almost completely linked with $|D'| = 1$ and $r^2 \geq 0.996$, while $-10,288\text{C}>\text{T}$ was not linked with the other three SNPs, having an $r^2 < 0.8$. Promoter haplotypes were constructed as shown in Fig. 1C. Two common promoter haplotypes, ht1 and ht2, were found with frequencies of 0.708 and 0.219, respectively. A rare promoter haplotype, ht3, with an allele frequency of 0.071, was also found. It was found that ht1 was composed of the major alleles of the four SNPs, C of $-10,288\text{C}>\text{T}$, C of $-6,426\text{C}>\text{G}$, G of $-4,361\text{G}>\text{C}$, and G of $-765\text{G}>\text{A}$, while ht2 was composed of minor alleles of each of the SNPs. Haplotype ht3 is composed of the minor T allele of $-10,288\text{C}>\text{T}$ and the major alleles of the other three SNPs and may be the result of the recombination of ht1 and ht2 between the loci of $-10,288\text{C}>\text{T}$ and $-6,426\text{C}>\text{G}$ (Fig. 1C). Ht4 and ht5, with extremely low population frequencies of 0.0008 and 0.0007, respectively, were not further analyzed, because their effects on the population are negligible.

Effects of β -catenin promoter haplotypes on its mRNA expression levels

β -catenin mRNA levels in the peripheral blood cells were compared among a subset of subjects, whose total RNAs were extracted

Table 1
General characteristics of the study subjects.

Variables	Control (n = 400)	Asthma (n = 284)	p-value
Sex (male:female)	218:182	136:148	0.104 ^b
Age	51.38 $\pm$ 13.42 ^a	52.99 $\pm$ 15.46	0.196 ^c
BMI (kg/m ²)	23.79 $\pm$ 2.78	23.88 $\pm$ 3.19	0.617 ^d
FEV1 (L)	3.12 $\pm$ 0.73	2.36 $\pm$ 0.81	0.000 ^d
FEV1 (% of predicted value)	98.44 $\pm$ 11.92	88.84 $\pm$ 21.15	0.000 ^d
FVC (L)	3.92 $\pm$ 0.90	3.41 $\pm$ 0.97	0.138 ^d
FEV1/FVC (%)	79.82 $\pm$ 7.23	67.15 $\pm$ 14.17	0.000 ^d
Total IgE (U/mL)	112.45 $\pm$ 108.85	394.86 $\pm$ 447.78	0.008 ^d

P-values less than 0.05 were denoted in boldface font.

^a Mean $\pm$ SE.

^b p-value was obtained by Chi-square test.

^c p-value was obtained by ANCOVA adjusted for sex.

^d p-value was obtained by ANCOVA adjusted for sex and age.

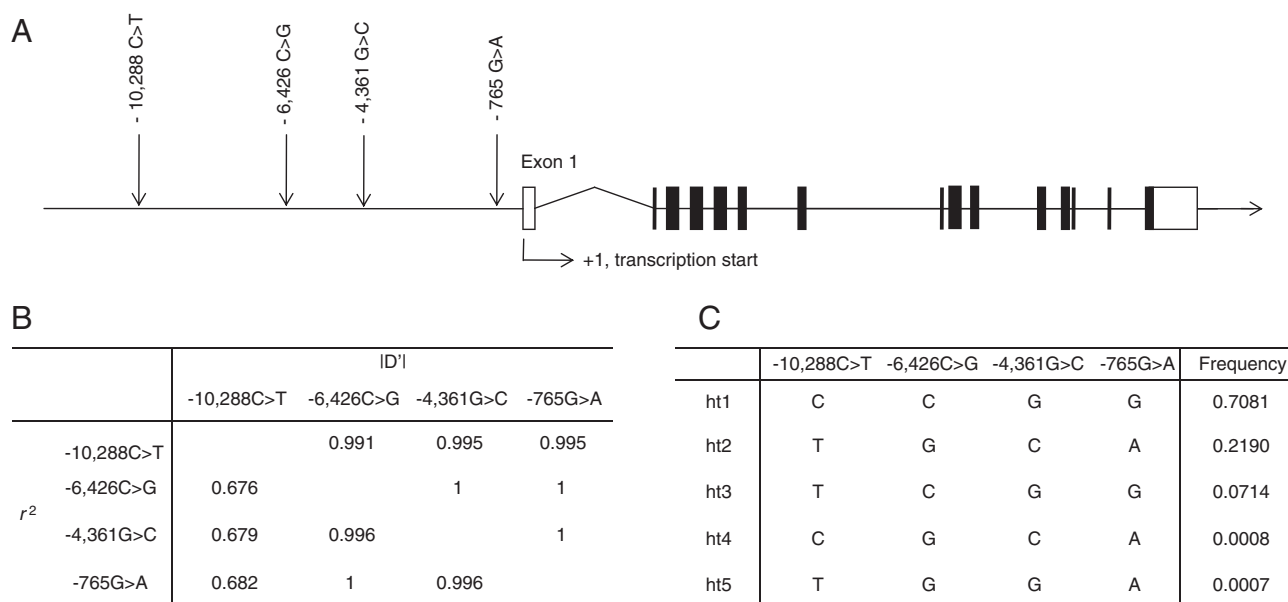


Fig. 1. SNPs and haplotypes of the promoter region of the β -catenin gene in Korean subjects. (A) Gene map and the location of SNPs in the promoter region of the β -catenin gene on chromosome 3p21. Coding exons are marked by black blocks, and the 5' and 3' UTRs by white blocks. The first base of the transcription site was denoted as nucleotide +1. (B) LD coefficients among four SNPs in 684 Korean subjects. (C) Promoter haplotypes of β -catenin and their frequencies in 684 Korean subjects.

from peripheral blood, to examine the effects of promoter haplotypes on mRNA expression levels. Ht1 carriers, heterozygotes ($-/ht1$) or homozygotes ($ht1/ht1$), had significantly decreased β -catenin mRNA levels compared with non-carriers ($-/-$), as shown in Fig. 2A. On the other hand, homozygotes for ht2 ($ht2/ht2$) had significantly higher mRNA expression levels compared with non-carriers ($-/-$) and heterozygote carriers ($-/ht2$), as shown in Fig. 2B. No significant effects on mRNA expression levels were found for ht3. The homozygote carriers, $ht3/ht3$, were not found among the subset of subjects from whom total RNA was obtained, because of the low frequency of the ht3 haplotype. Ht3 heterozygotes ($-/ht3$) had similar mRNA expression levels with non-carriers ($-/-$), as shown in Fig. 2C.

Associations of β -catenin promoter haplotypes with asthma risks

Three promoter haplotypes of β -catenin, ht1, ht2, and ht3, were then analyzed for their associations with asthma risk by logistic regression analyses using the covariates of gender, age, and haplotype. The results are summarized in Table 3, and detailed results of the logistic regression analyses are provided in Supplementary Data 2. For the ht2 haplotype, homozygote carriers ($ht2/ht2$) were more frequently found in asthma patients (6.0%) when compared with the control subjects (2.8%), suggesting that it is associated with increased asthma risk. Logistic regression analyses showed that homozygote carriers of ht2 ($ht2/ht2$) showed a significantly increased asthma risk with an odds ratio (OR) of 2.340 at a 95% confidence interval (CI) of 1.067–5.132 ($P=0.034$) compared with non carriers ($-/-$), while heterozygote carriers of ht2 ($-/ht2$) showed no significantly changed asthma risks compared with non-carriers ($-/-$). For the ht1 haplotype, on the other hand, non-carriers ($-/-$) were more frequently found in asthma patients (9.9%) when compared with control subjects (5.8%), suggesting that the ht1 haplotype is associated with decreased asthma risk. Logistic regression analyses showed that heterozygote carriers ($-/ht1$) showed an OR of 0.532 at a 95% CI of 0.292–0.970 ($P=0.039$), and homozygote carriers ($ht1/ht1$) showed an OR of 0.591 at a 95% CI of 0.327–1.070 ($P=0.083$). The lack of significance in homozygotes ($ht1/ht1$) may be due to the insufficient number of subjects enrolled in this study. All ht1 carriers, which include both heterozygotes ($-/ht1$) and homozygotes ($ht1/ht1$), had

decreased asthma risks compared with non-carriers, with an OR of 0.563 ($P=0.050$) (data not shown). Ht3 had no association with asthma risks.

Discussion

There have been some reports concerning the involvement of the β -catenin in immune responses induced by bacteria and lipopolysaccharide [5,6], suggesting the possibility that its genetic polymorphisms are associated with human immune disorders. Until now, however, no genetic association study of β -catenin in relation to the asthma of human subjects has been reported, and this is the first such report.

Most genetic polymorphism studies of β -catenin have been conducted in relation to cancer. Mutations of β -catenin have been commonly observed in some types of cancer including endometrioid ovarian cancer, hepatoblastoma, Wilms' kidney tumors, and some colorectal cancers [14]. These mutations usually affect serine/threonine and adjacent amino acids in the N-terminal region of the β -catenin protein and prevent its phosphorylation-mediated degradation, leading to uncontrolled β -catenin accumulation, which results in tumorigenesis [15]. In this study, however, these coding region mutations accompanying amino acid changes were not included, because of their strong tumorigenic effects and their very low potential for involvement in chronic diseases such as asthma. Instead, we focused on the promoter polymorphisms of β -catenin, which affect its mRNA expression levels mildly but significantly (Figs. 1 and 2).

Table 2
Characteristics of SNPs identified in the promoter region of the β -catenin gene.

SNP	Position	Locus from transcription start site	Nucleotide change	Minor allele frequency ^a	HWE p-value ^a
rs7630377	Promoter	-10,288	C>T	0.291	0.334
rs9859392	Promoter	-6,426	C>G	0.221	0.449
rs9870255	Promoter	-4,361	G>C	0.220	0.478
rs3864004	Promoter	-765	G>A	0.221	0.449

^a Obtained from the genotyping of the 684 Korean subjects recruited in this study.

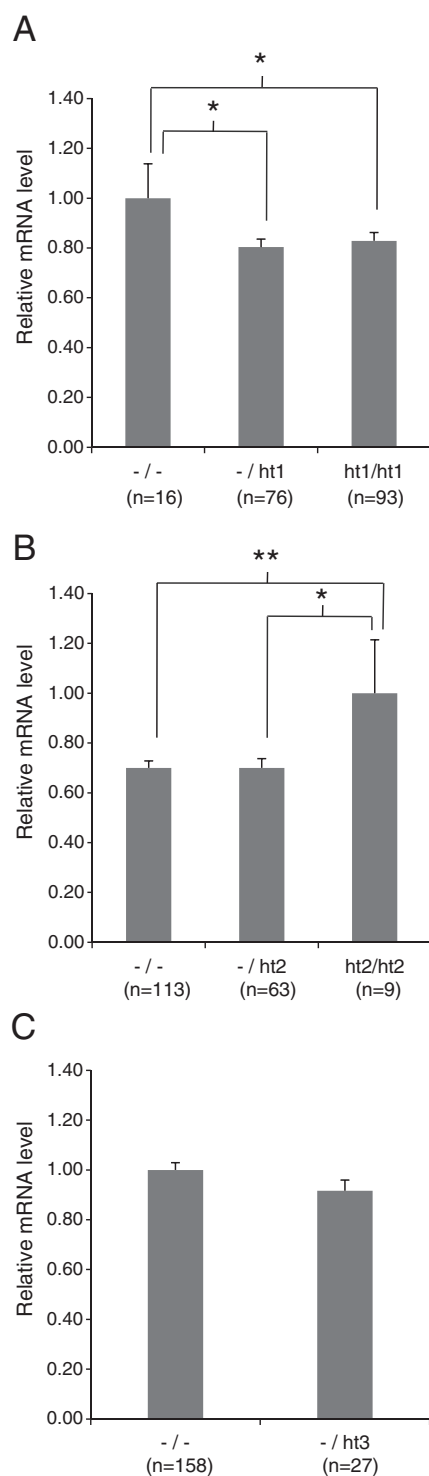


Fig. 2. Effects of the promoter haplotypes of β -catenin on the mRNA expression levels in the peripheral blood nucleated cells of human subjects. β -catenin mRNA levels in the peripheral blood nucleated cells obtained from a subset of 185 subjects were compared according to their promoter haplotypes using ANCOVA adjusted for age and gender (n: number of subjects).

By comparing Fig. 2 and Table 3, it was found that the effects of promoter haplotypes on mRNA expression levels were identical to their effects on asthma risks: Ht1 decreased both β -catenin mRNA expression and asthma risk, and ht2 increased both β -catenin mRNA expression and asthma risk, while ht3 had no effects on

Table 3

Asthma risks of Korean subjects according to β -catenin promoter haplotypes.

Haplotype	Asthma (N = 284)	Control (N = 400)	P-value	OR (95% CI)
ht1[CCGG] -/-	28 (9.9%)	23 (5.8%)	0.039	0.532 (0.292–0.970)
-/ht1	115 (40.5%)	179 (44.8%)		
ht1/ht1	141 (49.6%)	198 (49.5%)	0.083	0.591 (0.327–1.070)
ht2[TGCA] -/-	166 (58.5%)	250 (62.5%)	0.592	1.093 (0.790–1.510)
-/ht2	101 (35.6%)	139 (34.8%)		
ht2/ht2	17 (6.0%)	11 (2.8%)	0.034	2.340 (1.067–5.132)
ht3[TCGG] -/-	250 (88.0%)	339 (84.8%)	0.214	0.748 (0.473–1.183)
-/ht3	33 (11.6%)	59 (14.8%)		
ht3/ht3	1 (0.4%)	2 (0.5%)	0.766	0.693 (0.062–7.790)

Asthma risks were analyzed by logistic regression analyses using the covariates of age, gender and haplotypes.

Significant associations were denoted in boldface font.

either. These data show a relation between β -catenin expression and asthma risk, and suggest a possible functional role of β -catenin in asthma development.

When haplotypes of β -catenin were analyzed for possible association with the response to anti-asthma treatment, no significant associations were found ($P > 0.05$), suggesting that the promoter polymorphisms of β -catenin may be not associated with the response to anti-asthmatic treatment, even though more study is needed.

Conclusion

The results of this study showed that β -catenin promoter haplotypes, which affect its expression levels, were significantly associated with asthma risk in Korean subjects. Even though the association should be replicated by other population studies, this study is the first report on the genetic association of the β -catenin with asthma of human subjects, and provides important clues on the roles of β -catenin in the development of asthma and other immune disorders.

Conflict of interest

The authors declare that there are no conflicts of interest in this article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.clinbiochem.2012.05.002>.

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SUPPLEMENTARY DATA 1. Primer/probe sets used for genotyping of SNPs

SNP	Product No.
rs7630377	ABI (C 267808_10)
rs9859392	ABI (C 1408775_10)
rs9870255	ABI (C 258825_10)
rs3864004	ABI (C 11900129_1)

SUPPLEMENTARY DATA 2. Logistic regression analysis of haplotypes for asthma risk.

Logistic regression analysis of ht1 haplotype for asthma risk.

Covariate	Wald	Degree of freedom	p-value	Odds Ratio	95% Confidence Interval
Gender (female)	2.090	1	0.148	1.254	0.923 – 1.703
Age (yr)	1.434	1	0.231	1.006	0.996 – 1.017
ht1 haplotype	4.251	2	0.119	-	-
-/ht1 vs -/-	4.248	1	0.039	0.532	0.292 – 0.970
ht1/ht1 vs -/-	3.010	1	0.083	0.591	0.327 – 1.070

- / - : non-carrier of ht1

- / ht1: heterozygote carrier of ht1

ht1/ht1: homozygote carrier of ht1

Logistic regression analysis of ht2 haplotype for asthma risk.

Covariate	Wald	Degree of freedom	p-value	Odds Ratio	95% Confidence Interval
Gender (female)	2.056	1	0.152	1.251	0.921 – 1.700
Age (yr)	1.596	1	0.206	1.007	0.996 – 1.017
ht2 haplotype	4.544	2	0.103	-	-
-/ht2 vs -/-	0.288	1	0.592	1.093	0.790 – 1.510
ht2/ht2 vs -/-	4.497	1	0.034	2.340	1.067 – 5.132

- / - : non-carrier of ht2

- / ht2: heterozygote carrier of ht2

ht2/ht2: homozygote carrier of ht2

Logistic regression analysis of ht3 haplotype for asthma risk.

Covariate	Wald	Degree of freedom	p-value	Odds Ratio	95% Confidence Interval
Gender (female)	2.129	1	0.144	1.256	0.925 – 1.707
Age (yr)	1.600	1	0.206	1.007	0.996 – 1.017
ht3 haplotype	1.613	2	0.446	-	-
-/ht3 vs -/-	1.543	1	0.214	0.748	0.473 – 1.183
ht3/ht3 vs -/-	0.089	1	0.766	0.693	0.062 – 7.790

- / - : non-carrier of ht3

- / ht3: heterozygote carrier of ht3

ht3/ht3: homozygote carrier of ht3