

# Polymorphisms of the IL-18 promoter and bronchial asthma

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**Abstract.** To study the association of polymorphisms of the IL-18 promoter (-607C/A and -137G/C) with bronchial asthma, 120 subjects with bronchial asthma were selected as the experimental group (49 cases with bronchial asthma and allergic rhinitis as experimental group 1 and 71 cases with bronchial asthma without allergic rhinitis as experimental group 2) and 120 healthy individuals were selected as the control group. A polymerase chain reaction sequence-specific primer (PCR-SSP) was used to identify the genotypic polymorphisms between the experimental and control groups. The results revealed that the frequencies of the CC, CA and AA genotypes at -607C/A in experimental group 1 were 18.37, 40.82 and 40.82%, respectively; these frequencies were 32.39, 54.92 and 12.68%, respectively, in experimental group 2. There was a statistically significant difference in the genotype distribution between experimental groups 1 and 2 ( $\chi^2=12.81$ ;  $P<0.05$ ). The allele frequencies of genotypes C and A were 38.78 and 61.22%, respectively, in experimental group 1 and 59.86 and 40.14%, respectively, in experimental group 2. The allele frequencies at -607C/A were significantly different between experimental groups 1 and 2 ( $\chi^2=10.32$ ;  $P<0.05$ ). Frequencies of genotypes GG, GC and CC at -137G/C in experimental group 1 were 48.98, 46.94 and 4.08%, respectively, and 80.28, 14.08 and 4.08% in experimental group 2 with significant difference ( $\chi^2=15.73$ ,  $P<0.05$ ). Allele frequencies of G and C in experimental group 1 were 72.45 and 27.55%, and 87.32 and 12.68% in experimental group 2 with statistically significant difference ( $\chi^2=8.42$ ,  $P<0.05$ ). In conclusion, polymorphisms of the IL-18 gene promoter at -607C/A and -137G/C were associated with allergic rhinitis in bronchial asthma, indicating that polymorphisms of the IL-18 gene promoter at -607C/A and -137G/C differentially affect the pathogenesis of asthma and allergic rhinitis.

## Introduction

Bronchial asthma (referred to as asthma) is a common chronic inflammatory disease of the airways characterized by variable and recurring bronchospasm that causes periodic attacks of coughing, wheezing, shortness of breath and chest tightness (1). A small number of patients also present with chest pain as a main symptom. These symptoms may be initiated when patients are exposed to irritant gases or allergens and they are often exacerbated at night and/or in the morning (2). The principal pathological features of asthma include reversible bronchoconstriction, increased airway reactivity and increased inflammatory response (3). Studies have shown that the imbalance of different types of helper T cells (Th), particularly the imbalance of Th1/Th2 cells, may be the main cause of asthma. Specifically, increased numbers of Th2 cells and the secretion of inflammatory factors may be key factors in airway inflammation (4,5). Interleukin-18 (IL-18) regulates the immune response of Th1/Th2 cells, and polymorphisms of the IL-18 gene promoter are associated with diseases such as rheumatoid arthritis (6). This study investigated the association of asthma with polymorphisms of the IL-18 promoter at sites -607C/A and -137G/C by measuring these polymorphisms in asthma patients and in healthy individuals.

## Materials and methods

**Case records.** The experimental group samples were collected from asthma patients (n=120) diagnosed in The People's Hospital of Lishui (Zhejiang, China) from March 2007 to June 2011. The subjects comprised 59 men and 61 women and their median age was 25.58 years ( $\pm 7.78$ , range 15-37 years). Among the asthma patients, 49 were cases of allergic rhinitis in asthma (experimental group 1) and 71 were without allergic rhinitis (experimental group 2). Healthy individuals (n=120) who had received a health examination in the Physical Examination Center in the hospital were selected as a control group during the same time period. These individuals comprised 61 men and 59 women of median age, 36.89 years ( $\pm 7.67$ , range 15-37 years). No statistically significant differences in age or gender existed between the experimental and control groups. The Ethics Committee of Lishui University approved the study.

**Genotype identification of IL-18 promoter at -607C/A and -137G/C.** A polymerase chain reaction sequence-specific primer (PCR-SSP) (7) was used to identify the genotypes of the IL-18 promoter at -607C/A and -137G/C. Primer 5 primer

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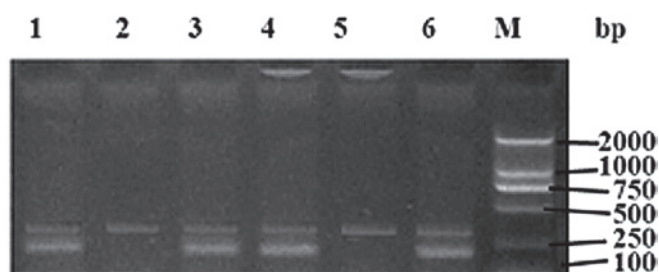


Figure 1. Electrophoresis of the IL-18 promoter region -607C/A site polymorphism. Lanes 1 and 2, CC genotype; lanes 3 and 4, CA genotype; lanes 5 and 6, AA genotype; M, DL2000 DNA marker.

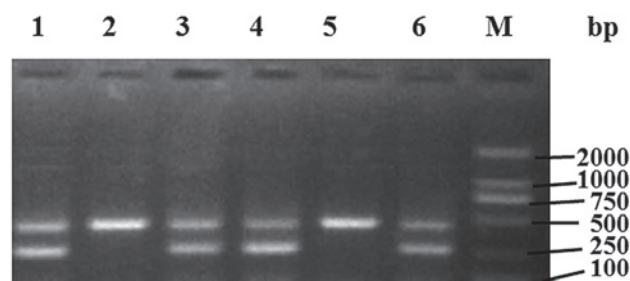


Figure 2. Electrophoresis of the IL-18 promoter region -137G/C site polymorphism. Lanes 1 and 2, GG genotype; lanes 3 and 4, GC genotype; lanes 5 and 6, CC genotype; M, DL2000 DNA marker.

design software was used to design specific F1, F2, common reverse and control forward primers of the promoter at -607C/A and -137G/C, respectively. Samples of venous blood (5 ml) were obtained from all patients for extraction of genomic DNA by the phenolic-chloroform-isoamyl alcohol method (8). Polymerase chain reaction (PCR) was adapted to amplify the specific segment. The primer sequences for the promoter at the -607C/A site were: specific forward F1: 5'-GTTGCA GAAAGTGTA AAAATTATTAC-3'; specific forward F2: 5'-GTTGCAGAAAGTGTA AAAATTATTAA-3'; common reverse: 5'-CTTTGCTATCATTCCAGGAA-3'; and internal control forward: 5'-TAACCTCATTCCAGGACTTCC-3'. The primer sequences for the promoter at the -137G/C site were: specific F1 forward: 5'-CCCCAACTTTTACGGAAG AAAAG-3'; specific F2 forward: 5'-CCCCAACTTTTACGG AAGAAAAC-3'; common reverse: 5'-CCAATAGGACTG ATTATTCGCA-3'; and internal control forward: 5'-AGG AGGGCAAATGCACTGG-3'. The primers were synthesized by Takara Biotech Co., Ltd. (Dalian, China). The specific forward primers F1 and F2 were used for all PCR reactions of each sample. The PCR reaction system included: 21  $\mu$ l genotype DNA; 2.5  $\mu$ l 10X PCR buffer (Promega Corp., Madison, WI, USA); 1.5  $\mu$ l dNTP (25 mmol/l, Promega Corp.); 0.25  $\mu$ l Taq DNA polymerase (2.5 U/ml, Promega Corp.); 1.5  $\mu$ l  $MgCl_2$  (25 mmol/l, Promega Corp.); 0.5  $\mu$ l specific forward primer F1 or F2; 0.5  $\mu$ l common reverse primer; 0.5  $\mu$ l internal control forward primer; and water to a volume of 25  $\mu$ l. PCR reaction conditions at -607C/A were as follows: 7 cycles of denaturation for 30 sec at 94°C; annealing for 40 sec at 64°C; extension for 45 sec at 72°C; then 30 cycles of denaturation for 60 sec at 94°C; annealing for 60 sec at 57°C; extension for 60 sec at 72°C; and a final extension for 5 min at 72°C. PCR reaction conditions at -137G/C were as follows: 5 cycles of denaturation for 5 min; denaturation for 30 sec at 94°C; annealing for 40 sec at 68°C; and extension for 60 sec at 72°C; then 30 cycles of denaturation for 60 sec at 94°C; annealing for 60 sec at 62°C; and extension for 60 sec at 72°C; and a final extension for 5 min at 72°C.

Genotypes were determined from the bands obtained by agarose gel electrophoresis. The genotype CC at -607C/A gave a primer F1 product with two bands (301 and 196 bp) and a primer F2 product with one band (301 bp). The genotype CG yielded primer F1 and F2 products with two bands (301 and 196 bp). The genotype GG yielded a primer F1

product of one band (301 bp) and a primer F2 product of two bands (301 and 196 bp) (Fig. 1). For the genotype CC at -137G/C, the primer F1 product produced two bands (261 and 446 bp) and the primer F2 product yielded one band (446 bp). For genotype GC, the products of primers F1 and F2 each produced two bands (261 and 446 bp). For genotype CC, the primer F1 product produced one band (446 bp) and the primer F2 product yielded two bands (261 and 446 bp) (Fig. 2).

**Statistical analysis.** SPSS 13.0 for Windows (IBM, Armonk, NY, USA) statistical software was used for statistical analysis. The  $\chi^2$  test was adopted to analyze the data and  $P < 0.05$  was considered to indicate a statistically significant result.

## Results

**Polymorphism analysis of the IL-18 gene promoter at -607C/A.** The genotype distributions and allele frequencies of the IL-18 gene promoter at -607C/A between the experimental and control groups conformed to the Hardy-Weinberg equilibrium. No statistically significant differences in the genotype distributions and allele frequencies between the experimental and control groups were identified ( $P > 0.05$ ). The frequencies of genotypes CC, CA and AA in experimental group 1 were 18.37, 40.82 and 40.82%, respectively, and in experimental group 2 were 32.39, 54.92 and 12.68%, respectively. Therefore, there was a statistically significant difference in the genotype distribution at -607C/A between experimental groups 1 and 2 ( $\chi^2 = 12.81$ ;  $P < 0.05$ ; Table I). The allele frequencies of C and A in experimental group 1 were 38.78 and 61.22%, respectively, and in experimental group 2 were 59.86 and 40.14%, respectively. There was a significant difference in the allele frequency at -607C/A between experimental groups 1 and 2 ( $\chi^2 = 10.32$ ;  $P < 0.05$ ).

**Polymorphism analysis of the IL-18 gene promoter at -137G/C.** The genotype distributions and allele frequencies of the IL-18 gene promoter at -137G/C between the experimental and control groups conformed to the Hardy-Weinberg equilibrium. No statistically significant differences were identified in the genotype distributions and allele frequencies between the experimental and control groups ( $\chi^2 = 15.73$ ;  $P > 0.05$ ; Table II). The frequencies of genotypes GG, GC and CC in experimental group 1 were 48.98, 46.94 and 4.08%,

Table I. Genotype and allele frequencies of IL-18 gene -607C/A site polymorphism.

Groups	n	Genotype frequency			Allele frequency	
		CC	CA	AA	C	A
Control group	120	30	49	41	109	131
Experimental group	120	32	59	29	123	117
Experimental group 1	49	9	20	20	38	60
Experimental group 2	71	23	39	9 <sup>a</sup>	85	57 <sup>a</sup>

<sup>a</sup>P<0.05 vs. experimental group 1.

Table II. Genotype and allele frequencies of IL-18 gene -137G/C site polymorphism.

Groups	n	Genotype frequency			Allele frequency	
		GG	GC	CC	G	C
Control group	120	83	32	5	198	42
Experimental group	120	81	33	6	195	45
Experimental group 1	49	24	23	2	71	27
Experimental group 2	71	57	10	4 <sup>a</sup>	124	18 <sup>a</sup>

<sup>a</sup>P<0.05 vs. experimental group 1.

respectively, and in experimental group 2 were 80.28, 14.08 and 4.08%, respectively. Therefore, there was a significant difference in the genotype distribution at -137G/C between experimental groups 1 and 2 ( $\chi^2=15.73$ ;  $P<0.05$ ). The allele frequencies of G and C in experimental group 1 were 72.45 and 27.55%, respectively, and in experimental group 2 were 87.32 and 12.68%, respectively. Therefore, there was a statistically significant difference in the allele frequency at -137G/C between experimental groups 1 and 2 ( $\chi^2=8.42$ ;  $P<0.05$ ).

## Discussion

Studies have shown that an imbalance of Th1/Th2 cell function may cause asthma (9). Apter and Szefer (10) indicated that Th1 and Th2 cells had similar *in vivo* effects; for example, both were able to induce bronchial asthma, and cytokines from Th1 and Th2 cells may cooperate to aggravate bronchial asthma symptoms. This contrasts with the earlier theory that Th1 and Th2 cytokines have contrasting effects on airway hyper-responsiveness (10). IL-18 is a member of the IL-1 family and is synthesized and secreted by a variety of immune effector cells. As a Th1-type cytokine, IL-18 is able to induce the occurrence of Th1-type cytokines, including IFN- $\gamma$  and TNF- $\alpha$ , and contribute to the damage of tissues. However, IL-18 is also able to stimulate the expression of Th2 cytokines (11). IL-18 improves IgE and IgG1 expression in bronchioloalveolar eosinophils and increases cytokine release through the promotion of eosinophil activation. Therefore, IL-18 may trigger allergic inflammation by causing the accumulation of eosinophils and stimulating

IgE (12). The plasma IL-18 levels of children with asthma are significantly higher than those of healthy children (13,14), thus IL-18 may be associated with the occurrence of bronchial asthma.

The current study analyzed the genotypes of the IL-18 gene promoter at -607C/A and -137G/C in patients with bronchial asthma and healthy individuals. The results indicated that there were no statistically significant differences in the genotype distributions and allele frequencies at -607C/A and -137G/C between subjects with bronchial asthma and healthy subjects. However, there were significance differences in genotype distributions and allele frequencies at -607C/A and -137G/C in patients with bronchial asthma and allergic rhinitis ( $P<0.05$ ).

IL-18 is located at chromosome 11q22.2-22.3, which has two promoters upstream of exons 1 and 2. The analysis of the IL-18 sequence revealed that the -137G/C site, located at the GATA3 binding site, is able to induce the expression of Th2 cytokines, while inactivation of GATA3 may reduce the airway inflammation in asthmatic mice. Furthermore, the presence of allele G is required for increased IL-18 expression following cell stimulation by PMA, but substitution with allele C attenuates this effect (15).

Pawlik *et al* (6) also found an association between polymorphisms of the IL-18 promoter at site -607C/A and severe bronchial asthma. The results of our study revealed that polymorphisms of the IL-18 gene promoter at -137G/C and -607C/A are not correlated with bronchial asthma without allergic rhinitis, but correlate with bronchial asthma with allergic rhinitis. As such, our results are not entirely consistent with the previous studies possibly to ethnic/geographic

differences in the study populations. Further studies are required in diverse ethnic regions to elucidate the correlation of IL-18 promoter polymorphisms with asthma.

In brief, our results have shown that polymorphisms of the IL-18 gene promoter at -137G/C and -607C/A do not correlate with bronchial asthma, but correlate with allergic rhinitis in bronchial asthma. Polymorphisms of the IL-18 promoter at -137G/C and -607C/A may have unique effects on the pathogenesis of asthma and allergic rhinitis.

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