Association of Interleukin-18 Gene Polymorphism With Asthma in Chinese Patients

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Like other allergic diseases, asthma results from multiple conditions. Asthmatic beginning and severity are mediated by both environmental and genetic factors. In asthma studies, important work is realization of the genetic background and identification of genetic factors resulting in asthma development and phenomena. Here, we investigated whether interleukin (IL)-18 single nucleotide polymorphisms (SNPs) are involved in Chinese asthma patients. IL-18 (IL-18) SNP was detected by polymerase chain reaction (PCR)-based restriction analysis in 201 patients with asthma and 60 normal controls. Significant differences

were found in the genotype distribution of IL-18 SNP between asthma patients and controls ($P=0.000003$). Allelic frequency of the IL-18 gene distinguished asthma patients from controls ($P=0.000066$). The results revealed a significant difference between asthma patients and normal controls in IL-18 SNP and a statistical correlation between IL-18 polymorphisms (105A/C) and asthma formation. We concluded that Chinese who carry the C/C homozygote of the IL-18-105A/C gene polymorphism in coding regions may have a higher risk of developing asthma. *J. Clin. Lab. Anal.* 22:39–44, 2008. © 2008 Wiley-Liss, Inc.

Key words: asthma; interleukin-18 (IL-18); single nucleotide polymorphisms (SNPs)

INTRODUCTION

Asthma is regarded as a multifactorial condition. The pathogenic mechanisms of asthma are thought to be complex and caused by an interaction of multiple susceptibility genes and environmental factors. Evidence indicates that environmental factors acting early in life and interacting with specific genes are important events for the development of the chronic, persistent form of the disease (1). Studies of these gene–environment interactions might help identification of risk factors and development of preventive strategies for asthma at an early age. Some loci and candidate genes have been studied to exhibit linkage and association of asthma and asthma-associated phenotypes, bronchial hyperresponsiveness, atopy, and high immunoglobulin E (IgE) levels, to microsatellite markers and single nucleotide polymorphisms (SNPs) within specific cytokine/chemokine and IgE regulating genes, but only a few genes with

significant risk have been mapped. However, the clinical meaning of the genetic variations within these genes remains largely unknown (2,3). A previous report indicated that the relevance of combinations of multiple genetic and environmental factors in the occurrence and natural history of allergic disorders are very relative (4). Hence, identification of gene–environment interactions will be a very important issue; i.e., how the environment

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alters the immunologic, genetic, and epigenetic mechanisms. It was proposed that many of the genes related to IgE synthesis and airway remodeling in asthma are persistent or reminiscent fetal genes that may not be blocked during late pregnancy or early infancy (5). Variation in the infectious environment and in the pattern of microbial exposure of children, preventing the shift of allergen-specific responses from the T-helper type 2 (Th2) to the Th1 phenotype, may be crucial for the increase in the prevalence of atopic disorders (1,6,7). Furthermore, epidemiologic observations have showed that the prevalence of Th1-dominated autoimmune diseases is also increasing (8).

In the previous investigation, asthma was thought of as a Th2 disease with some cytokine releases, including IL-3, IL-4, and IL-5 (9). Moreover, rising evidence has shown that other cytokines, which were traditionally classed as Th1, are also associated with the inflammatory response in asthma. Allergic diseases such as asthma and atopic dermatitis have increased in the last decade, so they have become a more serious public health problem. Allergy is known as a multifactorial condition and allergic onset and severity are induced by both genetic and environmental factors (10). Besides, the cytokine IL-18 has been shown to be involved in cellular immunity through both natural killer (NK) cell activation and Th1 induction (11–14). It can also inhibit IgE synthesis by directly affecting B cells when stimulated with IL-12 through the induction of endogenous interferon (IFN)- γ (15). Previous studies have indicated that IL-18 could stimulate the release of histamine and IL-4 from basophils (16), and IL-13 from T cells and NK cells (17). It also enhances IL-8 production by eosinophils (18) and induces naive T cells to differentiate into Th2 cells (19). Taken together, these experiments show that IL-18 has both allergy-promoting and antiallergic functions (20). A surfeit of IL-18 was recently found in allergic patients with atopic dermatitis or asthma (21–25). However, IL-18 was also raised in patients with other diseases in which dominant Th1 cells play a key role of immunity (26–30). These studies indicate that some detailed mechanisms of asthma formation are still controversial. Recent experiments have demonstrated that the 105A/C polymorphism of the IL-18 gene may be associated with the pathogenesis of asthma (31), but associations of some gene with a disease reported in previous studies could not be observed in different populations (32,33). That is, the same SNP but with distinct genetic backgrounds could either increase or decrease the risk of disease susceptibility. Recently, the connection between IL-18 SNP and susceptibility to asthma has been reported in Japanese patients (31). Nevertheless, this finding could not answer if IL-18 SNP could link to the risk of developing asthma

in the Chinese population. To address this question, we investigated the distribution and potential effects of IL-18 SNP on asthma susceptibility in the Chinese patients from Taiwan.

MATERIALS AND METHODS

Study Population

The patient group consisted of 101 asthmatic children 3–18 years old, and 100 asthmatic adults 19–70 years old (105 males and 96 females; age range 3–70 years; average age 21 ± 11.2 years) with bronchial asthma (atopy) were enrolled in our study. Patients with atopy were positive for allergen according to the radio-immunoassay test (RAIST) and pulmonary function test showed obstructive defect. Bronchial asthma was diagnosed for patients with symptoms of wheezing dyspnea, chronic cough, and chest tightness in early morning or night, with the positive RAIST. Pulmonary function tests included forced expiratory volume in 1 sec (FEV1)/forced vital capacity (FVC) and FEV1. Results over the 80% predicted values were regarded as normal. The control group consisted of 60 healthy volunteers (22 males and 38 females; age range 16–28 years; average age 21 ± 2.5 years) who had no history of allergy and routine pulmonary function test presented as normal. Informed consents were acquired from all individuals that participated in our study.

Detection of IL-18-105A/C Polymorphism by Restriction Fragment-Length Polymorphism (RFLP)

Blood leukocytes were used as the source of deoxyribonucleic acid (DNA) in asthma patients and controls. DNA was isolated using a Genomaker reagent kit (Biosbio, Taipei, Taiwan). Genotyping was performed using polymerase chain reaction (PCR) and carried out in a total reaction volume of 50 μ L containing genomic DNA, 2–6 pmol of each primer, 1 $\times$ Taq polymerase buffer (1.5 mM $MgCl_2$), and 0.25 units of AmpliTaq DNA polymerase (Perkin Elmer, Foster City, CA). The PCR analyses were performed using a GeneAmp PCR system 2400 (Perkin Elmer, Wellesley, MA). The PCR conditions comprised an initial denaturation step at 94°C for 5 min; 35 amplification cycles at 94°C for 30 sec, 60°C for 30 sec, and 72°C for 45 sec; and a final extension step at 72°C for 7 min. The primers (5'-TGT-TTA-TTG-TAG-AAA-ACC-TGG-AAT-T-3' and 5'-CCT-CTA-CAG-TCA-GAA-TCA-GT-3') were used for PCR amplification of the coding region of the IL-18 gene. PCR products were digested with the restriction enzyme *TaqI* (New England Biolabs, Beverly, MA) at 65°C overnight, separated on 3% (wt/vol)

agarose gel and detected by ethidium bromide staining. The enzyme digested the PCR products to yield two products, 123 and 25 bp. The polymorphism was divided into digestible (C/C homozygote), indigestible (A/A homozygote), and A/C heterozygote.

Statistical Analysis

The various parameters were compared using the χ^2 test. $P < 0.05$ was considered statistically significant. Odds ratios (ORs) and 95 confidence intervals (CIs) are given. SPSS software (version 10.0; SPSS Inc., Chicago, IL) was used to perform the calculations.

RESULTS

Three Different RFLP Patterns for IL-18-105A/C

DNA samples from asthma patients and the controls were prepared and applied to PCR. The 148-bp PCR products were assayed by RFLP. Theoretically, two fragments of 123 and 25 bp should be present if the product can be digested by *TaqI*. Figure 1 revealed digestible, indigestible homozygous, and heterozygous sites for the IL-18 gene SNP.

Different Frequencies for IL-18-105A/C in the Asthma Patients and Controls

The genotype distribution and allelic frequencies for the IL-18 gene in the asthmatic adult patients and controls are presented in Table 1. Significant differences were found in the genotype distribution of IL-18 SNP between all patients with asthma and controls

Fig. 1. The patterns of RFLP obtained from the three genotypes after *TaqI* digestion of the 148-bp PCR product for IL-18 gene. The RFLP indicated a digested fragment in lane 1 (123 bp), an indigestible fragment in lanes 2 and 3 (148 bp), and a heterozygous band in lane 4. M = Marker.

($P = 0.000003$). The distribution of the A/A homozygote in all patients with asthma (77.6%) was higher than that in controls (51.7%). Besides, the allelic frequency of the IL-18 gene was statistically significant to distinguish all asthma patients from controls ($P = 0.000066$, χ^2 test). Further, we assayed asthmatic adult patients and asthmatic child (pediatric) patients. Table 2 demonstrates that the frequency of the A allele for adult asthmatic patients was (75.0%), which was significantly higher than for controls (51.7%) ($P = 0.004025$, χ^2 test). Significant differences were found in the genotype distribution of IL-18 SNP between the asthmatic adult patients and controls ($P = 0.002374$; χ^2 test). For asthmatic child patients (Table 3), the frequency of the A allele was 90.1%, which was significantly higher than that for controls (74.2%) ($P = 0.000343$; χ^2 test). Significant differences were also found in the genotype distribution of IL-18 SNP between the asthmatic adult patients and controls ($P = 0.002374$, χ^2 test).

IL-18-105A Associated With Higher Risk in Developing Asthma

In addition, the OR of the A/A homozygote for the risk of asthma was 3.019 (95% CI, 1.635–5.576). The OR of the “A/C+C/C” genotypes for the risk of asthma

TABLE 1. Distribution of IL-18-105A/C genotypes among all asthma patients and healthy control subjects

	Asthma patients n (%)	Normal controls n (%)	P^a
Genotype			0.000003
A/A	156 (77.6)	31 (51.7)	
A/C	45 (22.4)	27 (45.0)	
C/C	0 (0)	2 (3.3)	
Allelic frequency			0.000066
Allele A	357 (88.8)	89 (74.2)	
Allele C	45 (11.2)	31 (25.8)	

^a P values were calculated by χ^2 test.

TABLE 2. Distribution of genotypes among the asthmatic adult patients and healthy control subjects

	Asthma patients n (%)	Normal controls n (%)	P^a
Genotype			0.004025
A/A	75 (75.0)	31 (51.7)	
A/C	25 (25.0)	27 (45.0)	
C/C	0 (0)	2 (3.3)	
Allelic frequency			0.002374
Allele A	175 (87.5)	89 (74.2)	
Allele C	25 (12.5)	31 (25.8)	

^a P values were calculated by χ^2 test.

TABLE 3. Distribution of genotypes among the asthmatic child patients and healthy control subjects

	Asthma patients n (%)	Normal controls n (%)	<i>P</i> ^a
Genotype			0.000343
A/A	81 (80.2)	31 (51.7)	
A/C	20 (19.8)	27 (45.0)	
C/C	0 (0)	2 (3.3)	
Allelic frequency			0.000153
Allele A	182 (90.1)	89 (74.2)	
Allele C	20 (9.9)	31 (25.8)	

^a*P* values were calculated by χ^2 test.

TABLE 4. Odds ratio and 95% CI of IL-18 gene 105A/C polymorphism

Genotype	Odds ratio (95% CI)
A/A	3.019 (1.635–5.576)
A/C	1 (reference)
A/C+C/C	0.931 (0.478–1.815)
Per copy of allele A	2.763 (1.654–4.616)
Per copy of allele C	1 (reference)

CI = confidence interval.

was 0.931 (95% CI, 0.478–1.815). The OR per copy of the “A” allele for the risk of developing asthma was 2.407 (95% CI, 1.416–4.091). The OR per copy of the “C” allele for the risk of developing asthma was 0.8709 (95% CI, 0.450–1.687) (Table 4).

DISCUSSION

This investigation is the first study to assay the IL-18 gene in asthma in the Chinese population. We demonstrate that IL-18 SNP is associated with asthma in the Chinese population in Taiwan. Besides, because the significance of statistical analysis was dominant, it could be concluded that patients with A/A homozygote in their IL-18-105 region have higher risk in developing asthma.

In this study, the “A” allele of the IL-18 gene 105A/C polymorphism was overrepresented in asthma patients, indicating an association with atopic asthma. This polymorphism, therefore, is a good genetic marker for further studies of the causes of atopic asthma. To date, only a few gene SNPs have been studied (1,34–37) and a great amount of the genetic background of asthma still needs to be identified. We selected cytokines as candidate genes because they have several proteins that are important components in the pathogenesis of many diseases. The positive results encourage us to seek other cytokine network genes for association with asthma disease and it is expected that they may provide further

information. Previous studies have indicated that the functions of IL-18 can induce allergic sensitization in mice (38,39) and obviously raise Th2 cytokine production, IgE levels, eosinophil recruitment in the airways, and airway mucus. In recent studies, several individual investigations related the functions of IL-18 expression and serum levels in allergic diseases have been published. Raised serum IL-18 levels were studied in the patients with allergic asthma (22) and acute asthma (21), atopic dermatitis (40), and allergic rhinitis (41). In the procedure of Th2 differentiation, IL-18 and T-cell receptor-mediated stimulation was induced by allergens such as house dust mites, which could trigger naive CD4⁺ T cells to grow into IL-4-generating cells in vitro (42). Besides, IL-18 could raise specific IgE generation in CD4⁺ T cells, IL-4, CD154, and STAT6 (19). Higa et al. (31) demonstrated that the IL-18-105A allele haplotype is associated with asthma, but it was not evident that the “A” allele was associated with expression level of IL-18. The authors explained that 105A/C might directly affect the modulation of transcription of the gene or RNA stabilization or that 105A/C is linked to another polymorphism, which alters the functional property of IL-18 or its gene regulation. To prove the points, further studies are necessary. Taken together, IL-18 can trigger cytokine production (43–45), but it possesses wide kinds of influences on other immune cells that contain stimulation of T cell differentiation and inhibition of IgE synthesis (38,46). Besides, IL-18 can modulate Th1 and Th2 response (20), so the roles of IL-18 in allergic inflammation and asthma are contradictory. Hence, the immunological effects of IL-18 still need to be identified and studied, although our data also indicate that the IL-18 gene polymorphism is a candidate genetic marker to screen for asthma.

In conclusion, we report the association between the IL-18 SNP and the risk of asthma in Chinese patients in Taiwan. Our study suggests that Chinese patients in Taiwan who carry the A/A homozygote of the A/C polymorphism in the IL-18-105A gene may have a higher risk of developing asthma. We expect that our investigation could contribute to realization of the pathogenic mechanisms of asthma and help to build predictive markers for asthma.

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