



Association of single nucleotide polymorphisms in interleukin 12 (*IL-12A* and *-B*) with asthma in a Chinese population

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ABSTRACT

Increasing evidence has indicated that genetic variants may contribute to immune dysregulation and susceptibility to noninfectious inflammatory diseases. Cytokines, including interleukin 12 (*IL-12*), play a key role in the regulation of the immune system. The aim of this study was to investigate whether single nucleotide polymorphisms (SNP) in *IL-12A* and *IL-12B* were associated with asthma in a Chinese population. Genotype characteristics were determined in 197 asthma patients and 369 controls by the polymerase chain reaction–restriction fragment length polymorphism method and DNA sequencing assay. The genotype and allele frequencies of *IL-12A* rs568408 demonstrated significant differences between cases and controls ($p < 0.001$). The AC genotype of rs3212227 was associated with a significantly decreased risk of asthma compared with the AA genotype ($p = 0.036$). The subjects carrying combined genotypes (rs568408 AG and rs3212227 AC/CC) at both loci had a 2.05-fold increased asthma risk compared with those carrying all other genotypes ($p = 0.001$). In contrast, individuals carrying combined genotypes of rs568408 GG and rs3212227 AC/CC were associated with a significantly decreased risk of asthma compared with those carrying the combined genotypes of rs568408GG and rs3212227AA ($p = 0.009$). No significant difference was reported for rs2243115 between cases and controls. These results suggest that the SNPs in *IL-12A* rs568404 and *IL-12B* rs3212227 may individually and jointly contribute to the risk of asthma in a Chinese population.

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1. Introduction

Asthma, the most common chronic respiratory disease affecting children and young adults, is a complex disease with variable phenotype and aberrant T-helper (Th) 2 cytokine profile. Asthma is characterized by reversible airflow obstruction, airway inflammation, remodeling, and hyperresponsiveness [1,2]. At its simplest, asthma is divided into 2 subtypes: allergic and nonallergic [3]. Over the past 30 years, there has been a dramatic increase in the number of patients with asthma [4], which affects more than 300 million people around the world [5].

Asthma involves the interaction of genetic and environmental components. The contribution to the susceptibility to asthma with heritability was estimated to vary between 36 and 79% [6,7]. The understanding that genetics play a role in the pathogenesis of asthma has been recognized for more than 100 years [4]. The exact mechanism of asthma, however, is still poorly understood. Asthma is characterized as not 1 disease but many, with various unidentified causes underlying its development and manifestation. Recently, genome-wide association studies [8,9] have confirmed a

strong genetic component to asthma. Difficulty remains, however, in identifying specific causal genes and determining whether ethnic disparities should be attributed to the genetic control of certain genes for this complex disorder. In recent years, more than 200 asthma candidate genes have been proposed by different approaches, such as human association and positional cloning [10,11].

Cytokines play a pivotal role in the regulation of immune response and the polymorphic nature of the cytokine genes may confer flexibility on the immune response [12]. Recent evidence suggests that deficiency in the Th1 cytokine pathway may underlie susceptibility to asthma [13]. *IL-12* is an important immunoregulatory cytokine with an antagonistic effect of the Th1/Th2 cytokine balance and provides a functional link between innate and acquired immune responses [14]. *IL-12* is derived from dendritic cells, Langerhans cells, mononuclear phagocytic cells, and mast cells. *IL-12* stimulates interferon- γ production and activates and induces proliferation and cytokine production of natural killer cells [15].

Previous studies have indicated that the *IL-12B* single nucleotide polymorphism (SNP; rs3212227) was significantly associated with the risk of a wide range of autoimmune and inflammatory diseases, such as psoriasis [16], type 1 diabetes [17], asthma severity pheno-

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Table 1
Demographics of patients with asthma and controls

Variables	Controls <i>n</i> = 369 (%)	Asthmatic patients <i>n</i> = 197 (%)	<i>p</i> value
Age (years, mean $\pm$ SD)	38.21 $\pm$ 12.66	32.41 $\pm$ 11.11	NS
Sex			
Male	178 (48.2)	86 (43.7)	NS
Female	191 (51.8)	111 (56.3)	

n = Number of subjects; % = percentage of sex composition; SD = standard deviations; NS = not significant.

type in Caucasians [18], Crohn's disease [19], and rheumatoid arthritis [20]. Hirota et al. reported that the *IL-12B* rs321227 polymorphism was significantly associated with a risk of childhood atopic asthma and was associated with a high total immunoglobulin-E level in a Japanese population [21]. However, no study to date has examined the association between *IL-12B* gene polymorphism and the risk of asthma in a Chinese population. The *IL-12B* polymorphism was therefore selected to investigate whether the polymorphism was related to asthma risk. Additionally, given the complexity of asthma, gene-gene interaction may play a crucial role in asthma development. Because *IL-12B* was selected, rs2243115 and rs568408 were chosen in *IL-12A* to detect the effect of *IL-12A* and *IL-12B* interaction on the risk of asthma.

Based on these studies, it was hypothesized that SNPs in *IL-12A* and *IL-12B* genes may be involved in asthma development in a Chinese population. A hospital-based case-control study was carried out to investigate the association between *IL-12A* and *IL-12B* polymorphisms and susceptibility to asthma.

2. Subjects and methods

2.1. Patients and controls

A total of 197 unrelated patients with asthma were recruited from West China Hospital, Sichuan University, from July 2005 to March 2008. The patients (86 males and 111 females), with an average age of 32.41 years, exhibited typical clinical symptoms of asthma. The diagnostic criteria of asthma were based on American Thoracic Society guidelines [22]. Three hundred sixty-nine control subjects were used (178 males and 191 females with an average age of 38.21 years). All subjects answered negatively to questions regarding the presence of respiratory symptoms and exhibited negative results for each of the standard diagnostic laboratory tests, such as a normal chest radiogram and a forced expiratory volume in 1 second (FEV₁) greater than 75% of predicted value [23]. A 2-mL venous blood sample was collected from each subject and used for genotyping assays. This study was performed with the approval of the Ethics Committee of Sichuan University. The clinical parameters are summarized in Table 1.

Table 2
Genotype frequencies of 3 SNPs in *IL-12A* and *IL-12B* between patients with asthma and controls

Polymorphisms	Controls (<i>n</i> = 369) <i>n</i> (%)	Patients (<i>n</i> = 197) <i>n</i> (%)	OR(95% CI)	<i>p</i> value
<i>IL-12A</i> rs568408				
GG	296 (80.2)	129 (65.5)	1.0 (ref)	<0.001
AG	73 (19.8)	68 (34.5)	2.14 (1.45–3.16)	
<i>IL-12A</i> rs2243115				
TT	324 (87.8)	163 (82.7)	1.0 (ref)	0.08
GT	44 (11.9)	34 (17.3)	1.54 (0.95–2.50)	
GG	1 (0.3)	0 (0.0)	—	0.10
GT/GG	45 (12.2)	34 (17.3)	1.50 (0.93–2.44)	
<i>IL-12B</i> rs3212227				
AA	133 (36.0)	86 (43.6)	1.0 (ref)	0.036
AC	181 (49.1)	78 (39.6)	0.67 (0.46–0.97)	
CC	55 (14.9)	33 (16.8)	0.93 (0.56–1.55)	0.77
AC/CC	236 (64.0)	111 (56.4)	0.73 (0.51–1.04)	0.08

n = Number of subjects; % = frequency of genotype; ref = reference.

2.2. Blood sample and DNA isolation

A peripheral venous blood sample of 2 mL was drawn from each individual by standard venopuncture. The blood sample was collected in sterile anticoagulant tubes. Genomic DNA was isolated using a commercially available kit (Bioteke Corporation, Beijing, China) according to the manufacturer's instructions.

2.3. Genotyping

IL-12 polymorphisms (rs2243115 and rs568408 in *IL-12A* and rs3212227 in *IL-12B*) were identified using polymerase chain reaction–restriction fragment length polymorphism analysis. The primers for rs2243115, rs568408, and rs3212227 restriction enzymes were based on current literature found in *Clinical Cancer Research* [24]. Restriction fragments were separated on 5% polyacrylamide gel and visualized by silver staining to determine the genotypes. To confirm the accuracy of genotyping, partial samples were examined by DNA sequencing.

2.4. Statistical analysis

Hardy–Weinberg equilibrium was tested with a goodness-of-fit χ^2 test. The associations between genotype and allele frequencies of *IL-12A* and *IL-12B* and risk of asthma were estimated by computing the odds ratios (ORs) and their 95% confidence intervals (CIs). Statistical significance was set at *p* < 0.05, and SPSS statistical software, version 11.5, was used for all statistical analyses.

3. Results

The genotype and allele frequencies of the 3 SNPs (*i.e.*, rs2243115 and rs568408 in *IL-12A* and rs3212227 in *IL-12B*) are summarized in Tables 2 and 3. The genotype frequencies agreed with the Hardy–Weinberg equilibrium in each group. The frequencies of the GG and AG of rs568408 were 65.5 and 34.5% in cases and 80.2 and 19.8% in controls, respectively. There was a significant difference in the distribution of genotypes between cases and controls (OR = 2.14, 95% CI: 1.45–3.16). The A allele of rs568408 was associated with a significantly increased risk of asthma compared with the G allele (OR = 1.90, 95% CI: 1.33–2.71). The frequencies of the AA and AC of rs3212227 were 43.6 and 39.6% in asthma patients and 36.0 and 49.1%, respectively, in controls. The AC genotype was associated with a notably decreased risk of asthma compared with the AA genotype (OR = 0.67, 95% CI: 0.46–0.97). However, no significant association was observed between the *IL-12A* rs2243115 T/G polymorphism and risk of asthma.

The combined effects of *IL-12A* rs568408 and *IL-12B* rs3212227 on the risk of asthma were also examined. As shown in Table 4, individuals carrying rs568408GG and rs3212227 AC/CC combined genotypes were associated with a significantly decreased risk of asthma compared with those carrying rs568408GG and rs3212227

Table 3
Allelic frequency of 3 SNPs in patients with asthma and controls

SNPs	Alleles	Controls	Patients	OR (95% CI)	p value
rs568408	G	665 (90.1)	326 (82.7)	1.00 (ref)	<0.001
	A	73 (9.9)	68 (17.3)	1.90 (1.33–2.71)	
rs2243115	T	692 (93.8)	360 (91.4)	1.00 (ref)	0.13
	G	46 (6.2)	34 (8.6)	1.42 (0.90–2.25)	
rs3212227	A	447 (60.6)	250 (63.5)	1.00 (ref)	0.34
	C	291 (39.4)	144 (36.5)	0.89 (0.69–1.14)	

AA (OR = 0.57, 95% CI: 0.38–0.87). However, patients carrying rs568408 AG and rs3212227 AC/CC combined genotypes had a significantly higher risk of asthma compared with individuals carrying all other genotypes (OR = 2.05, 95% CI: 1.31–3.19).

4. Discussion

Asthma is a complex disease, and it may be a syndrome rather than 1 disease entity, in which different pathways eventually result in airway obstruction in a variety of phenotypes [25].

IL-12 is a 70-kDa heterodimer composed of 2 disulfide-linked subunits of 35 (IL-12A) and 40 kDa (IL-12B). The subunit p35 is homologous to IL-6, whereas the subunit p40 is homologous to the receptor for IL-6 [15,26,27]. The 2 genes for these subunits, *IL-12A* encoding IL-12p35 located on 3p12–q13.2 and *IL-12B* encoding IL-12p40 located on 5q31–33, are unlinked [13,27]. The production of IL-12 requires the coordinated expression of both p40 and p35, whereas p35 can only be secreted when associated with p40 [28,29]. There is an increasing volume of literature published to date on the association of *IL-12B* gene rs3212227 with inflammatory diseases [17–20]. Based on the literature, *IL-12B* gene rs3212227 was selected to investigate whether the polymorphism was associated with the risk of asthma. It was reported that the AC genotype of rs3212227 was associated with a significantly decreased risk of asthma. The finding was consistent with the study by Hirota et al. [21], who reported that the rs321227 polymorphism was significantly associated with the risk of childhood atopic asthma in a Japanese population. However, the result was inconsistent with the results of Noguchi et al. [30], who investigated whether 1188 A/C rs3212227 alleles were preferentially transmitted to asthma-affected or rhinitis-affected children within families by conducting transmission disequilibrium tests. In this study, the case and control subjects were from genetically unrelated Chinese families. The different study design and different sample source are most likely responsible for the conflicting results. Alternately, environmental factors may contribute to the pathogenesis of allergic diseases [4]; therefore, environmental exposures are a potential explanation for the conflicting results. Replicated studies are warranted to confirm this finding.

Additionally, the association of *IL-12A* polymorphisms and asthma risk in a Chinese population was investigated. It was reported that the AG genotype of rs568408 A/G was associated with a significantly increased risk of asthma, indicating that potentially

functional polymorphisms in *IL-12A* rs568408 might play an important role in the etiology of asthma.

Gene–gene interaction was also considered in the pathogenesis of disorders. Asthma is a complex disease with variable phenotype. Complex diseases are affected by multiple genes and gene–gene interaction, which have an important role in the development of diseases [31]. In this study, the combined effects of multiple SNPs from different genes (*IL-12A* and *IL-12B*) on the risk of asthma were examined. It was reported that the combined genotypes of rs568408 and rs3212227 (i.e., rs568408GG/rs3212227 AC/CC, rs568408AG/rs3212227 AC/CC) were associated with susceptibility to asthma. These associations suggested that rs568408 AG had a more dangerous effect on the risk of asthma than any other genotype and that the individuals carrying rs568408 AG and rs3212227 AC/CC combined genotypes were more prone to suffer from asthma, although the pathogenic effect of rs568408 AG was diluted by rs3212227 AC/CC. Our data presented the possibility that the combined effect of cytokine polymorphisms might have a potential functional effect on the production of IL-12A and IL-12B and eventually affect susceptibility to asthma. Although statistical interaction does not always indicate the existence of a biologic interaction [32], gene–gene interaction analysis may prove an effective method to find an important predisposing factor in a complex disease study, which is not available from single SNP analysis.

This study has some limitations. Detailed information on the age of onset of asthma for patients was not available, which potentially impacts the study results with regard to genetic susceptibility to asthma between patients. Additionally, environmental factors were not considered when examining the effects of gene–gene interaction on susceptibility to asthma. Both age of onset data and environmental factors are possible sources of error and account for some deviation among the results.

In conclusion, this is the first study to investigate an association between the *IL-12A* and *IL-12B* polymorphisms and asthma in a Chinese population. It was reported that rs568408 and rs3212227 polymorphisms may be related to asthma susceptibility in a Chinese population. Additionally, individuals carrying rs568408 AG and rs3212227 AC/CC genotypes may have an elevated risk of asthma, whereas subjects carrying rs568408 GG and rs3212227 AC/CC genotypes may have a decreased risk of asthma. Nevertheless, further validation is warranted to diversify the study group to include additional populations.

Table 4
Combined analysis between rs568408 polymorphism in *IL-12A* and rs3212227 polymorphism in *IL-12B* and asthma risk

Genotypes	Cases (n = 197) n (%)	Controls (n = 369) n (%)	OR (95% CI)	p value
Combined genotypes				
rs568408GG and rs3212227AA	65 (33.0)	109 (29.5)	1.0 (ref)	0.25
rs568408AG and rs3212227AA	21 (10.7)	24 (6.5)	1.47 (0.76–2.84)	
rs568408GG and rs3212227AC/CC	64 (32.4)	187 (50.7)	0.57 (0.38–0.87)	0.009
rs568408AG and rs3212227AC/CC	47 (23.9)	49 (13.3)	1.61 (0.97–2.66)	0.06
Dichotomized genotypes				
All other genotypes	150 (76.1)	320 (86.7)	1.0 (ref)	0.001
rs568408 AG and rs3212227 AC/CC	47 (23.9)	49 (13.3)	2.05 (1.31–3.19)	

n = Number of subjects; % = frequency of genotype; ref = reference.

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