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Interleukin-17A and-17F single nucleotide polymorphisms associate with susceptibility of asthma in Chinese Han population

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Abstract

Interleukin 17 (IL-17) plays important roles in the progression of asthma. Genetic variants in the IL-17 may influence the immunopathogenesis of many diseases. Many studies have investigated the relevance of IL-17 polymorphism with cancers or immune diseases, including asthma. In this study, single nucleotide polymorphisms (SNPs) of IL-17 were explored by PCR-RFLP and verified by sequencing method. The frequencies of genotypes and alleles were analyzed. Haplotypes were analyzed with the SHEsis online program. The relationship between the genotypes of SNPs and IgE level was also investigated. The False Discovery Rate (FDR) correction was performed (P -adjusted<0.05). The frequencies of A allele, GA and (GA+AA) genotype of rs3748067 were significantly higher in asthma patients. As for rs763780, the C allele in patients was more frequent than healthy controls. In addition, we found C carriers (CT+CC) were significantly higher in asthma patients. We further found

that the haplotype CT for IL-17F (rs763780/rs2397084) was associated with an increased susceptibility of asthma, but this association did not survive after FDR correction. The level of serum total IgE in mutant group (GA+AA) of rs3748067 was significantly higher than the wild genotype (GG) group and control group. These results suggested that IL-17 SNPs, but not haplotypes may be associated with the susceptibility of asthma in Chinese Han population from central China.

Key Words: IL-17A, IL-17F, Single nucleotide polymorphism, Asthma

1. Introduction

Asthma is one of the most common chronic inflammatory respiratory disease, which characterized by airway hyper-reactivity, eosinophilic inflammation in airway and elevated immunoglobulin E (IgE) levels. Aberrant T-helper (Th)2 cells and the cytokines that it secreted play an important role in the pathology of asthma [1]. However, with the development of research on pathogenic role of various Th cells, some limitations have been recognized in the Th2 based inflammation model.

In recent years, Th17, a new subset of Th cells, has been found to be pro-inflammatory, which involved in neutrophilic inflammation and extensive studies have to make contribution for understanding the role of Th17 cells in asthma [2-4]. Among the cytokines produced by Th17, IL-17A and IL-17F have been correlated with a mass of inflammatory and autoimmune diseases, which demonstrated to be a key role in recruitment and activation of neutrophile and other immune cells [5].

As we all known, the susceptibility to asthma is determined by the combined effect of genetic and environmental factors [6]. Several studies demonstrated that

asthma was associated with a number of genes, such as IL-4, IL-13, IL-18, ADAM33, IL-17A, IL-17F [7,8], etc. However, the same gene may have different susceptibility in different population. Recent genetic studies have linked IL-17F SNPs to the susceptibility of immune-inflammatory diseases such as autoimmune thyroid diseases, multiple sclerosis, ulcerative colitis, lung cancer and tuberculosis [9-11]. Besides, IL-17A (rs3748067) has been found as a susceptibility factor for brucellosis [12]. Moreover, IL-17F (rs763780) and IL-17F (rs2397084) both have impact on the susceptibility to rheumatoid arthritis [13,14]. However, the relationship between asthma and these three SNPs in Chinese Han population from central China is still unclear. In this study, we examined three SNPs in Chinese Han asthma patients: one SNP in the IL-17A gene, namely, rs3748067 and two SNPs in the IL-17F gene, namely, rs763780 and rs2397084. We also evaluated the association of genotypes and allele frequencies between asthma patients and healthy controls. In addition, we analyzed the relationships between above genotypes of SNP and the level of total serum IgE.

2. Subjects and methods

2.1. Patients and subjects. In this case-control study, we recruited 221 asthma patients (118 females; 103 males) and 223 healthy controls (110 females; 113 males) from the Department of laboratory Respiration Diseases, Union Hospital, Wu Han, China. All participants were questioned to obtain clinical and family histories, as well as data on smoking habits. The results of skin prick test of patients were collected from medical records or a patient consultation questionnaire. The inclusion criteria

were: 1) region: central China; 2) ethnicity: Han; 3) pulmonary function test: positive in bronchial dilation test; and 4) no wheezing, dyspnea, chest distress, or cough symptoms caused by other diseases. In our study, all patients mainly came from Hubei, Hunan, Henan provinces in central China. We excluded patients who had a history of other allergic, other autoimmune diseases, severe complications and tumor diseases. Control subjects were collected from people who accepted regular check up at the medical examinations center in our hospital. The inclusion criteria were: 1) region: central China; 2) ethnicity: Han; 3) pulmonary function test and imaging: normal; 4) without clinical symptoms; and 5) no family hereditary disease, allergic diseases, autoimmune diseases.

2.2. Asthma diagnosis and ethics statement. All the asthma patients were diagnosed according to the Chinese guideline for the prevention and management of bronchial asthma developed by Chinese Medical Association respiratory asthma group (2008). Some were newly diagnosed asthma patients, the others were recurrent asthma patients. All the asthma patients met this standards, which included: 1) recurrent wheezing, shortness of breath, chest tightness or cough; 2) when the asthma attacked, wheezing sound could be heard in the lungs; 3) The symptoms was relieved after specific treatments; 4) no wheezing, dyspnea, chest distress, or cough symptoms caused by other diseases; 5) patients should have at least one of the following tests positive if they had atypical clinical symptoms: the positive for bronchial provocation test or exercise test, the positive for bronchial dilation test, and the intraday variability for peak expiratory flow (PEF) was more than 20%. Our study was approved by the

ethics committee at Tongji Medical College, Huazhong University of Science and Technology. All subjects were Chinese Han population from the same area in Middle China and gave their informed consents.

2.3. Genomic DNA extraction. Ethylene diamine tetraacetic acid (EDTA) anti-coagulated venous blood samples were collected from all participants. Genomic DNA was extracted from leukocytes in peripheral blood using Genomic DNA isolation kit (TIANGEN Biotech, Beijing) strictly in accordance with the protocol provided by the kit.

2.4. Genotyping. Genotyping for rs3748067 G>A in the IL-17A and for rs763780 T>C, rs2397084 T>C in the IL-17F were performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The primers, restriction enzymes (New England Bio labs, Beverly, MA, USA) and product sizes of PCR-RFLP for IL-17 polymorphic sites were listed in Table 1. The primers were synthesized by the company of QingKe (Wuhan). PCR reactions were performed in a total volume of 50 μ L containing 10 $\times$ buffer 5 μ L, 2.5mmol/L of each dNTP 4 μ L, 25mmol/L of MgCl₂ 3 μ L, 10 μ mol/L of each primer 2.5 μ L, 10ng/ μ L of genomic DNA 2 μ L and 2U of Taq DNA polymerase (Fermentas, USA), which was increased to a final volume of 50 μ L by addition of distilled water. The PCR reaction condition for IL-17A (rs3748067) was as follows: initial denaturation at 95°C for 5min, 38cycles of denaturation at 95°C for 30s, annealing at 57°C for 30s, extension at 72°C for 20s and a final 7min extension at 72°C. The PCR profile for IL-17F (rs763780) was as follows: 35cycles of denaturation at 94°C for 30s, annealing at 53°C for 30s, extension at 72°C

for 20s and a final extension at 72°C for 7min. The protocol of PCR for IL-17F (rs2397084) was as follows: denaturation at 94°C for 5min, 39cycles of denaturation at 94°C for 30s, annealing at 58°C for 30s and a final extension at 72°C for 7min. The PCR products were purified by PCR purification kit (Takara, Japan). All digested products were analyzed by 2 or 3.5% agarose gel electrophoresis. The gel was stained with Gold View and visualized under an ultraviolet illuminator. Besides, the PCR products were sent to the company of QingKe (Wuhan) for direct sequencing, using an ABI 3730XL DNA Analyzer (Applied Biosystems).

Table 1. PCR-RFLP primers, restriction enzymes and product size for IL-17 SNP sites.

Polymorphic site	Enzyme	Primer sequences (5'→3')	Product sizes(bp)
1249 (G/A, rs3748067)	EcoR I	F: GGGCTGAACTTTTCTCATACTTAGA R: GAGACATTGTCTTCAGACTACAATG	212
7488 (T/C, rs763780)	Nla III	F: GTTCCCATCCAGCAAGAGAC R: AGCTGGGAATGCAAACAAAC	412
7383 (T/C, rs2397084)	Ava II	F: GTGTAGGAAGCTTGGGCTGCATCAAT R: AGCTGGGAATGCAAACAAAC	470

2.5. Determination of Serum IgE Levels. The serum levels of IgE were determined by immuno-chemiluminescence method according to the instructions of the IgE kit (Shenzhen New Industry Biomedical Engineering Co., Ltd, Shenzhen, China) and measured by Maglumi 2000 PLUS automatic chemical luminous analyzer. A level of serum IgE above 190IU/mL meant a positive result.

2.6. Statistical analysis. The Chi-square (χ^2) test or Fisher exact test was used to evaluate the frequencies and genotypes for IL-17 and to estimate the Hardy-Weinberg equilibrium between asthma patients and controls. By using the software packages SPSS 17.0 (SPSS Inc, Chicago, Illinois, USA), unconditional

univariate and multivariate logistic regression analysis were performed to obtain the crude and adjusted odds ratio (OR) and their 95% confidence intervals (CIs).

Haplotypes were analyzed by the SHESIS online program

(<http://analysis.bio-x.cn/myAnalysis.php>), a powerful software platform [15,16].

The statistical analysis of the results for IgE was performed using SPSS17.0. The *t* test was used to determine the difference between patients and controls. To control the error type I, we had performed FDR (False Discovery Rate) correction. $P < 0.05$ in a two-tailed test was considered statistically significant.

3. Results

3.1. Demographic Characteristics of patients and controls. The characteristics of the patients and the controls were listed in Table 2. The gender ratio (female to male) for asthma patients is 1.14 : 1 and 0.97 : 1 in the controls. The mean age was 37.49 ± 10.57 years for asthma patients and 36.09 ± 9.24 years for control group. There was no significant difference between asthma patients and healthy controls (HC) in the gender ratio, age, and smoking. However, the number of Eosinophil% and serum IgE in asthma patients were higher than control group ($P < 0.001$), which were tested by Department of Clinical laboratory in our hospital. The highest positive rate of inhalational allergens of patients was the dermatophagoides farinae and pteronyssinus (69.39%), then were polyvalent fungus (32.65%) and followed by canine or feline scurf (20.41%), cockroach (17.35%), and ambrosia artemisiaefolia (10.2%). The highest positive rate of food allergens of patients was fish, shrimp or crab (32.65%), then were milk (9.18%) and mutton or

beef (4.08%).

Table 2. Demographic characteristics of adult asthma patients and controls

Variables	Controls (n=223)	Asthma patients (n=221)	P value ^a
Age (years) (mean $\pm$ SD)	36.09 $\pm$ 9.24	37.49 $\pm$ 10.57	NS
Gender, No(%)			
Female	110 (49.33)	118 (53.39)	NS
Male	113 (50.67)	103 (46.61)	
Smoking, No(%)			
Smoker	45 (20.18)	41 (18.55)	NS
Nonsmoker	178 (79.82)	180 (81.45)	
Aeroallergen testing, No(%)			
<i>Dermatophagoides farinae</i> and <i>pteronyssinus</i>	-	68 (69.39)	-
Cockroach	-	17 (17.35)	-
Canine or feline scurf	-	20 (20.41)	-
<i>Ambrosia artemisiaefolia</i>	-	10 (10.20)	-
Polyvalent fungus	-	32 (32.65)	-
Mutton or beef	-	4 (4.08)	-
Milk	-	9 (9.18)	-
Fish, Shrimp or crab	-	32 (32.65)	-
Allergic rhinitis, No(%)			
Yes	-	112 (50.68)	-
No	-	109 (49.32)	
Family history of asthma, No(%)			
Yes	-	58 (26.24)	-
No	-	163 (73.76)	
Eosinophil, %	1.57 $\pm$ 0.82	4.99 $\pm$ 4.12	<0.001
FEV ₁ /FVC ratio (%)	85.97 $\pm$ 2.65	75.28 $\pm$ 11.25	<0.001
IgE			
1-190IU/mL, No(%)	214 (95.96)	92 (41.63)	<0.001
>190IU/mL, No(%)	9 (4.04)	129 (58.37)	<0.001
average value(IU/mL)	46.52	577.34	<0.001

^a Values were analyzed by the *t* test, or χ^2 test

NS, nonsignificant; IgE, immunoglobulin E; FEV₁, forced expiratory volume in the first second; FVC, forced vital capacity

3.2. Genotypes and allele frequencies of three SNPs in the IL-17

between asthma patients and the controls. The distributions of three SNPs in control group were in agreement with the Hardy-Weinberg equilibrium ($P>0.05$). The genotype and allele frequency of the three SNPs selected in the IL-17 was compared between 221 asthma patients and 223 control subjects, which was shown in Table 3.

For the IL-17F rs2397084 site, the frequencies of TT, CT genotypes were 96.83% and 3.17% in asthma patients, and 99.10% and 0.90% in the controls respectively. Neither patients nor controls had a genotype of CC. Although the frequency of CT genotype in patients was higher than HC, there was no statistically significant difference between asthma patients and HC (3.17% vs 0.90%, $P=0.090$)

For the IL-17A rs3748067 site, the frequencies of GG, GA and AA genotypes were 79.19%, 19.91% and 0.9% in asthma patients, and 91.93%, 7.62% and 0.45% in the controls respectively. The frequencies of A allele, GA and (GA+AA) were significantly higher in asthma than the controls (10.86% vs 4.26%, $P<0.001$; 19.91% vs 7.62%, $P=0.001$; 20.81% vs 8.07%, $P<0.001$). When we used the G allele as the reference, the A allele was related to a higher risk of asthma (OR, 2.74; 95% CI, 1.58-4.74). Next, when the GG genotype was used as the reference, we found that the GA genotype was also associated with a higher risk of asthma (OR, 3.03; 95% CI, 1.67-5.50). In addition, we found that A variant (GA+AA) was associated with a higher risk of asthma (OR, 2.99; 95% CI, 1.67-5.35).

For the IL-17F rs763780 site, the frequencies of TT, CT and CC genotypes were 69.23%, 30.32% and 0.45%, respectively in asthma patients and 78.03%,

21.52% and 0.45% in the controls. Although the frequency of CT in patients was higher than control group, there was no significant difference between asthma patients and controls ($P=0.107$). However, we found C carriers (CT+CC) was significantly higher in asthma patients than HC (30.77% vs 21.97%, $P=0.035$). When we used the T allele as the reference, we found that the C allele was only related to borderline risk of asthma (OR, 1.47; 95% CI, 0.99-2.17). +

Table 3. The association of observed genotypic and allelic frequencies of IL-17 SNPs in asthma patients and controls.

SNP		Controls (%) n=223	Cases (%) n=221	P value ^a	OR (95% CI) ^b
IL-17A	General genotype				
rs3748067 (G/A)	GG	205 (91.93)	175 (79.19)		1.00 (ref)
	GA	17 (7.62)	44 (19.91)	0.001	3.03 (1.67-5.50)
	AA	1 (0.45)	2 (0.90)		2.34 (0.21-26.06)
	GA+AA	18 (8.07)	46 (20.81)	0.000	2.99 (1.67-5.35)
	Allele				
	G	427 (95.74)	394 (89.14)		1.000 (ref)
	A	19 (4.26)	48 (10.86)	0.000	2.74 (1.58-4.74)
IL-17F	Genotype				
rs2397084 (T/C)	TT	221 (99.10)	214 (96.83)		1.000 (ref)
	CT	2 (0.90)	7 (3.17)	0.090	3.61 (0.74- 17.59)
	CC	0	0		—
	Allele				
	T	444 (99.55)	435 (98.42)	0.091	1.000 (ref)
	C	2 (0.45)	7 (1.58)		3.57 (0.74- 17.29)
IL-17F	Genotype				
rs763780 (T/C)	TT	174 (78.03)	153 (69.23)		1.000 (ref)
	CT	48 (21.52)	67 (30.32)	0.107	1.59 (1.03-2.44)
	CC	1 (0.45)	1 (0.45)		1.14 (0.07-17.29)
	CT+CC	49 (21.97)	68(30.77)	0.035	1.59 (1.03- 2.44)
	Allele				
	T	396 (88.79)	373 (84.39)		1.000 (ref)
	C	50 (11.21)	69 (15.61)	0.054	1.47 (0.99- 2.17)

CI, confidence interval; OR, odds ratio; SNPs, single nucleotide polymorphisms; ref,

reference. Bold text indicates $P < 0.05$.

a Value was determined by χ^2 test or fisher's exact test.

b Logistic regression analyses were used for calculating odds ratios and OR was adjusted by age and gender.

3.3. Influence of IL-17 haplotypes on the susceptibility of Asthma

Haplotype analysis was performed to evaluate the correlations of two SNPs in IL-17F promoter between asthma patients and healthy controls. In this analysis, two main haplotypes (CT and TT) were discovered which accounted for more than 98% of the total number of haplotypes (Table 4). The frequency of TT haplotype was lower in asthma patients than controls, and the haplotype TT appeared a decreased risk of asthma (82.8% vs 88.3%, OR=0.67, 95%CI 0.46-0.99, $p=0.047$). However, after FDR correction, these associations did not remain significant. The frequencies of CT haplotype was higher in asthma patients than controls (15.6% vs 11.2%, OR=1.48, 95%CI 1.00-2.19, $p=0.047$). After FDR correction, these associations also did not remain significant. In addition, we performed the haplotypes analysis of IL-17F polymorphisms (rs763780, rs2397084) in asthma patients and in controls stratified by gender. The haplotypic frequency of CT was showed a significant difference in female patients and controls (19.5% vs 11.4%, OR=1.90, 95%CI 1.12-3.22) rather than males patients (Table 5).

Table 4. Haplotypes analysis of IL-17F polymorphisms (rs763780, rs2397084) in Asthma patients and controls.

Haplotype	Control (freq, %)	Asthma (freq, %)	<i>P</i> value	OR (95% CI)	FDR adjusted p-value
C T	50.00(11.2)	68.95(15.6)	0.047	1.48 [1.00~2.19]	0.094

T T	394.00(88.3)	366.05(82.8)	0.047	0.67 [0.46~0.99]	0.094
T C	2.00 (0.5)	6.95 (1.5)	-	-	
C C	0.00 (0.0)	0.05 (0.1)	-	-	

CI, confidence interval; OR, odds ratio. FDR: False Discovery Rate, Bold text indicates $P < 0.05$.

Table 5. Haplotypes analysis of IL-17F polymorphisms (rs763780, rs2397084) in asthma patients and controls stratified by gender.

Haplotype	Female				Male			
	Control (freq, %)	Asthma (freq, %)	<i>P</i> value	OR(95%CI)	Control (freq, %)	Asthma (freq, %)	<i>P</i> value	OR(95%CI)
C T	25.0(11.4)	46.00(19.5)	0.016	1.90(1.12~3.22)	25.00(11.1)	22.57(11.0)	0.97	1.01(0.55~1.85)
T T	194.00(88.2)	188.00(79.7)	0.016	0.53(0.31~0.89)	200.00(88.5)	178.43(86.6)	0.97	0.99(0.54~1.81)
T C	1.00(0.5)	2.00(0.8)	-	-	1.00(0.4)	4.57(2.2)	-	-
C C	-	-	-	-	0.00(0.0)	0.43(0.2)	-	-

CI, confidence interval; OR, odds ratio. Bold text indicates $P < 0.05$.

3.4. Correlation of variants in IL-17 with expression level of serum

IgE We analyzed the levels of serum IgE in the subgroup of IL-17 polymorphisms. Considering the serum total IgE in the healthy control group were mostly within 70 IU/mL, we used 1.85IU/mL transformed by log₁₀ as the boundary and divided patients or controls into two groups. For the IL-17F rs763780 site, the results showed that there was no correlation between the mutant type (CT+CC) group and the levels of serum total IgE in the risk of asthma (Table 6). For the IL-17A rs3748067 site, we found that the risk factor of (GA+AA) was significantly associated with a high risk of asthma, especially in those with high levels of serum IgE (OR=5.01, 95%CI 1.45-17.27) (Table 7). In addition, the correlation was further supported by the results that the distribution of serum total IgE. For the 46 cases of mutant genotypes

(GA+AA), the proportion of IgE concentration in the normal range accounted for 28.26%, the proportion of IgE concentration between 190 and 2000 IU/mL accounted for 52.18%, and the proportion of IgE concentration more than 2000 IU/mL accounted for 19.56%. However, in the 175 cases of wild genotype (GG), the percentage was 73.71%, 26.29%, and 0%, respectively (Table 8). We also found that the mean level of IgE in the mutant group (GA+AA) was significantly higher than the wild genotype (GG) group and control group (Fig.1).

Table 6. The correlation between rs763780 polymorphism and serum total IgE in the risk of asthma

SNP	Log ₁₀ IgE (IU/mL)	Asthma (n=221)		Control (n=223)		OR (95%CI)	P value
		CT+CC	TT	CT+CC	TT		
rs763780	<1.85 ^a	30	75	40	143	1.43 (0.83-2.48)	0.20
	≥1.85	39	78	9	31	1.72 (0.75-3.97)	0.19

a. Log₁₀-transformed by the level of 70 IU/mL IgE.

Table 7. The correlation between rs3748067 polymorphism and serum total IgE in the risk of asthma

SNP	Log ₁₀ IgE (IU/mL)	Asthma (n=221)		Control (n=223)		OR (95%CI)	P value
		GA+AA	GG	GA+AA	GG		
rs3748067	<1.85 ^a	10	84	15	167	1.33(0.57-3.08)	0.51
	≥1.85	36	91	3	38	5.01(1.45-17.27)	0.01

a. Log₁₀-transformed by the level of 70 IU/mL IgE.

Table 8. The relationship between rs3748067 genotypes and distribution of serum total IgE

IgE concentration (IU/mL)	(GA+AA) genotype (%) n=46	GG genotype (%) n=175	Control n=223
>3200	6 (13.04)		

2000-3200	3 (6.52)		
400-2000	10 (21.74)	18 (10.29)	
190-400	14 (30.44)	28 (16.00)	9 (4.93)
0-190	13 (28.26)	129 (73.71)	214 (95.07)

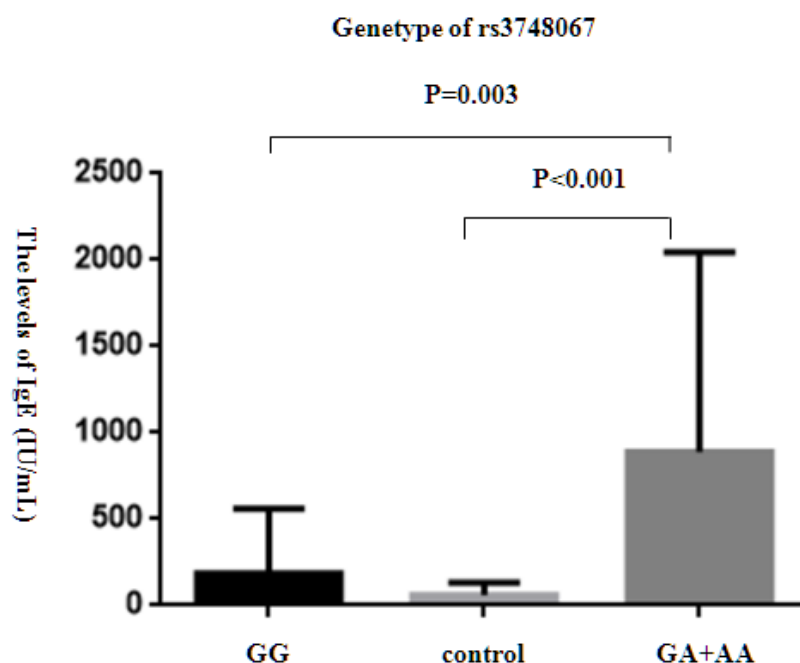


Fig.1. IL-17A rs3748067 polymorphism was found to be significantly associated with the serum levels of IgE.

Note: This IgE kit had a detection range of 0-3200IU/mL and the serum with the concentration exceeding 3200 IU/ml should be diluted and measured again to estimate the exact concentration.

4. Discussion

Asthma is a chronic airway inflammatory disorder of the airways which involved in many cells and cellular elements. There were many evidences suggest that high percentage of T-helper (Th)2 cells and its cytokines posed an important interconnection to the pathology of asthma [17,18]. However, along with the development of research on pathogenic role of various Th cell, some limitations have

been recognized to exist in the Th1/Th2 paradigm. In recent years, Th17 cells were identified to be differentiated from naive CD4⁺ T cells [19], and it has been found involving in neutrophilic inflammation, and playing an important role in asthma. IL-17 is a significant cytokine of Th17 cells. It has demonstrated that IL-17 was associated with many inflammatory and autoimmune diseases [20, 21]. There are six members in IL-17 cytokine family, including IL-17A, IL-17B, IL-17C, IL-17D, IL-17E and IL-17F [22]. IL-17A and IL-17F are two crucial effective members of the IL-17 gene family. They are both located on chromosome 6p and this genomic region has been found related to asthma by multiple genome scans [23]. They are candidate genes for asthma. The latest research shows that high levels of IL-17A and IL-17F are found in induced sputum, bronchial biopsies obtained, and bronchoalveolar lavage (BAL) fluid from patients with moderate or severe asthma. In addition, the level was positively correlated with the severity of asthma [24]. IL-17A and IL-17F increased airway neutrophil infiltration, airway mucous cell metaplasia, and airway remodeling, suggesting an important role of these cytokines in asthma pathogenesis. The variants of IL-17A and IL-17F gene might affect its expression. To the best of our knowledge, we first investigated the association between IL-17 SNPs (rs3748067, rs763780 and rs2397084) and asthma risk in Chinese Han population from central China. Besides, in this study, we first used the method of restriction length polymorphism to analyze the polymorphic sites, and then validated the sites by direct sequencing. This method improved the accuracy of our analysis of polymorphic sites.

Our study revealed that the SNP rs3748067 (1249 G>A) was associated with risk

of asthma. The SNP rs3748067 located at position of the IL 17A 3'-UTR, which could regulate mRNA expression post-transcriptionally by binding to the 3'-UTR of targeted genes. This area might be important in disease susceptibility [25]. Although there was no record of SNP rs3748067 in researches of asthma till now, other diseases were suggested to be associated with them. Ranji Hayashi [26] et al. reported rs3748067 minor carrier (CT+TT) had decreased risks for the development of ulcerative colitis (UC). Ruhui Han [27] et al. found that the genotype of rs3748067 AA was associated with a decreased risk of coal workers' pneumoconiosis (CWP), particularly among subgroups of smokers and CWP cases with stage I. In addition, Xukui Zhang [28] et al. and Yang LJ [29] et al. observed a significant interaction among rs3748067 and H. pylori infection on the risk of gastric cancer. Rs3748067 AA genotype also could be considered as susceptibility factors for brucellosis [30]. In our study, we found that the frequency of GA genotype and A allele were significantly higher in asthma patients than the controls, and the subjects carried A allele were associated with a significantly higher risk of asthma. Our study also indicated that the minor allele A of rs3748067 was associated with elevated IgE levels. However, the underlying mechanisms of this association have yet to be determined. Many studies suggested that IL-17 was associated with IgE levels. Allergen-specific IgE plays an essential role in the pathogenesis of allergic asthma. Nobuaki Mizutani [31] et al. elucidated the contribution of IL-17 to an IgE-mediated late-phase asthmatic response and airway hyperresponsiveness (AHR). The exact mechanism of the SNP in regulating IgE expression required further investigation.

For the rs763780, the IL-17F 7488T/C (rs763780) variant in exon 3, which causes a His-to-Arg substitution at amino acid 161 (H161R), it has recently been studied in ulcerative colitis, rheumatoid arthritis, tuberculosis, autoimmune thyroid diseases, cancers, multiple sclerosis [10-14]. Many studies assessed the association of rs763780 polymorphism and susceptibility of asthma in different populations. However, the results were still ambiguous. In an earlier study, Mio Kawaguchi [32] et al. indicated that IL-17F sequence variant (His161Arg) was associated with protection against asthma and antagonized wild-type IL-17F activity in Japanese population. In contrast, a study among a Chinese Population revealed that the rs763780 TC heterozygote was associated with an increased risk of asthma in which this association was exclusive to males [33]. On the other hand, several studies showed evidence supporting a lack of association between asthma and the SNP of rs763780 in Saudi Arabia [34], childhood asthma in Tunisia [35], and in a Korean Population [36]. All in all, these results about the association between IL-17F (rs763780) polymorphism and asthma susceptibility were inconclusive and inconsistent. Therefore, we performed a study to obtain further research of the association of IL-17F rs763780 polymorphism with asthma susceptibility. Although the frequency of rs763780 genotype CT had no significant difference between asthma patients and controls, we found C carriers (CT+CC) were significantly higher in asthma patients than controls. These results indicated that rs763780 C carriers might be associated with a higher risk of asthma. Such results were in accordance with the findings of our Chinese counterparts [33].

Besides, for rs2397084 polymorphism, there was no significant results to uncover the relationship between this polymorphism site and asthma susceptibility.

Next, in our study, we used haplotype analysis to evaluate the correlations of two SNPs (rs763780, rs2397084) in IL-17F promoter between asthma patients and healthy controls. In this analysis, although the frequency of haplotype TT in asthma patients was lower than in controls, the decline had no difference after FDR correction. Meanwhile, the frequency of haplotype CT in asthma patients was significantly higher than controls, which showed an increased risk of asthma. However, this increase also had no difference in statistics after FDR correction. Therefore, IL-17 haplotypes may not be associated with the susceptibility to asthma in Chinese Han population from central China. In addition, we made the haplotypes analysis of IL-17F polymorphisms (rs763780, rs2397084) in asthma patients and controls be stratified by gender. The haplotypic frequency of CT showed a significant difference in female patients and controls rather than males. However, this phenomenon was not clear and we would increase the sample size of female patients in subsequent studies to verify this conclusion.

Our study had a few potential limitations. Firstly, the sample size needed to be enlarged in order to increase the frequencies of rare genotypes and haplotypes and confirmed the relationship between IL-17 SNPs and the susceptibility of asthma. For example, the sample size of patients with CT genotype for rs2397084 SNPs of IL-17 was relatively small, which might limit the statistical power to find an association between groups. Besides, we did not detect the level of serum IL-17 in asthma

patients. In addition, gene reporter assays would be utilized to investigate the influence of gene transcription in IL-17 promoter. Finally, some factors, such as the causes, frequency, and season of asthma attack, were not analyzed.

In conclusion, our findings supported the association between IL-17 SNPs and the risk of asthma in Chinese Han population from central China. GA genotype of rs3748067 and the C carries (CT+CC) of rs763780 were associated with a higher risk of asthma. We also indicated that the minor allele A of rs3748067 was associated with elevated IgE levels. However, we found that there was no association between rs2397084 SNPs and the susceptibility of asthma patients. Summarizing, our study showed that the haplotypes CT of IL-17F were not associated with the susceptibility of asthma in Chinese Han adults. Further prospective studies including larger sample size are necessary to confirm our findings.

Conflict of interest

The authors declare that they have no competing interests.

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