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Association Analysis of *TIM-1* -232G>A and 5383_5397 Insertion/Deletion Polymorphisms With Childhood Asthma and Total Serum Immunoglobulin E Levels in Middle China

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Abstract

Background: One of the members of the T cell immunoglobulin (Ig) domain and mucin domain (*TIM*) gene family, *TIM-1*, located in the chromosome 5q31-33 region, has been associated with the development of T helper (T_H) 2-biased immune responses and may be selectively expressed in T_H2 cells. Previous studies have also shown an association between polymorphisms in the *TIM-1* gene and asthma or asthma-related phenotypes.
Objective: The aim of the present study was to analyze the association between the *TIM-1* polymorphisms -232G>A and 5383_5397 insertion/deletion (ins/del) and susceptibility to asthma in a group of patients from middle China.
Methods: Polymerase chain reaction (PCR)-restriction fragment length polymorphism analysis and PCR-polyacrylamide gel electrophoresis were used to detect -232G>A and 5383_5397 ins/del genotypes in 302 asthmatic children and 206 controls. Serum total IgE was measured by chemiluminescence and specific IgE to common aeroallergens by immunoblot analysis.
Results: We found no association between the -232G>A polymorphism and asthma or total serum IgE levels or statistically significant differences between asthma and control subjects in terms of genotype and allele frequency for the 5383_5397 ins/del polymorphism. We did, however, detect a difference in total serum IgE levels for 5383_5397ins/ins genotypes in individuals with atopic asthma ($P<.05$) in that they had higher IgE levels than those with del/del and del/ins genotypes.
Conclusion: Our results suggest that the 5383_5397 ins/ins genotype in the *TIM-1* gene is associated with elevated serum total IgE levels, particularly in individuals with atopic asthma. Further studies are needed to confirm such an association.

Key words: Asthma. *TIM-1.* Polymorphism. Serum total immunoglobulin E.

Resumen

Antecedentes: Uno de los miembros de la familia de genes del dominio de inmunoglobulina (Ig) de células T y dominio mucina (TIM), *TIM-1*, localizado en la región cromosómica 5q31-33, se ha asociado con el desarrollo de la respuesta inmunitaria con predisposición a T helper 2 (T_H2) y podría expresarse selectivamente en las células T_H2. Estudios previos han demostrado una asociación entre polimorfismos del gen *TIM-1* y el asma o fenotipos relacionados con el asma.
Objetivos: El objetivo del presente estudio fue analizar la asociación entre el polimorfismo *TIM-1* -232G>A y 5383_5397 inserción/deleción (ins/del) y la susceptibilidad para el asma en un grupo de pacientes de China media.
Métodos: Se emplearon el análisis de polimorfismo de longitud de fragmentos de restricción- reacción en cadena de la polimerasa (RCP) y RCP-electroforesis en gel de poliacralimida para la detección de los genotipos del -232G>A y del 5383_5397 ins/del en 302 niños asmáticos y 206 controles. Se midió la IgE total sérica por quimioluminiscencia y la IgE específica a aeroalérgenos comunes mediante análisis de inmunoblot.

Resultados: Encontramos que no existía asociación entre el polimorfismo -232G>A y el asma o los niveles de IgE total sérica o diferencias estadísticamente significativas entre el asma y los sujetos control con respecto al genotipo y la frecuencia alélica para el polimorfismo 5383_5397 ins/del. Encontramos, sin embargo, una diferencia en los niveles de IgE total sérica entre los genotipos del polimorfismo 5383_5397 ins/del en individuos con asma atópica ($P<0,05$), en aquellos con genotipo 5383_5397 ins/ins) los niveles de IgE eran superiores comparados con los genotipos del/del y del/ins.
Conclusiones: Nuestros resultados sugieren que el genotipo 5383_5397 ins/ins en el gen *TIM-1* está asociado con los niveles de IgE total sérica, particularmente en los individuos con asma atópica. Son necesarios más estudios para confirmar dicha asociación.

Palabras clave: R: asma. *Tim-1*. Polimorfismo. Immunoglobulina E sérica total.

Introduction

Asthma is an obstructive inflammatory disease caused by a combination of genetic and environmental factors [1,2]. A growing body of evidence suggests that $CD4^+$ T cells may play an important role in the development of allergen-induced airway hyperreactivity and asthma pathogenesis. Naive $CD4^+$ T helper (T_H) cells can differentiate into T_H1 and T_H2 cells, which produce different cytokines and perform distinct functions [3-5]. These cells can also regulate clonal expansion and function for each other. The balance between T_H1 and T_H2 cells is vital in the immune response. If this balance is interrupted, both kinds of cells can cause pathologic effects and disease. The predominant induction of T_H2 cells can inhibit autoimmune diseases and mediate asthma and other allergic diseases, while that of T_H1 cells is responsible for delayed hypersensitivity and organ-specific autoimmune diseases [6,7].

It has been previously reported that the gene family of T cell immunoglobulin (Ig) domain and mucin domain (TIM) proteins, expressed in T cells, plays an important role in regulating T_H1- and T_H2-cell-mediated immunity [8]. The *TIM* gene family is located on human chromosome 5q31-33, which has been repeatedly suggested to be linked to asthma susceptibility and asthma-related phenotypes [9-13]. In humans, the *TIM* gene family has just 3 members, *TIM-1*, *TIM-3*, and *TIM-4*. (The *TIM-2* gene is found only in mice). The encoding product of all members of the family is a type I membrane protein characterized by identical structural motifs including a signal peptide, Ig domain, mucin domain, transmembrane region, and intercellular tail with phosphorylation sites [6,8]. *TIM-1* is also referred to as KIM1 or HAVCR-1 as it was initially cloned as kidney injury molecule 1 and hepatitis A virus cellular receptor. KIM-1 and HAVCR-1 have been found to be expressed in the kidney and liver, respectively [14,15]. As a membrane protein of T cells, TIM-1 is believed to be associated with the development of T_H2-biased immune responses and to be possibly selectively expressed in T_H2 cells [16,17]. One recent study reported that TIM-1 was significantly increased in pulmonary tissues and peripheral blood mononuclear cells in asthmatic mice after ovalbumin challenge [18], and in comparing the coding region sequences for *TIM-1* genes in 2 strains of mice another study reported variants in *TIM-1* and an association between *TIM-1* polymorphisms and the T cell and airway phenotype regulator (Tapr) locus [8]. *TIM-1* has, therefore, become a good candidate susceptibility gene for T_H2-driven allergic diseases such as asthma.

A number of population studies that have investigated the association between asthma and several recently identified polymorphisms in both the coding and promoter regions of *TIM-1* [19,20]. While other genetic markers such as the exon 4 variations in the *TIM-1* gene have been intensively studied to assess whether or not they play a role in the pathogenesis of allergic diseases, the results have not been consistent [21-24]. An association between the 5383_5397 insertion/deletion (ins/del) polymorphism in the *TIM-1* exon 4 region and susceptibility to asthma has been described in some but not all populations [20-25]. Furthermore, Chae et al [19] identified several polymorphisms in the *TIM-1* promoter region in a Korean population and also found a promoter polymorphism, –1637A>G, to be associated with susceptibility to rheumatoid arthritis. *TIM-1* promoter region polymorphisms, have, accordingly, become important candidate markers in disease-association analysis.

The aim of this study was to investigate a possible association between both the -232G>A polymorphism in the gene promoter region of *TIM-1* and the 5383_5397ins/del polymorphism in exon 4 with childhood atopic or nonatopic asthma in a population from middle China. We also investigated the relationship between the genotypes of each polymorphism and total serum IgE levels in asthma patients.

Material and Methods

Study Subjects

A total of 302 children with asthma were enrolled from pediatric outpatient clinics at the affiliated Union Hospital of Huazhong University of Sciences and Technology in Wuhan, China. Asthma was diagnosed according to the criteria of the American Thoracic Society [26] and the children had experienced recurrent episodes of at least 2 of the following symptoms: cough, wheeze, and dyspnea. Atopic asthma was defined according to the following criteria: *a)* at least 1 symptom of asthma, including cough, wheezing, breathlessness, and chest tightness; *b)* an improvement of at least 15% in baseline forced expiratory volume in 1 second (FEV_1) after bronchodilator use; and *c)* a total serum IgE level of over 120 IU/mL determined by direct chemiluminescence, and a positive test for at least 1 of 7 allergen-specific IgE antibodies. Because it is usually not possible to determine FEV_1 in children

Table 1. Clinical and Demographic Characteristics of Patients and Controls

Characteristics	Healthy Controls (n=206)	Patients With Nonatopic Asthma (n = 96)	Patients With Atopic Asthma (n = 206)	*P* Value[a]
Age, mean (SD) (range), y	10.1 (3.61) (4-16)	8.5 (4.67) (3-15)	8.9 (4.28) (3-14)	.61
Sex, male/female, No.	122/84	57/39	108/98	.31
Total IgE log10 value, mean (SD)	1.34 (0.64)	1.53 (0.48)	2.32 (0.53)	<.001

Abbreviations: IgE, immunoglobulin E; log10, logarithm base 10.
[a] Determined by χ^2 or 1-way analysis of variance where appropriate.

under 5 years old, in these patients, we diagnosed and classified asthma according to the frequency and severity of symptoms (cough, wheeze, and dyspnea). We enrolled 206 age-matched healthy individuals at the hospital as controls. These controls did not have any of the above conditions in their previous physical check-up or past history and furthermore had no symptoms or history of respiratory disease, chronic skin disease, or first-degree relatives with asthma or allergy. All the children enrolled in the study were living in the same area of the Hubei province in middle China. Written informed consent to participate in the study was obtained from all the parents. The study was approved by the ethics committee at the school of medicine at our university prior to genetic analysis. The clinical characteristics of the patients and controls are shown in Table 1. Genomic DNA was extracted from peripheral blood leukocytes using a DNA extraction kit (TaKaRa Biotechnology Co. Ltd., Tokyo, Japan) according to the manufacturer's directions.

Serum Concentrations of Total and Allergen-Specific IgE

Total serum IgE was measured using the ACS-180 PLUS chemoluminescence system (Bayer Diagnostic, New York, USA). Allergen-specific IgE was detected qualitatively in vitro using the Euroassay test kit (EUROIMMUN Medizinische Labordiagnostika AG, Lübeck, Germany), with results analyzed for human antibodies to 7 different inhalation allergens including grass mix, birch pollen, mugwort pollen, housedust mite, cat hair, dog hair/dander, and *Cladosporium herbarum*. Total serum IgE levels were analyzed as a quantitative trait following logarithmic transformation.

Typing of the TIM-1 -232 G>A Gene Polymorphism

The -232G>A polymorphism was genotyped by polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) analysis. The primer pair 5'-TGGGAAGTCAGGGGCTGTTTCTGT**C**G-3' and 5'-GCAGACAGGCTGGTTGGTA-3' was used to amplify the genomic region surrounding the single nucleotide polymorphism, -232G>A. The upstream primer contained a mismatched nucleotide C instead of G (shown underlined and bold) to incorporate a restriction endonuclease site for Taq I. PCR was performed in a total volume of 25 μL solution containing 50 ng of genomic DNA, 200 μM of each dNTP (mixture of dATP, dTTP, dCTP, and dGTP), 0.2 μM of each primer, 1.5 mM of magnesium dichloride, 10 mM of Tris hydrochloride (pH, 8.3) and 1 U of Taq DNA polymerase (Fermentas International Inc., Burlington, Canada). Cycling conditions included initial denaturation at 95ºC for 5 minutes followed by 35 cycles at 95ºC for 40 seconds, 56ºC for 40 seconds, 7ºC for 60 seconds, and a final extension at 72ºC for 10 minutes. To avoid contamination, negative controls without genomic DNA were included in each PCR reaction; 10 μL of the 232 base pair (bp) PCR fragment generated was then digested by adding 5 units of TaqI (TaKaRa Biotechnology Co. Ltd) and incubated overnight at 65ºC. This enzyme digested the PCR product into 208- and 24-bp fragments when the A allele was present. Digestion products were separated on 8% polyacrylamide gel electrophoresis (PAGE) gel at 100 V for 3 hours and visualized by ethidium bromide staining with a UV transilluminator.

Typing of TIM-1 5383_5397 ins/del Gene Polymorphism

The 5383_5397 ins/del polymorphism was genotyped by PCR-PAGE analysis. The primer pair 5'-GAACGAGCACCACTGTTCCA-3' and 5'-TTACCTGGTTCATGGTTCTG-3' was designed according to a previously published method [25] and used to amplify the genomic region. PCR was carried out under the same conditions described in the section on -232G>A genotyping, with the exception that the annealing temperature was set at 55° C for 45 seconds. The PCR products were separated on 8% denatured polyacrylamide gel. The ins allele yielded a single band of 244 bps and the del allele yielded one of 229 bps.

Statistical Analysis

Allele frequencies at each locus were calculated and Hardy-Weinberg equilibrium was estimated using the Pearson χ^2 test. Differences in allele frequencies and genotype distribution of each polymorphism between patients and controls were also analyzed using the χ^2 test or the Fisher exact test. Allele and genotype frequencies, crude odds ratios with 95% confidence intervals, multivariate logistic regression analysis, and significance values were analyzed using version 13.0 of the SPSS statistical package (SPSS Inc., Chicago, Illinois, USA). Pairwise linkage disequilibrium and haplotype analyses were performed using the SHEsis online software [27]. IgE levels were transformed to log10 values to provide a normal

distribution for statistical analysis. Standard analysis of variance (ANOVA) and analysis of covariance (ANCOVA) for quantitative traits were used to compare mean serum IgE levels between genotype groups. A *P* value of less than .05 was considered to be statistically significant.

Results

The characteristics of the 2 groups of patients with asthma and the controls are summarized in Table 1. The distribution by sex was similar in the 3 groups. Those with atopic asthma had higher total serum IgE levels than both those with nonatopic asthma and healthy controls ($P<.001$). The allergen-specific IgE test results showed that there were 3 major environmental allergens in atopic asthmatic children from middle China: housedust mite (86%), birch and Mugwort pollen (28%) and cat or dog hair/dander (15%).

The genotype distributions of the *TIM-1* -232G>A and 5383_5397ins/del polymorphisms were in agreement with Hardy-Weinberg equilibrium ($P>.05$) in both patients and controls. The frequency of genotypes and alleles of the 2 polymorphisms are shown in Table 2. These frequencies were compared between asthma patients and controls using the Pearson χ^2 test or the Fisher exact test. No statistically

Table 2. Genotype and Allele Frequencies of *TIM-1* Polymorphisms -232G>A and 5383_5397ins/del in Patients and Controls

Position[a]	Genotype	No. (%) in Controls	No. (%) in Patients With		Crude Odds Ratio (95% CI)[b]	*P*[c]	*P*[d]
			Nonatopic Asthma	Atopic Asthma			
-232G>A							
	GG	117 (0.568)	53 (0.552)	108 (0.524)	1.00		
	AG	82 (0.398)	39 (0.406)	86 (0.417)	1.14 (0.76-1.67)	.90[c]	.41[d]
	AA	7 (0.034)	4 (0.04)	12 (0.058)	1.86 (0.71-4.89)		
	G	316 (0.767)	145 (0.755)	302 (0.733)	1.00	.75[e]	.26[d]
	A	96 (0.233)	47 (0.245)	119 (0.267)	1.20 (0.87-1.64)		
5383–5397 ins/del							
	del/del	126 (0.612)	58 (0.604)	116 (0.563)	1.00		
	ins/del	75 (0.364)	36 (0.375)	78 (0.379)	1.13 (0.75-1.69)	.97[c]	.19[d]
	ins/ins	5 (0.0243)	2 (0.021)	12 (0.058)	2.60 (0.89-7.63)		
	del	327 (0.793)	152 (0.791)	310 (0.752)	1.00	.95[e]	.16[d]
	ins	85 (0.206)	40 (0.208)	102 (0.248)	1.27 (0.91-1.75)		

Abbreviations: CI, confidence interval; del, deletion; ins, insertion.
[a] Calculated from the translation start site.
[b] Patients with atopic asthma versus controls.
[c] Patients with nonatopic asthma versus controls by Fisher exact test.
[d] Patients with atopic asthma versus controls by χ^2 test.
[e] Patients with nonatopic asthma versus controls by χ^2 test.

Table 3. Adjusted Odds Ratios with 95% Confidence Interval (CI) Between Atopic Asthma and *TIM-1* Genotypes With Adjustment for Age and Sex

Position[a]	Genotype	No. (%) in Controls	No. (%) in Patients With Atopic Asthma	Adjusted Odds Ratio (95% CI)[b]	*P*
-232G>A	GG	117 (0.568)	108 (0.524)	1.00	
	AG	82 (0.398)	86 (0.417)	1.11 (0.74-1.66)	.62
	AA	7 (0.034)	12 (0.058)	1.68 (1.65-4.52)	.36
5383_5397ins/del	del/del	126 (0.612)	116 (0.563)	1.00	
	ins/del	75 (0.364)	78 (0.379)	1.07 (0.71-1.62)	.74
	ins/ins	5 (0.0243	12 (0.058)	2.16 (0.72-6.49)	.17

[a] Calculated from the translation start site.
[b] With adjustment for age and sex.

Table 4. Haplotype Analysis of the 2 *TIM-1* Gene Polymorphisms[a]

-232G>A	5383_5397ins/del	Haplotype Frequencies		χ^2	*P*
		No. (%) in Controls	No. (%) in Patients With Asthma		
A	del	24.98 (0.061)	38.61 (0.064)	0.045	0.83
A	ins	71.02 (0.172)	118.39 (0.196)	0.902	0.34
G	del	302.02 (0.733)	423.39 (0.701)	1.235	0.27
G	ins	13.98 (0.034)	23.61 (0.039)	0.183	0.67

Abbreviations: del, deletion; ins, insertion.
[a] Overall: $\chi^2 = 1.31$, degrees of freedom = 3, P = .73.

Table 5. Association Between Total Serum Immunoglobulin (Ig) E levels and *Tim-1* -232G>A and 5383_5397 ins/del Polymorphism Genotypes

Polymorphism	Genotype	Total Serum IgE, log10 values[a]			
		Nonatopic Asthma	Atopic Asthma	*P*[b]	*P*[c]
-232G>A					
	GG	1.50 (0.46)	2.31 (0.49)		
	AG	1.56 (0.44)	2.26 (0.53)	.74	.67
	AA	1.42 (0.27)	2.37 (0.54)		
5383_5397ins/del					
	del/del	1.53 (0.46)	2.34 (0.52)		
	ins/del	1.50 (0.43)	2.28 (0.51)	.94	.014
	ins/ins	1.47 (0.50)	2.74 (0.26)		

Abbreviation: log10, logarithm base 10.
[a] Results expressed as means (SD) for different genotypes.
[b] Compared using analysis of variance for nonatopic asthma.
[c] Compared using analysis of variance for atopic asthma.

Table 6. Analysis of Covariance of the Effect of 5383_5397ins/del on Total Serum Immunoglobulin (Ig) E Levels in Patients With Atopic Asthma Following Adjustment for Age and Sex

Position	Genotype	No.	Serum total IgE (log 10 values)			
			Adjusted Mean[a]	95% CI	F	P[b]
5383_5397ins/del						
	del/del	116	2.34	2.25-2.43		
	ins/del	78	2.28	2.17-2.39	4.49	.0112
	ins/ins	12	2.75	2.46-3.04		

Abbreviations: CI, confidence interval; log10, logarithm base 10.
[a] Covariates appearing in the model are evaluated at the following values: age = 8.84, sex = 1.48 (calculated by male = 1, female = 2)
[b] Compared using analysis of covariance after adjustment for age and sex.

significant differences were detected between patients with either atopic or nonatopic asthma and controls for either of the 2 polymorphisms ($P>.05$ in all cases). Multivariate logistic regression analysis adjusted for age and sex also failed to show an association between either of the polymorphisms and susceptibility to atopic asthma (Table 3). Linkage disequilibrium analysis showed a high degree of disequilibrium for -232G>A and 5383_5397ins/del (D'=0.76), but no significant haplotypes were discovered for either of the polymorphisms (Table 4).

There was, however, a significant difference in total serum IgE levels between three 5383_5397ins/del genotypes

in children with atopic asthma when explored with ANOVA (F=4.325, $P<.05$). The mean (SD) total serum IgE log10 values for patients with the del/del, del/ins and ins/ins genotypes were 2.34 (0.52), 2.28 (0.51), and 2.74 (0.26), respectively. We observed an association between the ins/ins genotype and elevated plasma IgE levels in the atopic asthma group (Table 5). This association was still present using ANCOVA after adjustment for age and sex (F=4.49, $P=.012$) (Table 6). Conversely, there was no significant association between the -232G>A polymorphism and total serum IgE levels in either patients or healthy controls.

Discussion

Asthma is a complex disorder involving immunologic, genetic, environmental, and other factors. Allergic sensitization appears to be the strongest identifiable predisposing factor for asthma [28]. Nonallergic individuals, however, are also affected by asthma. Allergic asthma is characterized by prominently elevated IgE production and marked recruitment of mast cells, eosinophils, and basophils into the airway epithelium [29]. While previous studies have shown a linkage between asthma and 5q31-33 regions of the human genome [9-13], no specific genes or polymorphisms in this region have been definitively implicated in the pathogenesis of the disease. Asthma is thought to arise from an imbalance in T_H1-T2 PT2 immune regulation [30]. Therefore, the genes involved in the regulation of $CD4^+$ T cell differentiation might be asthma susceptibility genes. *TIM-1*, one of the recently identified genes in the 5q31-33 region, has been implicated in the regulation of T_H1 and T_H1 immune responses. It employs TIM-4 as its ligand, and TIM-1-TIM-4 interaction regulates T-cell proliferation [31]. In 1 study, the use of antibody to TIM-1 plus antigen substantially increased the production of both interleukin 4 and interferon-γ in unpolarized T cells, prevented the development of respiratory tolerance, and increased pulmonary inflammation [32].

Several recent studies analyzing the association between *TIM-1* polymorphisms and asthma susceptibility in different populations have yielded contradictory results [20-25]. One study, for example, showed that 5383_5397ins/del, located in the extracellular mucin-like domain of TIM-1, resulted in amino acid changes (157ins/del MTTTVP) and was possibly involved in regulating the activity of the TIM-1 protein [21]. The mucin-like domain is required for efficient TIM-1 ligand combinations. Because 157ins MTTTVP lengthens this critical region by 12% to 14%, this variation may affect the activity of the TIM-1 receptor [21]. Chae et al [22,33] showed that the 5383_5397ins/del polymorphism was significantly associated with atopic dermatitis susceptibility and rheumatoid arthritis in a Korean population, and McIntire et al [21] reported that it was associated with protection against atopy, but only in individuals exposed to the hepatitis A virus. In contrast, Gao et al [23] observed the association of the presumed protective allele with asthma independent of HIV status in an African American population. In addition, polymorphisms in the *TIM-1* promoter region might influence TIM-1 expression by changing the binding site of certain transcription factors. Moreover, TIM-1 is selectively expressed in T_H2 cells and directs T_H2 cytokine production, and inappropriate or excess T_H2-biased immune responses are thought to underlie the pathophysiology of asthma [34]. The evaluation of *TIM* gene family promoter polymorphisms regulating the susceptibility to asthma is thus of great value. A recent study found that 1 particular promoter polymorphism, –416G>C, was associated with asthma susceptibility and had a functional impact on transcription of the *TIM-1* gene in a Northern Chinese population [34]. It is important to determine whether these findings are replicable in other ethnic or independent populations and to examine the roles of associated variants in the development of the asthma phenotype.

The current study focused on the genetic associations between asthma and *TIM-1* polymorphisms -232G>A and 5383_5397ins/del. We found no significant association between the -232G>A promoter polymorphism and either atopic asthma or nonatopic asthma ($P>.05$) or between the 5383_5397ins/del polymorphism and asthma, which is in agreement with findings by Noguchi et al [20] and Chae et al [22]. On examining a Japanese population using family-based analysis, Noguchi et al found no association between 5383_5397ins/del and atopic asthma and Chae et al found none between 5383_5397ins/del and asthma in a Korean population. Our study suggests that the *TIM-1* gene does not contribute significantly to childhood asthma in our population (from middle China) either.

Total serum IgE levels and allergen-specific IgE levels, which are key predisposing factors for childhood asthma, appear to be determined by a combination of genetic and environmental factors [35]. There is significant evidence for a linkage between IgE phenotypes and chromosome 5q [36]. In the present study, we found a significant difference in total serum IgE levels between 3 genotypes of 5383_5397ins/del in our group of patients with atopic asthma. Those who were homozygous for the ins allele had a higher IgE level than those with the del/del or del/ins genotype, suggesting that the 5383_5397ins/del polymorphism may contribute to the genetic regulation of total serum IgE levels. Considering the influence of sex and age on total serum IgE levels, we conducted ANCOVA to remove this influence. After appropriate adjustment, the results confirmed the association between 5383_5397ins/del and total serum IgE levels (F=4.49, $P=.012$) but only in patients with atopic asthma. This may be explained by the presence of a gene-environment interaction in which 5383_5397ins/del acts as a basic modifier of atopy in individuals with different degrees of environmental allergen exposure [37]. This association is not corroborated by the findings of Noguchi et al [20], who failed to find an association between 5383_5397ins/del and total serum IgE levels using quantitative transmission disequilibrium analysis in a Japanese population. The divergent results may perhaps be due to differences in genetic and environmental factors.

In conclusion, we observed no association between 2 *TIM-1* polymorphisms (-232 G>A and 5383_5397ins/del) and asthma susceptibility in middle China. Nonetheless, we do not exclude the possibility that other *TIM-1* polymorphisms might be associated with asthma. Indeed, the identification of significant associations between total serum IgE and 5383_5397ins/del polymorphisms in individuals with asthma suggests that the *TIM-1* gene might play a role in IgE production in patients

with atopic asthma. Further studies with larger samples are required to analyze these associations.

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