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The effect of genetic variation and mRNA expression of interleukin-17A gene on susceptibility to asthma

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ABSTRACT

Analyzing the genetic variation and mRNA expression of interleukin-17A (IL-17A) gene and its impact on asthma susceptibility was the purpose of this study. 120 asthma patients were selected as the asthma group, and another 120 healthy individuals who underwent physical examination were selected as the health group; Compare the cytokine levels and mRNA expression of IL-17A between two groups, as well as the clinical indicator total immunoglobulin E (TIgE) levels; The genotype and allele distribution frequency of IL-17A Single-nucleotide polymorphism locus rs2275913 and rs8193036 were compared between the two groups; Compare the serum IL-17A and TIgE levels of different genotypes at rs2275913 and rs8193036 loci; and logistic regression was used to evaluate the impact of IL-17A on asthma susceptibility. The serum levels of IL-17A, TIgE, and IL-17AmRNA expression in the asthma group were higher than those in the healthy group (P<0.05). There were three genotypes of AA, AG and GG at rs2275913 locus, and the frequency distribution between the two groups was significant (P<0.05), and the frequency of A Allele frequency in asthma group was higher than that in healthy group (P<0.05). There are three genotypes of CC, CT, and TT at the rs8193036 locus, and there was no significant difference in the frequency distribution between the two groups (P>0.05). There is no difference in the frequency distribution of alleles C and T between the two groups (P>0.05). The levels of IL-17A and TIgE in the rs2275913AA genotype were higher than those in the AG and GG genotypes (P<0.05); There was no difference in IL-17A and TIgE levels among different genotypes of rs8193036 (P>0.05). The rs2275913 polymorphism was associated with asthma susceptibility and is an independent risk parameter for asthma susceptibility. Upregulation of serum IL-17A and TIgE, as well as overexpression of IL-17A mRNA, were closely related to asthma susceptibility in asthma patients. The rs2275913 polymorphism had a significant role in increasing the risk of asthma, and variant allele A may be a susceptibility factor for increasing asthma risk.

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Introduction

Asthma is a chronic airway disease characterized by chronic inflammation and airway hyperresponsiveness. Asthma patients typically experience recurrent symptoms such as wheezing, difficulty breathing, chest tightness, and coughing. The severity of asthma and the duration of symptoms may vary, from mild to moderate to severe (1,2). Research shows that gene Single-nucleotide polymorphism (SNP) is closely related to asthma susceptibility. Analyzing the relationship between asthma and gene SNP can help medical personnel develop individualized intervention measures for specific genotypes and expression levels, so as to better manage and control asthma (3). Interleukin-17A (IL-17A) is an inflammatory mediator produced by lymphocytes and plays an important role in the pathogenesis and development of asthma. IL-17A is a cytokine in the immune system that can activate and regulate various inflammatory reactions. In the airways of asthma patients, IL-17A levels increase, promoting the occurrence and persistence of airway inflammation. Analyzing the genetic variation, mRNA expression, and susceptibility to the asthma of the IL-17A gene is of great significance for the prevention and treatment of asthma. However, based on existing data, research on the relationship between the IL-17A gene and asthma susceptibility is limited by incomplete research sites (5). This study included the rs2275913 and rs8193036 loci in the IL-17A gene to analyze the genetic variation and mRNA expression of the IL-17A gene and its impact on asthma susceptibility. The results are reported as follows.

Materials and Methods

General information

The research subjects selected 120 asthma patients admitted to the respiratory department of our hospital from December 2021 to December 2022, and served as the asthma group, including 66 males and 54 females; Age ranged from 25 to 56 (40.12 ± 10.01) years old. 120 healthy individuals who underwent physical examination during the same period were selected as the health group, including 63 males and 57 females; Aged 24-55 (39.88 ± 9.65) years old. There was no statistically significant difference in basic data between the two groups (P>0.05). This study has been approved by the medical ethics committee of the hospital, and all selected subjects have signed relevant documents.

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Inclusion criteria: (i) Diagnosed with bronchial asthma and in accordance with the relevant standards in the "Chinese Guidelines for the Prevention and Treatment of Bronchial Asthma" (6); (ii) All healthy individuals in the health group are healthy and normal, with no previous history of asthma or family history, allergic disease or family history; (iii) The clinical data is complete.

Exclusion criteria: (i) Lung diseases other than asthma such as pneumonia and bronchopulmonary dilation; (ii) other diseases can cause rs2275913 and rs8193036 mutations; (iii) Immune deficiency diseases not caused by asthma; (iv) Combined inflammatory bowel disease; (v) Combined with Connective tissue disease.

Measurement of serum IL-17A and TIgE

2 ml of peripheral blood was taken from patients and placed in EP tubes, enzyme-linked immunoassay was applied to measure IL-17A level by adsorption method, and the kits were purchased from RapidBio Lab, USA, and chemiluminescence method was applied to measure TIgE level, and the kits were purchased from HanMind Bio-Tech Co.

IL-17A genotyping detection

Take 2 ml of the patient's peripheral blood and put it into the EP tube. A DNA extraction kit was used to extract gene DNA. The kit was purchased from Beijing Tiangen Biochemical Technology Co., Ltd. (Beijing, China). The forward primer sequence of gene rs2275913 is 5'-GCA-TAACTTCTGGCAGCTGTA-3', and the reverse primer sequence is 5'-TGCCCCACGGT CCAGAAAATAC-3'. The primer was designed and synthesized by Shanghai Yingwei Jieji Biological Co., Ltd. (Shanghai, China). The forward primer sequence at the rs8193036 locus of the gene is 5'-CAG AAG ACC TAC ATG TTA CT-3', and the reverse primer sequence is 5'- GTA GCG CTATCG TCT CTC T-3'. The primers were designed and synthesized by Beijing Dingguo Biotechnology (Beijing, China). Polymorphisms of IL-17A rs2275913 and rs8193036 were detected by polymerase chain reaction-restriction Endonuclease fragment length (PCR-RFLP). The genotype was determined by using the BOX gel imaging system, ABI 3730XL sequencer and GeneMapper 4.0 software. PCR detection of IL-17AmRNA expression.

Statistical analysis

Data were processed by the statistical software Statistic Package for Social Science (SPSS) 22.0 (IBM, Armonk, NY, USA). The count data of gender, gene distribution frequency, etc. were described by (n(%)), and the x^2 test was performed; the measure data of IL-17A, TIgE level, etc. were described by ($\bar{x}$±s), and the t-test was performed; Logistic regression was used to analyse the effect of the IL-17A gene on the susceptibility to asthma, and the relative risk degree was assessed by OR value as well as the 95% confidence interval. P<0.05 suggests that the difference is statistically significant.

Results

Comparison of serum IL-17A and TIgE levels between the two groups

Serum IL-17A and TIgE levels in the asthma group were higher than those in the healthy group (P<0.05). See Table 1, and Figure 1 for details.

Comparison of genotype and allele distribution frequencies between two groups of IL-17A rs2275913 and rs8193036

There were three genotypes AA, AG and GG at rs2275913, and their distribution frequencies in the asthma group were 26.67%, 48.33% and 25.00% respectively, while those in the healthy group were 11.67%, 55.00% and 33.33% respectively. The frequency of AA Genotype frequency in asthma group was significantly higher than that in the healthy group, and the difference between the two groups was statistically significant (P<0.05), and the frequency of A Allele frequency in asthma group (51.67%) was higher than that in the healthy group (29.17%) (P<0.05); There are three genotypes of CC, CT, and TT

Table 1. Comparison of serum IL-17A and TIgE levels between the two groups ($\bar{x}$±s).

Group	Cases	IL-17A (ng/L)	TIgE (IU/mL)
Asthma Group	120	49.84±15.36	298.82±88.96
Healthy group	120	35.24±10.05	72.69±18.52
t	-	8.715	27.261
P	-	<0.001	<0.001

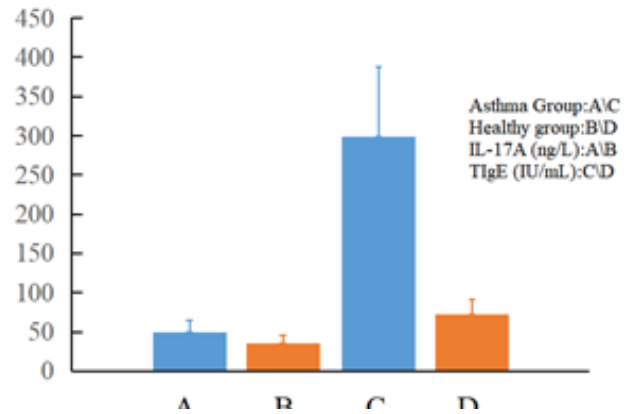

Figure 1. Comparison of serum IL-17A and TIgE levels between the two groups

Table 2. Comparison of genotype and allele distribution frequencies between two groups of IL-17A rs2275913 (n(%)).

Group	Cases	Genotype			Allele	
		AA	AG	GG	A	G
Asthma Group	120	32(26.67)	58(48.33)	30(25.00)	62(51.67)	58(48.33)
Healthy group	120	14(11.67)	66(55.00)	40(33.33)	35(29.17)	85(80.73)
x2	-	8.988			12.613	
P	-	0.011			<0.001	

Table 3. Comparison of genotype and allele distribution frequencies between two groups of IL-17A rs8193036 (n(%)).

Group	Cases	Genotype			Allele	
		CC	CT	TT	C	T
Asthma Group	120	25(20.83)	60(50.00)	35(29.17)	55(45.83)	65(54.17)
Healthy group	120	30(25.00)	68(56.67)	22(18.33)	65(54.17)	55(45.83)
x2	-	3.919			1.667	
P	-	0.141			0.197	

Table 4. Comparison of serum IL-17A and TIgE levels between different genotypes of rs2275913 and rs8193036 in the asthma group ($\bar{x}$±s).

Genotype	Cases	IL-17A (ng/L)	TIgE (IU/mL)
rs2275913			
AA	32	66.37±14.66	359.35±95.66
AG	58	45.68±10.22[a]	280.36±79.54[a]
GG	30	40.27±10.13[a]	269.94±68.33[a]
F		46.881	12.157
P		<0.001	<0.001
rs8193036			
CC	25	49.30±17.87	297.07±93.70
CT	60	50.69±14.51	287.42±80.13
TT	35	48.79±15.26	319.61±98.40
F		0.187	1.465
P		0.830	0.235

Note: [a] indicates a comparison with rs2275913AA, $P<0.05$.

Table 5. Logistic regression analysis of IL-17A gene and susceptibility to asthma.

Elements	B	Standard Error	Wald	Degree of freedom	Significance	Exp(B)	95 % confidence interval for EXP(B)	
							Lower limit	Upper limit
rs2275913A/G	0.487	0.195	6.258	1	0.012	1.628	1.111	2.385
rs8193036C/T	-0.298	0.194	2.349	1	0.125	0.742	0.507	1.087
(Constant)	-0.426	0.591	0.52	1	0.471	0.653	-	-

at the rs8193036 locus, with distribution frequencies of 20.83%, 50.00%, and 29.17% in the asthma group and 25.00%, 56.67%, and 18.33% in the healthy group, respectively. There was no statistically significant difference in frequency distribution between the two groups ($P>0.05$), and the comparison of allele C and T frequencies between the two groups was also not statistically significant ($P>0.05$). Please refer to Tables 2 and 3 for details.

Comparison of serum IL-17A and TIgE levels between different genotypes of rs2275913 and rs8193036 in asthma group

There was a statistically significant difference in the levels of IL-17A and TIgE among different genotypes of rs2275913 ($P<0.05$), with AA genotype having higher levels of IL-17A and TIgE compared to AG and GG genotypes ($P<0.05$); There was no statistically significant difference in IL-17A and TIgE levels among different genotypes of rs8193036 ($P>0.05$). Please refer to Table 4 for details.

Logistic regression analysis of IL-17A gene and susceptibility to asthma

Using asthma as the dependent variable (1=asthma group, 2=healthy group) and rs2275913 and rs8193036 genotypes as independent variables, logistic regression analysis was conducted. The results showed that rs2275913 gene polymorphism was an independent influencing factor for asthma susceptibility ($P<0.05$), while rs8193036 gene polymorphism was not significantly correlated with asthma susceptibility ($P>0.05$). Please refer to Figure 2 in Table 5 for details.

Comparison of IL-17A mRNA between the two groups

The expression level of IL-17A mRNA in the asthma group was (2.48 ± 0.23), which was higher than that in the healthy group (0.52 ± 0.12) ($t=82.805$, $P<0.001$). See Figure 3 for details.

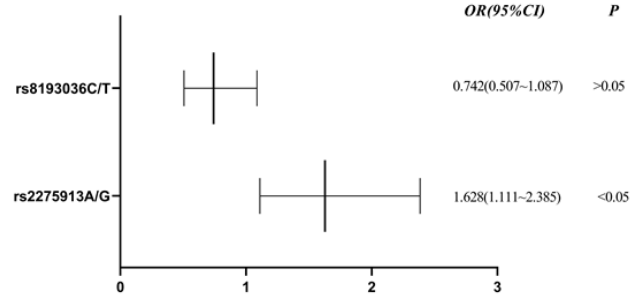

Figure 2. Forest plot of logistic regression analysis between IL-17A gene and asthma susceptibility.

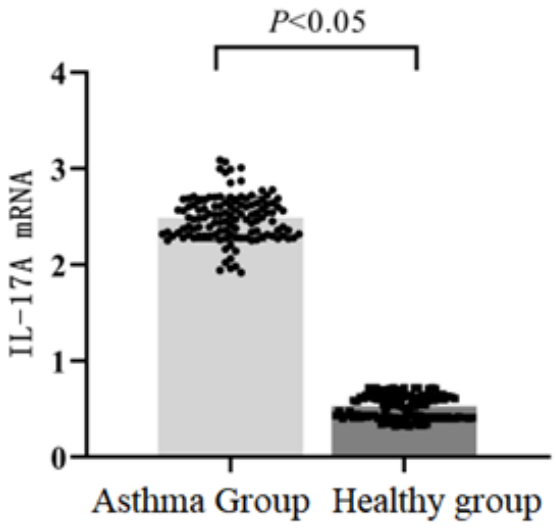

Figure 3. Comparison of IL-17A mRNA between the two groups.

Discussion

IL-17A is an inflammatory cytokine, which is closely related to the pathogenesis and susceptibility of asthma. IL-17A is involved in the regulation of immune and inflammatory responses, especially in the activation of neutrophils and lung inflammatory response (7,8). Studies have found that some SNPs of the IL-17A gene are associated with the risk of asthma, and these SNPs may affect the expression and function of IL-17A. At the same time, the mRNA expression level of IL-17A can also be used to evaluate the susceptibility to asthma. Measuring the mRNA expression level of IL-17A in the peripheral blood or airway of patients, can help to judge the susceptibility and prognosis of asthma (9). It can be seen that the genetic variation and mRNA expression level of IL-17A may be used as an auxiliary index to evaluate asthma susceptibility. However, there is no study to canonically define the genetic variation and mRNA expression level of l-17a and asthma susceptibility (10,11). Further research and validation are needed for IL-17A as an auxiliary index to assess asthma susceptibility. Further in-depth analysis is needed for some information about the genetic variation and mRNA expression of IL-17A and asthma susceptibility.

The results of this study showed that the serum levels of IL-17A and TIgE in the asthma group were higher than those in the healthy group, indicating that there was abnormal expression of serum IL-17A and TIgE levels in asthma patients. The reason may be that the immune system of asthma patients may react excessively to external stimuli, resulting in excessive enhancement of inflammatory response. IL-17A is involved in regulating the inflammatory response, and its overproduction may lead to an increased level of IL-17A in the serum of asthma patients. Total IgE in serum is a marker indicating an allergic reaction. Asthma patients are often accompanied by allergic diseases, such as hay fever, food allergy, etc. IL-17A can stimulate mast cell activation, resulting in increased synthesis and release of IgE in tissues, which leads to increased TIgE levels in serum (12,13). The expression level of IL-17A mRNA in the asthma group was (2.48 ± 0.23), which was higher than that in the healthy group (0.52 ± 0.12) (t=82.805, P<0.001). The reason may be that IL-17A can stimulate the activation and infiltration of other inflammatory cells (such as eosinophils, T cells, etc.), which play an important role in the process of airway inflammation in asthma patients, resulting in the increase of IL-17A in serum (14,15).

By analyzing the genotypes, it was found that there were AA, AG and GG genotypes at the rs2275913 locus, and their distribution frequencies were 26.67%, 48.33% and 25.00% in the asthma group, respectively. The A allele frequency in the asthma group (51.67%) was higher than that in the healthy group (29.17%); There were three genotypes of CC, CT and TT at rs8193036 locus, and their distribution frequencies were 20.83%, 50.00% and 29.17% in asthma group, respectively. There was no significant difference between the genotype expression of this locus and that of healthy people. Asthma is a complex disease, and its pathogenesis involves genetic and environmental factors. Gene polymorphisms can affect individual susceptibility to disease and pathological processes. The AA genotype of rs2275913 locus is more common in some patients with asthma, which may mean that this genotype may increase the risk of asthma in patients (16). Individuals with rs2275913 AA genotype may be more sensitive to immune responses to specific stimuli or antigens. IL-17A is an important inflammatory mediator, which can activate and promote the occurrence of inflammatory response. Therefore, AA genotype may lead to enhanced immune response to stimuli, thus triggering a more intense inflammatory response and resulting in increased levels of IL-17A and TIgE.

Logistic regression analysis was performed with asthma as the dependent variable (1=asthma group, 2=healthy group) and rs2275913 and rs8193036 genotypes as independent variables. The results showed that rs2275913 polymorphism was an independent influencing factor of asthma susceptibility. The reason may be that the genotype of this polymorphism site is related to the function of the immune system and inflammatory response. The genetic polymorphism of rs2275913 may affect the regulatory mechanism of the immune system. IL-17A is a cytokine that regulates the inflammatory response and participates in the activation of various immune cells and mediates the inflammatory response. Different genotypes may have differences in the production and activity of immune cells, thus affecting the intensity and stability of the inflammatory response. At the same time, the genotype of rs2275913 may affect the production of IL-17A and the intensity of the corresponding inflammatory response. IL-17A plays an important role in the inflammatory process, including regulating the infiltration of inflammatory cells and mediating the expression of inflammation-related genes. Different genotypes may lead to differences in the production and activity of IL-17A, and then affect the scale and duration of the inflammatory response (17-18). In addition, the genetic polymorphism of the rs2275913 locus may affect asthma susceptibility through the interaction with other genes. Asthma is a complex polygenic genetic disease, involving the interaction of multiple genes. The genotype of rs2275913 locus may interact with the variation of other genes to jointly affect the regulation of the immune system and inflammatory response, thus exerting an independent impact on asthma susceptibility.

Conflict of interests

The authors declared no conflict of interest.

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