

Effects of IL-13 gene polymorphism on the levels of serum IL-13 and total Ig-E in asthmatic children

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Background: The mechanism of asthma has not been clearly elucidated. This study was undertaken to explore the effect of IL-13 gene polymorphism on the levels of serum IL-13 and total IgE and to better understand the role of IL-13 gene polymorphism in the mechanism of pediatric asthma.

Methods: Restriction fragment length polymorphism (RFLP) method was used to detect +1923 site polymorphism of the IL-13 gene in intron 13 region and ELISA was used to detect the levels of serum IL-13 and total IgE.

Results: TT and TC gene frequencies were significantly higher in asthma group than in control group. CC type frequency was higher in the control group than in the asthma group. Serum IL-13 and total IgE levels were significantly higher in TT and TC gene types in the asthma group than in CC gene type in both groups.

Conclusion: IL-13 gene polymorphism may play an important role in the mechanism of asthma in children.

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Key words: IL-13; gene polymorphism; asthma; IgE

Introduction

Bronchial asthma is one of the most common chronic respiratory diseases during childhood. The mortality and morbidity of this disease are

increasing. Research into pathogenesis and mechanisms of asthma has long been a hot topic, and it has been shown that asthma is a genetic disease caused by multiple genes influenced by both inherited and environmental factors. As a chronic inflammatory disease characterized by hyperreaction in the airway, it is closely related to eosinophils, mast cells, T lymphocytes and their inflammatory mediators and/or cytokines.^[1-4] To understand the pathogenesis and mechanisms of asthma in children and to explore the possible relations of IL-13 gene polymorphism to asthma, we analyzed gene polymorphism at IL-13 intron 3 +1923 locus in 96 children with asthma, and tested the levels of serum IL-13 and total IgE.

Methods

Study subjects

Ninety-six children with asthma were treated at outpatient and inpatient departments from February 2001 to July 2002. They were 65 boys and 31 girls, aged from 1 to 9 years (average 2.59 ± 1.44 years). Nineteen children were diagnosed as having mild asthma, 50 middle asthma, and 27 severe asthma according to the diagnostic criteria set up by the 1998 National Pediatric Asthma Prevention and Treatment Cooperative Group of China.^[5] Control group comprised 53 healthy children, 40 males and 13 females, aged from 1 to 8 years (average 2.90 ± 1.45 years). The age, sex, nationality, residency, history of smoking, and parasites infection were not statistically different between the two groups. Children in both groups did not take any steroids or immune inhibitors one month before blood sampling.

Methods

Sample collection

In 3 ml of venous blood sample collected, 1 ml was antiangulated with EDTA-K2 for extracting genomic

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DNA, and 2 ml angulated. No hemolysis existed. The glass tubes were left still for 60 minutes and centrifuged at room temperature for 10 minutes at 1500 r/min. The serum was transferred to another clean and dry tube. The above procedure was repeated and the serum was transferred to a 1.5 ml Eppendorf tube. The samples were kept at -20°C .

Serum IL-13 and total IgE

Serum IL-13 and total IgE were tested using ELISA. The kit was bought from Sigma, USA. The test was made according to the instructions of the manufacturer.

Restriction fragment length polymorphism (RFLP) of the locus at IL-13 intron 3 +1923

DNA was extracted using small scale blood genomic DNA extraction kit (Shanghai Huasheng Biotechnical Company, Shanghai, China), and kept at -4°C . PCR primers were synthesized^[6] with sense primer: 5'-GGCTGAATATCCATGGTGTGTGTCC-3' and anti-sense primer: 5'-GGCTGAGGTCGGCTAGGCTGAAGAC-3'. In DNA amplification with 50 μl reaction volume, the following were added: template DNA 2 μg in 20 μl , ddH₂O 10.6 μl , 10 \times buffer 5 μl , MgCl₂ 2.8 μl (1.4 mmol/L), 100 mmol/L dNTP 1 μl (500 $\mu\text{mmol/L}$), 6.25 $\mu\text{mol/L}$ sense, and anti-sense primers 5 μl , respectively. Amplification program set at 94 $^{\circ}\text{C}$ 10 minutes, 94 $^{\circ}\text{C}$ 30 seconds, 58 $^{\circ}\text{C}$ 40 seconds, 72 $^{\circ}\text{C}$ 50 seconds for 38 times, and finally 72 $^{\circ}\text{C}$ for 7 minutes. Restriction endonuclease analysis was made for PCR products, when BsaAI 2 μl was added into PCR products 16 μl and 10 \times buffer 2 μl . The mixture was kept in 37 $^{\circ}\text{C}$ water bath for 4 hours. In electrophoresis at 100 V and 15 mA, PCR digested products were loaded on 2.5% agarose gel and observed using an UVP dense scanner. Results were judged for IL-13 intron 3 +1923 locus gene type: TT gene type manifested as a single band; 559 bp, CC type as 2 bands; 310 bp and 249 bp, TC type as 3 bands; 559 bp, 310 bp and 249 bp.

Statistical analysis

Student's *t* test was used for comparison of the two groups. The *F* test was used for comparison of multiple numbers. The *Q* test was used for one by one comparison. The counted data were analyzed by the chi-square test.

Results

Gene type frequency distribution of IL-13 intron 3 +1923 locus

Among the 96 children, 12 (12.5%) showed TT gene type, 43 (44.79%) TC gene type, and 41 (42.71%) CC gene type. In the control group, 0 belonged to TT type, 14 (26.42%) TC type, and 39 (73.58%) CC type. Both asthma and control groups showed a significant difference in gene frequency distribution at IL-13 intron 3 +1923 locus. TT and TC gene type frequencies were significantly higher in the asthma group than in the control group. CC type frequency was higher in the control group than in the asthma group (Table 1).

Table 1. Gene frequency at IL-13 intron 3 +1923 locus in the asthma and control groups

Group	Cases	TT	TC	CC
Asthma	96	12 (12.50%) [*]	43 (44.79%) [*]	41 (42.71%) ^{Δ}
Control	53	0 (0%)	14 (26.42%)	39 (73.58%)

* : $P=0.0054$; #: $P<0.05$; Δ : $P<0.001$.

Effect of IL-13 intron 3 +1923 locus gene type on the level of serum IL-13 and total IgE

As shown in Table 2, serum IL-13 and total IgE levels were significantly higher in TT and TC gene types in the asthma group than in CC gene type in both groups.

Discussion

IL-13 is a cytokine with multiple functions secreted by CD₄⁺ and TH₂ cells. Many studies have shown that IL-13 plays an important role in the mechanism of children's asthma, i. e. , it can directly stimulate the secretion and activity of B cell to synthesize overdue IgE in atopy children while increasing the opportunity of asthma episodes. Moreover, it is a chemical activator of eosinophils and can release a signal named eotaxin which attracts eosinophils to infiltrate into the inflammatory areas.^[7,8] In the early stage of hypersensitive reaction, IgE is able to activate eosinophiles in blood.^[9] The activated eosinophils are accumulated in the airways and release many strong base protein particles including eosinophiles cation proteins. These particles have strong detrimental and inflammatory effects on tis-

Table 2. Effect of IL-13 intron 3 +1923 locus gene type on the levels of serum IL-13 and total IgE (mean \pm SD)

Group	Asthma group			Control group	
	TT	TC	CC	TC	CC
IL-13 (ng/L)	15.61 \pm 3.08 [*]	10.78 \pm 2.34 ^{**}	6.84 \pm 1.39	6.36 \pm 0.72	3.83 \pm 1.31
Total IgE (k μ /L)	93.61 \pm 13.06 ^{Δ}	77.52 \pm 12.29 ^{Δ}	56.48 \pm 14.00	40.06 \pm 11.34	14.11 \pm 4.62

* : $P<0.01$; * * : $P<0.01$; Δ : $P<0.01$; \blacktriangle : $P<0.01$.

sues especially the mucosa of the airway, leading to inflammation and denudation of the mucosa of the airway.

In recent years, scientists in UK, the USA and Japan have investigated IL-13 gene polymorphism and found that a close relation of IL-13 gene polymorphism to asthma. However, the detailed mechanism has not yet been elucidated. In this study, RFLP method was used to detect the polymorphism of C/T at IL-13 intron 3 + 1923 locus in children with asthma and controls. The results showed that the expression of IL-13 was related to the polymorphism of IL-13 intron 3 + 1923 locus. When it was C at IL-13 intron 3 + 1923 locus, the level of IL-13 was lower. When it was T, the level of IL-13 was higher. These data suggest that there is a relationship between IL-13 gene polymorphism, serum IL-13 and IgE.

The possible mechanisms by which IL-13 gene polymorphism influence IL-13 expression are as follows. First, methylated DNA can directly interfere with the binding of transcription factor. When C is changed to T, such interference may disappear. Therefore, the speed of transcription and IL-13 expression increase. Second, methylcytidine may be combined by specific methylated DNA protein and block or replace the effects of transcription factors. The speed of transcription is decreased. If methyl cytidine is de-amino to form thymine, the blockage disappears and the speed of transcription increases. Third, intron, which is thought to have no function at all, is one of the major uncoding elements in eukaryote. But recent studies have indicated that intron has an important function for pre-RNA splicing. The point mutation in intron may cause abnormal splicing and form different proteins.^[10] Our findings in this study support that IL-13 gene polymorphism plays an important role in the pathogenesis of asthma in children.

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search Ethics, Nanjing, China.

Competing interest: None declared.

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