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Correlation of SOCS3 gene polymorphism with childhood asthma

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Abstract. – OBJECTIVE: To explore the correlation of the suppressor of cytokine signaling 3 (SOCS3) gene polymorphism with childhood asthma.

PATIENTS AND METHODS: A total of 204 asthma children (observation group) and 235 healthy children (control group) were enrolled. General clinical information of enrolled subjects was collected. Inflammatory factors and pulmonary function test indexes in each subject were examined. Moreover, the polymorphism of SOCS3 gene rs9914220 was detected with the TaqMan-MGB probe.

RESULTS: Asthma children in the observation group exhibited higher levels of interleukin-4 (IL-4), IL-17, and IL-33 than those of the control group ($p<0.05$). The forced expiratory volume in 1 second (FEV_1), FEV_1/forced vital capacity (FVC) ratio (%), peak expiratory flow (PEF), and FVC in the observation group were lower than those in the control group. However, the residual volume (RV), and RV/total lung capacity (TLC) ratio were higher in observation group than those in control group ($p<0.05$). Distribution frequency of the genotypes varied a lot between the two groups ($p<0.05$). However, we did not observe a significant difference in SOCS3 alleles between the two groups ($p>0.05$). According to the analysis of the genetic model, there were differences in dominant and cumulative models between the two groups ($p<0.05$), whereas the recessive model was not different between the two groups ($p>0.05$).

CONCLUSIONS: Levels of inflammatory factors and pulmonary functions help to effectively monitor the progression of childhood asthma, thus increasing the clinical diagnosis rate. The polymorphism of the SOCS3 gene rs9914220 site is correlated with the onset of childhood asthma.

Key Words:
Asthma in children, Inflammatory factors, Pulmonary functions, Suppressor of cytokine signaling 3, Single nucleotide polymorphism.

Introduction

Bronchial asthma is a kind of chronic non-specific inflammatory disease involving in multiple inflammatory cells, inflammatory mediators, and cytokines. The major pathological manifestations include the airway hyperresponsiveness and mucus hypersecretion. Asthma is a common respiratory disease, which is prevalent in children. It can not only cause respiratory tract abnormalities, but also affect the overall development of children. According to the epidemiological survey of childhood asthma conducted from September to December 2010 in Shanghai, the incidence of asthma reaches 5.81% among children in Shanghai, with a predicted rise each year[1]. Childhood asthma not only affects the growth and development of affected children, but also exerts a huge impact on their families and society. Early diagnosis and treatment are of great significance for childhood asthma[2]. Current studies have revealed that childhood asthma is closely associated with the local and systemic inflammatory responses. Detection of serum levels of inflammatory factors may contribute to the diagnosis of asthma[3,4]. Genetic factors also play important roles in the occurrence and development of childhood asthma. Suppressor of cytokine signaling 3 (SOCS3) is involved in the transduction of numerous signaling pathways, which is expressed *via* the induction of inflammatory factors and anti-inflammatory factors, thus serving as a key player in inflammatory diseases[5-7]. This study detected the polymorphism of the SOCS3 gene rs9914220 site in asthma children using the TaqMan-MGB probe. The correlation of SOCS3 gene polymorphism with childhood asthma was further explored. Our results provide a theoretical support for the genetic polymorphism of childhood asthma.

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Patients and Methods

Patients

Asthma children were selected from the Respiratory Medicine Department of Kaifeng Children's Hospital. Inclusive criteria were applied: (1) Patients satisfied the diagnostic criteria in *Guidelines for the Diagnosis and Prevention of Bronchial Asthma in Children* revised by the Respiratory Group, Pediatrics Society, Chinese Medical Association; (2) Deny of foreign body aspiration history; (3) Legal guardians of asthma children provided the informed consent; and (4) Those with favorable compliance and complete information. Exclusion criteria: (1) Patients with asthma due to other causes; (2) Dysfunctions of heart, kidney, liver or other major organs or (3) Patients who were complicated with mental diseases or other cognitive dysfunctions and could not cooperate. This study included 204 asthma children in observation group based on the above criteria, with 113 males and 91 females aged (7.5±1.4) years on average. Meanwhile, 235 healthy children in the same period were selected from the medical center of our hospital as the control group. There were 137 males and 98 females aged (8.5±1.3) years on average in the control group. All patieints were Chinese Han children, and their family members signed the informed consent. This investigation was approved by the Ethics Committee of Kaifeng Children's Hospital.

Collection of General Clinical Information

Name, age, sex, symptoms, signs, and examination reports of subjects were collected. 5 mL of venous blood was taken from the elbows of subjects and centrifuged at 4°C, 800 g for 5 min. The upper serum was dispensed into Eppendorf (EP) tubes (200 μL/tube) and stored at -80°C for later use. Serum levels of interleukin-4 (IL-4), IL-17, and IL-33 were determined *via* enzyme-linked immunosorbent assay (ELISA; R&D Systems, Minneapolis, MN, USA). Moreover, all subjects received pulmonary function tests. The attending physicians from the Respiratory Department conducted the above operations.

Extraction of Deoxyribonucleic Acid (DNA)

After 1 mL of venous blood was taken from the elbows of the subjects, DNA was extracted using a medium-amount whole blood genomic DNA extraction kit (BioTeke Corporation, Beijing, China) according to the instructions in the kit. Moreover, a TaqMan® single nucleotide polymorphism (SNP) genotyping assay kit (Thermo Fisher Scientific, Waltham, MA, USA) was employed to detect and analyze the genotypes of the samples (Table I).

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 20.0 software (IBM, Armonk, NY, USA) was used for statistical analysis. Measurement data were expressed as ($\chi \pm s$). The independent-samples *t*-test was employed for comparing the differences between the two groups. The likelihood-ratio chi-square (χ^2) test was performed to analyze whether the distribution of each genotype met the Hardy-Weinberg equilibrium law. R×C χ^2-test was applied for comparing the frequency of genotypes and alleles in each group. $p<0.05$ was considered statistically significant.

Results

Comparisons of Inflammatory Factor Levels

Subjects in the observation group had higher levels of IL-4, IL-17, and IL-33 than those of the control group ($p<0.05$) (Table II).

Comparisons of Pulmonary Function Test Indexes

Subjects in the observation group exhibited lower forced expiratory volume in 1 second (FEV_1), FEV_1/forced vital capacity (FVC) ratio (%), peak expiratory flow (PEF), and FVC, but

Table I. TaqMan®-MGB probe information of SOCS3 gene rs9914220.

SNP reference	rs9914220
Assay ID	C_30507543_10
SNP Type	Transition substitution, Intergenic/Unknown, Intragenic
Context sequence	CACCCCTGGTATGCACTGTGCCGTG[C/T]ACTGATTCTTGGGCAAGTTCAAATA

Table II. Comparisons of inflammatory factor levels between the two groups.

Group	No.	IL-4 (pg/L)	IL-17 (pg/L)	IL-33 (pg/L)
Observation group	204	32.08 ± 3.49	40.12 ± 5.00	127.82 ± 15.79
Control group	235	16.42 ± 2.21	21.31 ± 2.86	73.30 ± 8.20
t		5.341	4.578	3.721
p		0.012	0.024	0.038

higher residual volume (RV), and RV/total lung capacity (TLC) ratio than those of the control group ($p<0.05$) (Table III).

Genetic Equilibrium Test

The likelihood-ratio χ^2-test was conducted for the actual and theoretical frequency of three genotypes in the observation group and the control group. The frequency distributions of SOCS3 gene rs9914220 genotypes in both groups were consistent with Hardy-Weinberg equilibrium law ($p>0.05$) and comparable (Table IV).

Comparison of Genotype Distribution Frequency

The distribution frequency of genotype CC, CT, and TT in the observation group was 61.28%, 30.39%, and 8.33%, respectively, which was 61.28%, 35.74%, and 2.98% in the control group, respectively. The genotype distribution frequency was different between the two groups ($p<0.05$) (Table V).

Comparison of Allele Distribution Frequency

The distribution frequency of C and T alleles in observation group was 76.47% and 23.53%, respectively, which was 79.15% and 20.85% in control group, respectively. No significant difference in allele distribution frequency was observed between the two groups ($p>0.05$) (Table VI).

Analysis of the Genetic Model of SOCS3 Gene rs9914220

According to the genetic model analysis, there were significant differences in dominant and cumulative models between the two groups

Table III. Comparisons of pulmonary detection indexes between the two groups.

Group	No.	FEV_1 (L)	FEV_1/FVC ratio (%)	PEF (L/s)	RV (L)	RV/TLC ratio (%)	FVC (L)
Observation group	204	78.40 ± 10.72	65.42 ± 9.68	60.74 ± 8.52	3.57 ± 0.78	57.42 ± 11.32	58.42 ± 2.51
Control group	235	93.31 ± 10.28	81.50 ± 6.48	84.30 ± 8.51	2.17 ± 0.42	38.24 ± 8.21	85.41 ± 11.68
t		5.274	4.471	3.373	6.471	2.675	3.102
p		0.027	0.039	0.041	0.021	0.048	0.042

Table IV. Genetic equilibrium test of SOCS3 gene rs9914220 genotype.

Group	No.	CC		CT		TT		χ^2	*p*
		Actual frequency	Theoretical frequency	Actual frequency	Theoretical frequency	Actual frequency	Theoretical frequency		
Observation group	204	125	119.29	62	73.41	17	11.29	4.93	0.09
Control group	235	144	147.22	84	77.57	7	10.22	1.62	0.45

Table V. Comparisons of the genotype distributions of SOCS3 gene rs9914220 between the two groups.

Group	No.	Genotype [n (%)]			χ^2	*p*
		CC	CT	TT		
Observation group	204	125 (61.28)	62 (30.39)	17 (8.33)	6.668	0.036
Control group	235	144 (61.28)	84 (35.74)	7 (2.98)		

Table VI. Comparisons of the distributions of SOCS3 gene rs9914220 C/T alleles between the two groups.

Group	No.	Allele [n (%)]		χ^2	*p*
		C	T		
Observation group	204	312 (76.47)	96 (23.53)	0.910	0.340
Control group	235	372 (79.15)	98 (20.85)		

(p<0.05). However, we did not observe a remarkable difference in the recessive model between the two groups (p>0.05) (Table VII). It is suggested that the dominant and cumulative models were suitable for describing the genetic model of SOCS3 gene rs9914220 in childhood asthma.

Discussion

Childhood asthma is one of the common pediatric diseases, and its major cause is airway inflammatory response. Airway inflammation is caused by the interaction of interleukins, leukotrienes, and prostaglandins secreted by T lymphocytes, eosinophils, and other inflammatory cells. As a result, the airway remains hypersensitive to various stimulations, ultimately leading to the attack of asthma[8]. In this study, the observation group had higher levels of IL-4, IL-17, and IL-33 than those of the control group. Studies have manifested that IL-4 and IL-17 can induce the expressions of inflammatory and chemotactic factors. IL-33 is capable of activating NF-κB and MAPK signaling pathways through binding to the corresponding receptors, thereby regulating the inflammatory response[9-12]. Therefore, high levels of IL-4, IL-17, and IL-33 can promote the formation of airway hyperresponsiveness, thus triggering asthma. Based on the results of this study, we believed that high levels of IL-4, IL-17, and IL-33 were associated with the occurrence and development of childhood asthma, and they can serve as the monitoring indicators for disease judgment.

Pulmonary function test, as a kind of non-invasive detection technique, is applied to diagnose diseases in an accurate and quick way[13]. During the pathogenic process of asthma, the long-term inflammatory stimulation contributes to the airway constriction and swelling, leading to airway stenosis[14]. At the acute stage of asthma, the airway stenosis lowers the indicators like minute ventilation, but raises RV and airway resistance. Our study found that FEV_1, FEV_1/FVC ratio (%), PEF, and FVC in the observation group were lower than those in the control group, whereas RV and RV/TLC ratio were higher. It is suggested that asthma children in the observation group had evident airway function impairment and restricted exhalation flow due to airway hyperresponsiveness and airway stenosis, which was consistent with the pathological feature of asthma. Hence, during the diagnosis and treatment of childhood asthma, application of the pulmonary function test can provide auxiliary references for diagnosis and individual treatment.

Some studies[15-17] have revealed that the abnormal gene expression is closely associated with childhood asthma. SOCS3 is a member of the SOCS family, which locates on chromosome 17q25.3 and comprises 225 amino acids. SOCS3 is involved in the transduction of the Janus kinase (JAK)-STAY pathway and can be produced through the induction of transformation activators 1 and 3, with the JAKs as the target molecules. Studies[18,19] have demonstrated that SOCS3 can participate in human inflammations, immunity, occurrence and metastasis of tumors, and other related diseases. Upregulated SOCS3 in T cells is correlated with the severity of asthma, which facilitates anaphylaxis[20]. Therefore, it is presumed that SOCS3 generated through the transcription and translation of the SOCS3 gene may be correlated with the oc-

Table VII. Analyses of the genetic modes of SOCS3 gene rs9914220 in both two groups [n (%)].

	Item	Observation group	Control group	χ^2	*p*
Recessive mode	CC *vs.* CT+TT	125 (61.27)/79 (38.73)	144 (61.28)/91 (38.72)	0.000	1.000
Dominant mode	CC+CT *vs.* TT	187 (91.67)/17 (8.33)	228 (97.02)/7 (2.98)	6.058	0.014
Cumulative mode	CC *vs.* CT *vs.* TT	125 (61.28)/62 (30.39)/17 (8.33)	144 (61.28)/84 (35.74)/7 (2.98)	6.668	0.036

currence and development of childhood asthma. In this study, the SOCS3 polymorphic site rs9914220 (C/T) was selected, and the genotype frequency and allele frequency in both observation group and control group were analyzed with the TaqMan-MGB probe. The results showed that the genotype frequency of SOCS3 gene rs9914220 (C/T) was different between the two groups, indicating that the polymorphism of SOCS3 gene rs9914220 (C/T) was correlated with the incidence of asthma children. The genetic model of SOCS3 gene rs9914220 (C/T) was further analyzed. Dominant and cumulative models were different between the two groups, indicating that these two models were suitable for describing the genetic model of SOCS3 gene rs9914220 (C/T) in childhood asthma.

Conclusions

Polymorphism of SOCS3 gene rs9914220 (C/T) is a risk factor for childhood asthma, and the risk of cataract increases with the mutation of SOCS3 gene rs9914220 (C/T).

Conflict of Interest

The Authors declare that they have no conflict of interests.

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