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Correlations of LTα and NQO1 gene polymorphisms with childhood asthma

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Abstract. – OBJECTIVE: To explore the correlation of the lymphotoxin alpha (LTα) and nicotinamide adenine dinucleotide phosphate quinone oxidoreductase 1 (NQO1) gene polymorphisms with childhood asthma.

PATIENTS AND METHODS: A total of 102 asthma children (observation group) and 80 healthy children (control group) were enrolled. The information was collected via questionnaires and the polymorphisms of LTα rs2844484 and NQO1 rs2917666 were examined with the TaqMan-MGB probe.

RESULTS: Observation group had higher constituent ratios of contact with animal furs, personal history of infection, personal history of allergy, familial infection history and familial allergic history than those of control group ($p<0.05$). However, there were no differences in age, sex, passive smoking, purchase of new furniture and mask wearing between the two groups ($p>0.05$). The frequency of LTα rs2844484 genotype AA was significantly higher than that of genotype AG and GG ($p<0.01$), and NQO1 rs2917666 genotype CC showed notably higher frequency than that of genotype CG and GG ($p<0.05$). The frequency of LTα rs2844484 A allele was significantly higher than that of G allele ($p<0.01$), while NQO1 rs2917666 C allele had remarkably higher frequency than G allele ($p<0.05$). The comparisons of the recessive and additive modes revealed differences between the two groups ($p<0.05$). However, we did not observe significant difference in dominant mode between the two groups ($p>0.05$).

CONCLUSIONS: The risk factors for childhood asthma include the contact with animal furs, personal history of infection, personal history of allergy, familial infection history and familial allergic history. Polymorphisms of LTα and NQO1 genes are correlated with childhood asthma.

Key Words:
Childhood asthma, Risk factors, Lymphotoxin alpha, Nicotinamide adenine dinucleotide phosphate, Quinone oxidoreductase 1, Single nucleotide polymorphism.

Introduction

Bronchial asthma, a common allergic disorder among children, is a chronic airway inflammatory disease. It mainly involves mastocytes, T-lymphocytes, eosinophilic granulocytes. Clinically, it tends to occur with the symptoms of recurrent dyspnea, wheezing, cough and chest distress. According to an epidemiological survey, the current number of asthmatic patients in the world is approximately 0.334 billion[1]. And in 2010, the prevalence rate of asthma among Chinese children was about 0.42-5.73%, the mean of which was 2.32%[2,3]. In recent years, the incidence rate of childhood asthma has been increasing each year, which means a serious risk to the physical and mental health of children[4]. Therefore, it is of great significance to foresee the risk of asthma incidence, but there was no sufficient evidence for the identification of asthma susceptibility gene yet both at home and abroad[5,6]. Lymphoxin alpha (LTα), an immune-related factor, has similar molecular structure and active region to those of tumor necrosis factor-α (TNF-α). Moreover, it is closely linked to TNF-α. Recently, some studies have showed that TNF-α may be one of the crucial candidate sites for the susceptibility to bronchitis[7-9], but the correlations of LTα with asthma and other respiratory diseases have not been found out yet. Nicotinamide adenine dinucleotide phosphate quinone oxidoreductase 1 (NQO1) is a kind of phase II reactive enzyme. And its main functions are to reduce quinones, eliminate oxygen free radicals and improve the toxic effects of exogenous substances on cells. The current studies[10,11]

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have only suggested that NQO1 is correlated with the susceptibility to respiratory cancers. Its correlation with asthma, however, has not been proved yet. Therefore, in the hope of providing a theoretical support for the genetic polymorphism of asthma among children, we enrolled asthma children in our department with LTα and NQO1 genes as the candidates. It aimed to detect the polymorphisms of LTα rs2844484 and NQO1 rs2917666 sites with the TaqMan-MGB probe, and explore the correlation of LTα and NQO1 gene polymorphisms with the onset of childhood asthma.

Patients and Methods

Patients

This study included the asthma children admitted to and treated in Pediatrics Department of Jinan Zhangqiu District Hospital of TCM (Jinan, China). They were diagnosed based on the criteria in Guidelines for the Diagnosis and Prevention of Bronchial Asthma in Children (2016), with exclusion criteria as follows: 1) those complicated with other respiratory wheezing disorders, 2) those accompanied by the infectious diseases in other systems or with growth retardation 3) those suffering from dysfunctions of heart, kidney, liver or any other major organs. A total of 102 asthma children were selected as the observation group based on the above criteria, and they were aged 5.60±0.50 years old on average. Meanwhile, 80 healthy children were selected in our hospital as the control group and they were aged 5.70±0.30years old on average. All study patients were unrelated children of Han nationality, and they signed the informed consent. And this study was approved by the Ethics Committee of Jinan Zhangqiu District Hospital of TCM (Jinan, China).

Methods

Questionnaires

After the survey subjects, their guardians provided the informed consent and signed it. Besides, they were also informed of the content of this study, the biological samples and the news that general personal information would be collected, etc. The questionnaires were filled out truthfully. And their content involved demographic characteristics (such as sex and age), exposure to environments (such as exposure to passive smoking, purchase of new furniture and pet feeding), living habits (like wearing a mask), and medical history (including personal and familial histories of allergy and infection).

Extraction of Deoxyribonucleic Acid (DNA)

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

Statistical Analysis

The software called Statistical Product and Service Solutions (SPSS) 20.0 (Armonk, NY, USA) was used for statistical analysis. The measurement data were expressed as ($\overline{x}$±s). The independent-samples *t*-test was employed for the comparisons of measurement data between the two groups, and χ^2-test was adopted for the comparisons of count data. The likelihood-ratio χ^2 test was performed to analyze whether the genotype distribution met the Hardy-Weinberg equilibrium law. R×C χ^2-test was applied for the comparison of the frequency of genotypes and alleles in each group. $p<0.05$ suggested that there were significant difference in the statistics.

Results

Comparison of General Information

The constituent ratios of contact with animal furs, personal history of infection and allergy,

Table I. TaqMan®-MGB probe information of LTα rs2844484 and NQO1 rs2917666 sites.

SNP reference	rs2844484	rs2917666
Assay ID	C__2451915_10	C__15936599_10
SNP type	Intron	Intron
Context sequence	TATTAATTGGGATGTGTTTAGATTT[A/G] AGGTTAGGGTTACAGTTG GGGTTGA	GGGGTCCAGAGACTCAAGTTCCTAA[C/G] GAGGCTGAAGTAAGAGT GGGGGATT

Table II. Comparison of general information between the two groups.

Variable	Observation group	Control group	t/χ^2	p
Age	5.60±0.50	5.70±0.30	0.684	0.496
Sex				
Male	59 (57.84)	37 (46.25)		
Female	43 (42.16)	43 (53.75)	2.418	0.120
Passive smoking				
Yes	17 (16.67)	17 (21.25)		
No	85 (83.33)	63 (78.75)	0.620	0.431
Purchase of new furniture				
Yes	30 (29.41)	21 (26.25)		
No	72 (70.59)	59 (73.75)	0.222	0.637
Contact with animal furs				
Yes	28 (27.41)	6 (7.50)		
No	74 (72.55)	74 (92.50)	11.748	0.001
Mask wearing				
Yes	30 (29.41)	27 (33.75)		
No	72 (70.59)	53 (66.25)	0.392	0.531
Personal history of infection				
Yes	47 (46.08)	8 (10.00)		
No	55 (53.92)	72 (90.00)	27.672	0.000
Personal history of allergy				
Yes	70 (68.63)	4 (5.00)		
No	32 (31.37)	76 (95.00)	75.231	0.000
Familial infection history				
Yes	34 (33.33)	5 (6.25)		
No	68 (66.67)	75 (93.75)	19.533	0.000
Familial allergic history				
Yes	58 (56.86)	33 (41.25)		
No	44 (43.13)	47 (58.75)	4.372	0.037

and familial history of infection and allergy were higher in the observation group than those in the control group ($p<0.05$). However, there were no differences in age, sex, passive smoking, purchase of new furniture and mask wearing between the two groups ($p>0.05$) (Table II).

Genetic Equilibrium Test

The likelihood-ratio test on χ^2 was conducted for the practical and theoretical frequency of LTα rs2844484 and NQO1 rs2917666 genotypes in observation group and control group. The frequency distributions of LTα rs2844484 and NQO1 rs2917666 genotypes in both groups were consistent with Hardy-Weinberg equilibrium law ($p>0.05$) and they were comparable (Table III and IV).

Comparison of Genotype Distribution Frequency

The distribution frequency of the three genotypes of LTα rs2844484 was different between the two groups, and the frequency of genotype AA was significantly higher than that of genotype AG and GG ($p<0.01$). Besides, it also showed the difference in the distribution frequency of the three genotypes of NQO1 rs2917666 between the two groups, and genotype CC had higher frequency than genotype CG and GG ($p<0.05$) (Table V).

Comparison of Allele Distribution Frequency

The distribution frequency of LTα rs2844484 alleles was different between the two groups, and

Table III. Genetic equilibrium test of LTα rs2844484 genotype.

		AA		GA		GG			
Group	n	Actual frequency	Theoretical frequency	Actual frequency	Theoretical frequency	Actual frequency	Theoretical frequency	χ^2	p
Observation group	102	65	62.75	30	34.51	7	4.75	1.74	0.42
Control group	80	29	33.80	46	36.40	5	9.80	5.56	0.06

Table IV. Genetic equilibrium test of NQO1 rs2917666 genotype.

		CC		CG		GG			
Group	n	Actual frequency	Theoretical frequency	Actual frequency	Theoretical frequency	Actual frequency	Theoretical frequency	χ^2	p
Observation group	102	74	74.21	26	25.59	2	2.21	0.03	0.99
Control group	80	70	69.38	9	10.24	1	0.38	1.18	0.55

A allele had substantially higher frequency than G allele (p<0.01). It also showed the difference in the distribution frequency of NQO1 rs2917666 alleles between the two groups, and the frequency of C allele was higher than that of G allele (p<0.05) (Table VI).

Analyses of the Genetic Modes of LTα rs2844484 and NQO1 rs2917666

According to the genetic mode analysis, there were differences in recessive and additive modes between the two groups (p<0.05). However, the dominant mode showed no difference between the two groups (p>0.05), suggesting that the recessive and additive modes are suitable for describing the genetic modes of LTα rs2844484 and NQO1 rs2917666 (Table VII).

Discussion

Asthma is a common and frequently occurring disease. It can attack people at any age, but its incidence rate is higher in the group of children. The causes of this disease include environmental and individual genetic factors. And the results of the present study manifested that the observation group had higher constituent ratios of contact with animal furs, personal history of infection and allergy, familial history of infection and allergy than the control group (p<0.05). Animal furs can increase the concentrations of allergens in air, thereby leading to allergic asthma. Other scholars with similar results of studies also revealed that the close contact with pets might be the factor that affects the allergic respiratory diseases among children[12-14]. Asthma is closely associated with respiratory tract infections, of which the upper respiratory tract infection is often the inducing factor for asthma. And most of the asthmatic patients, especially children, normally exhibit aggravated symptoms after respiratory tract infections[15-18]. Additionally, asthma is a kind of allergic disease, which are generally hereditary. Once born, infants with the family history of asthma mostly have allergic constitution. These chil-

Table V. Comparisons of the genotype distributions of LTα rs2844484 and NQO1 rs2917666 between the two groups.

Gene	SNP code	Genotype	Observation group	Control group	χ^2	p
LTα	rs2844484	AA/AG/GG	65(63.73)/30(29.41)/7 (6.86)	29(36.25)/46 (57.50)/5 (6.25)	15.050	0.001
NQO1	rs2917666	CC/CG/GG	74(72.55)/26 (25.49)/2 (1.96)	70 (87.50)/9 (11.25)/1 (1.25)	6.132	0.047

Table VI. Comparisons of LTα rs2844484 and NQO1 rs2917666 alleles between the two groups.

Gene	SNP code	Genotype	Observation group	Control group	χ^2	p
LTα	rs2844484	A/G	160 (78.43)/44 (21.57)	104 (65.00)/56 (35.00)	8.119	0.004
NQO1	rs2917666	C/G	174 (85.29)/30 (14.71)	149 (93.13)/11 (6.87)	5.502	0.019

Table VII. Analyses of the genetic modes of LTα rs2844484 and NQO1 rs2917666 in the two groups [n (%)].

	Item	Observation group	Control group	χ^2	p
LTα rs2844484					
Recessive mode	AA vs. AG+GG	65 (63.73)/37 (36.27)	29 (36.25)/51 (63.75)	13.553	0.000
Dominant mode	AA+AG vs. GG	95 (93.14)/7 (6.86)	75 (93.75)/5 (6.25)	0.027	0.869
Additive mode	AA vs. AG vs. GG	65 (63.73)/30 (29.41)/7 (6.86)	29 (36.25)/46 (57.50)/5 (6.25)	15.050	0.001
NQO1 rs2917666					
Recessive mode	CC vs. CG+GG	74 (72.55)/28 (27.45)	70 (87.50)/10 (12.50)	6.067	0.014
Dominant mode	CC+CG vs. GG	CC+CG vs. GG	79 (98.75)/1 (1.25)	0.140	0.709
Additive mode	CC vs. CG vs. GG	74 (72.55)/26 (25.49)/2 (1.96)	70 (87.50)/9 (11.25)/1 (1.25)	6.132	0.047

dren are more sensitive to external stimulation, thus more susceptible to asthma. Therefore, pet feeding history, individual infection and allergic history, and familial infection and allergic history are the risk factors for childhood asthma. Early clinical prevention ought to be conducted for these children patients, so as to reduce the incidence rate of asthma. LTα is located on the MHC class III region of the short arm of chromosome 6. It is a kind of cytokine produced in parts of autoimmune diseases and tumors after the stimulation of mitogen and lymphocyte antigen. Since having similar structure to the TNF-α, LTα belongs to the tumor necrosis factor family, and it can affect cell apoptosis and regulate inflammatory immunity[19]. NQO1 is not only a key bi-electronic reductase but also an important cytosolic enzyme. NQO1, since located on autosome 16q22, can catalytically reduce the substrates of NADH/NADPH into more stable hydroquinones. Therefore, it can decrease the generation of free radicals, eliminate free radicals, protect normal cells from oxidative stress damage and prevent quinone carcinogenesis[20]. In this study, LTα rs2844484 (A/G) and NQO1 rs2917666 (C/G) were selected, and the genotype frequency and allele frequency in both of the observation group and the control group were analyzed with the TaqMan-MGB probe. The results showed that genotype AA had significantly higher frequency than genotype AG and GG, while the frequency of A allele was substantially higher than that of G allele. The three genotypes of NQO1 rs2917666 were also different between the two groups. The frequency of genotype CC was higher than that of genotype CG and GG, while C allele had higher frequency than G allele. The above results indicated that LTα and NQO1 genes are closely related to childhood asthma. Finally, we analyzed the genetic modes of LTα rs2844484 (A/G) and NQO1 rs2917666 (C/G), and discovered that the recessive and additive modes of these two gene sites were different between the two groups. However, there was no difference in dominant mode, indicating that recessive and additive modes are suitable for describing the genetic modes of LTα rs2844484 and NQO1 rs2917666.

Conclusions

The polymorphisms of LTα rs2844484 (A/G) and NQO1 rs2917666 (C/G) are correlated with the risk of childhood asthma, and it increases due to the dominant homozygous mutations of LTα rs2844484 (A/G) and NQO1 rs2917666 (C/G). Moreover, the recessive and additive modes are suitable for representing the genetic modes of LTα rs2844484 and NQO1 rs2917666.

Conflict of Interests

The Authors declare that they have no conflict of interests.

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