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Original Article

Correlations of IL6R gene polymorphism with pediatric asthma susceptibility

Zongjun Yu, Cuizhen Chen, Yan Ma

Department of Pediatrics, Shandong Provincial Third Hospital, Jinan City 250000, Shandong Province, P.R. China

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Abstract: Objective: To characterize the correlations between IL-6R gene polymorphism and pediatric bronchial asthma susceptibility, and to compare the serum levels of upstream IL-6 and downstream total IgE concentrations of IL-6R between children with bronchial asthma and healthy controls. Methods: A total of 260 patients were selected among the Han children with bronchial asthma admitted to the Department of Pediatrics in the Shandong Provincial Third Hospital and assigned to the case group; meanwhile, 300 healthy Han children were assigned to the control group. Blood samples were collected from peripheral veins of the enrolled children, and the DNA was extracted. TaqMan probes and ABI7300 PCR devices were applied to detect the allele and genotype frequencies of IL-6R rs12083537 and rs4845374 SNP loci in children of the two groups. The disparities in the distribution of allele and genotype frequencies between the two groups were compared with the application of the chi-square tests and the Fisher's exact tests. The Hardy Weinberg equilibrium was analyzed by the Stata software, version 11. The serum levels of IL-6 and total IgE concentrations were measured using the ELISA. Results: The genotype distribution of IL-6R rs4845374 and rs12083537 loci of all the children in both groups were in line with the Hardy Weinberg equilibrium. The IL-6R rs12083537 genotype (P=0.027) and allele (P=0.002) frequency distribution in children were markedly different in the two study groups; but the IL-6R rs4845374 genotypes (P=0.129) and allele (P=0.101) were similar; the AA genotype was a risk factor for pediatric bronchial asthma (P=0.019).The serum levels of IL-6 and total IgE concentrations among children in the case group were substantially higher than those in the control group (Both P<0.05), with considerably elevated serum total IgE concentrations in children with GG, AG and AA genotypes in the case group (All P<0.05). Conclusion: There was a correlation between polymorphism of IL-6R rs12083537 locus and pediatric bronchial asthma, and the AA genotype is one of the risk factors for asthma in childhood.

Keywords: Bronchial asthma, interleukin-6, gene polymorphism, disease susceptibility

Introduction

Bronchial asthma is a common respiratory disorder in childhood, with mobility and mortality increasing on a yearly basis. The disease is detrimental to the health of children [1, 2]. However, the pathogenesis of bronchial asthma in children is not fully known. Up to date, bronchial asthma is considered to be an allergic disease characterized by chronic airway inflammation. It primarily manifests a reduced production of Th1 cytokines and an increased secretion of Th2 cytokines, leading to increased IgE synthesis. This in turn may directly or indirectly induce airway inflammation and hyperresponsiveness in eosinophils and mastocytes [3-5]. The alleles of the promoter and regulatory regions of Th1 and Th2 cytokines vary greatly. The allele polymorphism may affect the synthesis of cytokines, which further complicates the pathogenesis of asthma. Growing attention has been paid to explore the association between the gene polymorphism of cytokines and the development of asthma [6, 7]. Multiple genes have been proven to be implicated in or regulate the immune response in organisms, or determine the differences in individual susceptibility [8, 9].

IL-6 is a specific cytokine secreted by Th2 cells, which plays crucial roles in inflammation and immune response [10, 11]. The results of previous studies have demonstrated that the serum level of IL-6 is positively correlated with the eosinophil level in the pathogenesis of asthma [12]. IL-6 can trigger activation of T lympho-

cytes and B lymphocytes, and induce the proliferation of IgE antibody and regulation of the synthesis and release of inflammatory mediators over the acute asthma period. An association exits between the serum level of IL-6 and bronchial asthma [13]. The biological role of IL-6 realizes mainly by binding to the IL-6 receptor (IL-6R) [14]. There are 232 single nucleotide polymorphism (SNP) loci of IL-6R gene in the Genbank database, but the data on the allele frequency distribution are not available for most of the loci. Hawkins et al reported a close association of the polymorphism of IL-6R rs4129267 locus with asthma, and IL-6R rs4129267 mutation from T allele to C may affect the interaction between IL-6 and receptor [15]. Other studies reported that the IL-6R rs4129267 loci polymorphism was related to asthma susceptibility [16]. Nevertheless, few reports cover the distribution of other IL-6R genetic polymorphism in Han children and its correlation with bronchial asthma. Therefore, in our present study, we selected IL-6 rs12083537 and rs4845374 loci from the SNPs database and enrolled Han children with asthma as subjects to investigate the correlation between IL-6 rs12083537 and rs4845374 polymorphism and pediatric bronchial asthma, with an aim to lay experimental basis for clinical interventions in asthma.

Materials and methods

Patients

From January 2014 to December 2016, 260 Han children with bronchial asthma admitted to the Department of Pediatrics in the Shandong Provincial Third Hospital were recruited and assigned to the bronchial asthma group (case group), including 182 male patients and 78 female ones, with a mean age of 6.3±1.4 years. Eligible children were those met the criteria for diagnosis of pediatric bronchial asthma. Additionally, 300 healthy Han children who had received physical examination in the same hospital were enrolled as controls (control group), including 213 males and 87 females, with a mean age 6.6±1.7 years. Healthy children were eligible for the study if they had no previous allergic disease such as bronchial asthma, allergic rhinitis, or eczema, nor a recent history of infection. Exclusion criteria were recurrent allergic disease, bronchiectasis, chronic obstructive pulmonary disease and interstitial lung disease, severe visceral dysfunction involving in the livers or the kidneys, or children who were unable to follow the study. The sex ratio and age distribution of children were well balanced among the children in the two groups (P>0.05); hence they were comparable. The patients or their guardians in both groups were informed of the study and provided written informed consent voluntarily. This study obtained approval from the Hospital Ethics Committee.

Methods

Genomic DNA extraction: A blood sample (5 ml) was collected from peripheral vein of each eligible child, followed by EDTA anticoagulation and protease K digestion. DNA was extracted from the sample and purified by salt induced precipitation before storage in the refrigerator at -80°C.

Primers design and synthesis: Primer sequence was designed based on the GenBank database. The rs4845374 locus forward primer was 5'-CGACTCTTACGATGATAAC-3', and the reverse primer 5'-ATGTGATCAAAGCCTGCCTGCA-3'; the rs12083537 locus forward primer was 5'-TCCGCGCTCAATTCGCTATT-3' and the reverse primer 5'-GCGTCCGTTGTGCCAGATTCA-3'. All the primers were then synthesized.

PCR reaction: The mixture containing 0.5 μL of dNTPs at a dose of 2.5 mol per liter, 0.4 μL of each primer, 2.5 μL of 10*PCR buffer, 0.3 μL of Taq DNA polymerase, 1 μL of DNA template, and 19.9 μL of ddH_2O were amplified in a 7300 PCR amplification device (ABI, US). The conditions for PCR amplification were predenaturing at 95°C for 4 min, denaturing at 95°C for 30 s, pre-annealing at 58°C for 40 s, extension at 72°C for 1 min, with 40 cycles in total.

SNP polymorphism for rs4845374 and rs12083537 loci in PCR-RFLP analysis: The PCR products and restriction endonuclease reaction system were mixed and reacted at 37°C for 15 h, and then isolated by 2% agarose gel electrophoresis, followed by judgement of the genotyping results. The wild type was GG genotype, the mutant type was AA genotype, and the heterozygous type was AG genotype.

Table 1. Genotype and allele frequency distribution of IL-6R rs12083537 locus

rs12083537	Case group (n=260/%)	Control group (n=300/%)	X^2	P
Genotype			7.152	0.027
G/G	151 (58.1)	220 (73.3)		
A/G	78 (30.0)	66 (22.0)		
A/A	31 (11.9)	14 (4.7)		
Allele			9.572	0.002
G	195 (75)	251 (83.7)		
A	65 (25)	49 (16.3)		

Table 2. Genotype and allele frequency distribution of IL-6R rs4845374 locus

rs4845374	Case group (n=260/%)	Control group (n=300/%)	X^2	P
Genotype			3.702	0.129
G/G	223 (85.8)	269 (89.6)		
A/G	29 (11.2)	25 (8.3)		
A/A	8 (3.1)	6 (2.0)		
Allele			4.118	0.101
G	233 (89.6)	288 (96.0)		
A	27 (10.4)	12 (4.0)		

Table 3. Values assigned to the variables

Variable	Assigned value
GG genotype	Control
AG genotype	No:0; Yes:1
AA genotype	No:0; Yes:1
Allele	0:G; 1:A

Sequencing verification: After PCR-RFLP analysis, 10 samples were randomly selected from each genotype, and the PCR products were sequenced, followed by verification by both forward and reverse sequencing.

Detection of serum levels of IL-6 and IgE concentrations: The serum levels of upstream IL-6 and downstream total IgE concentrations of IL-6R were detected for further assessment of the values of IL-6R gene polymorphism. A venous blood sample (5 mL) was drawn from each fasting patient in the case group and the control group. Ethylenediaminetetraacetic Acid (EDTA) was utilized as an anticoagulant, and the serum was centrifuged at 2000 rpm for 5 min before storage in a refrigerator at -80°C. The serum levels of IL-6 and total IgE concentrations were tested with the use of the enzyme linked immunosorbent assay (ELISA). The samples, standards, and the HRP-conjugated antibodies were orderly added to each micro-well pre-coated with IL-6 and IgE antibodies, incubated and washed thoroughly. After staining, the optical density (OD) value of each well at 450 nm was read using a microplate reader, and the standard curves were drawn, followed by calculation of the concentration of each sample.

Statistical analysis

All the experimental data were analyzed with the use of the SPSS software, version 19.0. Measurement data were presented as mean and standard deviation; the independent two-sample t-test was utilized for between-group comparisons. The Stata software (version 11) was employed to test whether the IL-6R rs4845374 and rs12083537 genotypes of children in the case group and the control group conformed to the Hardy-Weinberg (HWE) equilibrium. The allele and genotype frequency distribution of IL-6R rs4845374 and rs12083537 loci in the case group and the control group were compared using the chi-square test. Logistics regression analysis was conducted to evaluate the correlation between the IL-6R rs12083537 genotypes and bronchial asthma in children. A *P* value of less than 0.05 was deemed as significant difference.

Results

IL-6R genotypes and allele frequency distribution

The frequency distribution of IL-6R rs12083537 and rs4845374 genotypes in the two groups conformed to the HWE equilibrium. For rs12083537, the genotype and allele frequencies were strikingly different between the two groups (X^2=7.152, P=0.027; X^2=9.572, P=0.002), as shown in **Table 1**. The result of the IL-6R rs4845374 genotyping demonstrated small differences between the two groups in the genotype and allele frequency distribution (**Table 2**).

Risk factors for genotype for IL-6R rs12083537 locus in children with bronchial asthma in logistic regression analysis

Logistic regression analysis was employed to explore whether the genotypes of IL-6R rs-

Table 4. Risk factor for bronchial asthma in children

Variable	B	SE	Wald	P	OR	95% IC
AA genotype	1.486	0.391	9.302	0.019	2.892	1.869-3.611
AG genotype	0.237	0.785	7.041	0.071	1.266	0.881-1.588
G Allele	0.279	0.628	8.397	0.084	1.308	0.912-1.691

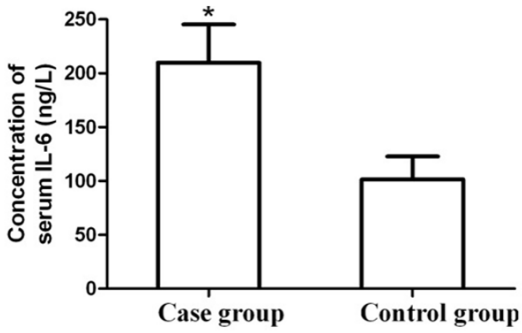

Figure 1. Comparison of serum levels of IL-6 between the two groups. Compared with the control group, *P<0.001.

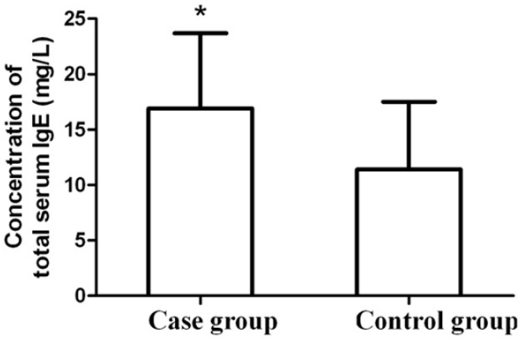

Figure 2. Comparison of serum total IgE concentrations between the two groups. Compared with the control group, *P<0.001.

12083537 locus is a risk factor for bronchial asthma in children, with bronchial asthma included as a dependent variable and genotypes and alleles of IL-6R rs12083537 locus as independent variables. The results showed that AA genotype was a risk factor for bronchial asthma in children (**Tables 3** and **4**).

Serum levels of IL-6 and serum total IgE concentrations

The results of the ELISA suggest that serum levels of IL-6 in patients differed significantly between the two groups (209.8±35.6 ng per liter in the case group vs. 101.5±21.4 ng per liter in the control group; t=22.472, P<0.001), so did the serum total IgE concentration (16.9±6.8 mg per liter vs. 11.4±6.1 mg per liter; t=21.792, P<0.001), as shown in **Figures 1** and **2**.

Comparison of serum levels of IL-6 and serum total IgE concentrations among children with different genotypes

The serum total IgE concentrations were basically similar in children with different genotypes within the same group (P>0.05), but the corresponding concentrations were significantly higher in children with different genotypes in the case group than those in the control group (P<0.05; **Table 5**).

The serum levels of IL-6 were higher in children with different genotypes in the case group than those in the control group (P<0.001); nevertheless, insignificant differences were seen in the serum levels of IL-6 in children with different genotypes between the two groups (P>0.05; **Table 6**).

Discussion

Pediatric bronchial asthma results from the influence of both genetic and environmental factors [17]. Approximately 70-80% of asthma attribute to genetic factors [18]. In essence, pediatric bronchial asthma is a multigene genetic disease with evident genetic susceptibility. Therefore, the analysis on the role of susceptible genes in the onset and development of pediatric asthma has become a hotspot and challenge in this field.

Inflammatory response plays a key role in the onset and development of bronchial asthma in children, and IL-6 contributes a lot to the inflammatory response. IL-6 is secreted by a wide range of cells, such as vascular endothelial cells, lymphocytes, and fibroblasts. IL-6/IL-6R signaling pathway regulates the immune responses of diverse subsets of CD4T cells. Macrophages and other innate immune cells can also interact smoothly with cell subsets via IL-6/IL-6R signaling pathway. IL-6R is primarily composed of 468 amino acids, including 449 amino acids in mature molecules, with 82, 28 and 339 amino acids in cytoplasmic domain, transmembrane domain and outer cytomem-

Table 5. Serum total IgE concentrations in children with different genotypes (mg/L)

Variable	Case	Genotype			F	P
		GG	AG	AA		
Case group	260	17.5±7.4	17.2±7.1	16.8±6.2	0.049	0.247
Control group	300	12.6±6.3	12.1±6.7	9.6±5.5	0.747	0.159
t		3.189	2.343	2.638		
P		0.002	0.029	0.015		

Table 6. Serum levels of IL-6 in children with different genotypes (mg/L)

Variable	Case	Genotype			F	P
		GG	AG	AA		
Case group	260	220.1±40.2	215.3±37.8	194.0±30.4	0.054	0.236
Control group	300	114.6±24.3	105.8±22.5	84.1±17,6	0.626	0.187
t		17.2739	22.162	19.472		
P		<0.001	<0.001	<0.001		

brane domain respectively, and 6 N glycosylation loci. The IL-6R gene polymorphism has shown to be associated with the asthma severity and lung function [19, 20]. In asthmatic patients, the IL-6R gene mutation is also associated with the sensitivity of bronchodilators including salbutamol [21, 22]. No prior researchers have reported the correlation between the IL-6R rs12083537 and rs4845374 polymorphism and bronchial asthma susceptibility in children described in this study.

The results of previous studies indicate that the associations between SNP polymorphism and pediatric bronchial asthma vary greatly in different areas. The polymorphism of IL-13 rs1295686 locus is related to children with asthma in the central part of China, but unrelated to children with the same disease in Shanghai [23]. Notably, the study site and the population stratification may contribute to the discrepancy in the results of analysis on genetic polymorphism. In our current study, we included the local Han children with asthma at the study site. We found that the distribution of IL-6R rs12083537 and rs4845374 genotype frequencies in the two groups accorded with the Hardy Weinberg equilibrium, indicating that they were in the genetic equilibrium. The distribution of IL-6R rs12083537 genotype and allele frequencies in children differed greatly between the two groups ($P<0.05$); nevertheless, the IL-6R rs4845374 genotype and allele frequencies were different slightly, indicating that the polymorphism of IL-6R rs12083537 locus was associated with bronchial asthma susceptibility in children. Further analysis confirmed that AA genotype was a risk factor for bronchial asthma attacks in children. This indicates that children with AA genotype have a markedly higher risk for asthma than other children. Moreover, the serum total IgE concentrations and the serum level of IL-6 were remarkably higher in the case group than those in the control group; they were also much higher in children with each genotype in the case group, suggesting that allele G mutation had no significant correlations with the serum IgE total concentration and IL-6 levels in children with bronchial asthma.

In conclusion, in our present study, we found that there was a correlation between IL-6R rs12083537 loci gene polymorphism in Han children and bronchial asthma susceptibility. However, there are some limits in this study. For example, it did not cover all the SNP loci of IL-6R, the sample size was small, and only the local Han children at the study site were included. Therefore, the sample size needs expanding or multicenter trials with larger sample size are required for further validation. In this way, we can provide more experimental evidence for early diagnosis of asthma in children and lay a theoretical basis for determining individuals susceptible to asthma and gene therapy.

Address correspondence to: Yan Ma, Department of Pediatrics, Shandong Provincial Third Hospital, No.12, Wuyingshan Road, Tianqiao District, Jinan City 250000, Shandong Province, P.R. China, Tel: +86-0531-85953277; Fax: +86-0531-85953277; E-mail: yanma69@126.com

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