Association of asthma and transforming growth factor-β_1 polymorphism in children

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***Background*: There are few reports on the relationship of transforming growth factor-β_1 (TGF-β_1) and asthma. This study was undertaken to explore the association of TGF-β_1 gene polymorphism with asthma in children.**

***Mothods*: Ninety-eight children with asthma and 52 normal children were enrolled. The TGF-β_1 gene -509C/T polymorphism in the 5′-flanking region was detected using the polymerse chain reaction-restric tion fragment length polymorphism (PCR-RFLP) technique. The total serum IgE level was examined by the sandwich ELISA method.**

***Results*: No significant differences were found in genotype distribution and allele frequencies between asthmatic individuals and normal controls. But significant differences were seen among severe asthma, mild asthma and normal controls. The serum IgE levels of TT genotype in asthmatic individuals were higher than those of CC or CT genetype.**

***Conclusions*: TGF-β_1-509C/T gene mutation is not a high risk factor of asthma, but it is closely correlated with asthma severity, and could be one of candidate genes in severe asthma. The level of serum total IgE is related to TGF-β_1-509C/T gene mutation homozygotes.**

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***Key words*: bronchial asthma; TGF-β_1 gene; polymorphism PCR-RFLP; children**

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Introduction

Asthma is a polygenic disease with increasing prevalence and mortality in recent years. Its pathogenesis is a complex one, relevant to factors of heredity, environment, behavior, psychology and immunity. The levels of TGF-β_1 are increased in the bronchoalveolar lavage fluid of asthmatics as compared with those of nonasthmatic individuals, and the levels increased after allergen challenge.[1-4] TGF-β_1 plays an important role in etiopathogenesis of asthma, which has attracted the attention of many scholars in recent years. This study was designed to detect TGF-β_1, C-509T polymorphism by polymerse chain reaction-restriction fragment length polymorphism (PCR-RFLP), and to explore the association of TGFβ and asthma, so as to provide a scientific basis for the prevention and control of the disease.

Methods

Subjects and specimens

Asthmatic group

A total of 98 asthmatic children who had been diagnosed and treated at our hospital according to the criteria for Pediatric Asthma Prevention and Treatment were enrolled.[5] They were divided into mild asthmatics (62 patients) and severe ones (36) according to their clinical manifestations and PEF values. They had not been given any corticosteroids and immune regulators 2 weeks ago.

Control group

Fifty-two normal children were selected randomly, who had been free from respiratory diseases and infections or any personal and family history of other diseases.

Collection of specimens

Two ml venous blood was taken, and 0.5 ml of it was anti-coagulated with 2% EDTA. Chromosome DNA was extracted from whole blood by using genome DNA

extraction kit and stored at −20℃. Another 1.5 ml was centrifuged at 2500 rpm at 4℃ to isolate sera, which were stored at −20℃ for the measurement of total IgE levels.

Methods

Measurement of IgE levels

IgE levels were measured by sandwich ELISA.

TGF-β_1-509T polymorphism

In amplification of TGF-β_1, gene fragments,[6] primers were synthesized by Beijing Saibaisheng Company, Beijing. Primer sequence: S1, 5′-GGG GAC ACC ATC TAC AGT G-3′, S2, 5′-GGA GGA GGG GGC AAC AGG-3′. Amplification reactions were performed in a volume of 50 μl, containing 100 ng genomic DNA, 20 pmol each primer, 10 mmol Tris-HCl (pH 8.3), 50 mmol KCl, 1.5 mmol $MgCl_2$, 200 μmol dNTP, and 1 U Taq DNA polymerase (Promega Company, USA). Cycling conditions were as follows: PCR reactions were initially denatured at 94℃ for 5 minutes, followed by 35 cycles of denaturation (94℃ for 30 seconds), 30 seconds of annealing at 60℃, and 30 seconds of extension at 72℃, and final extension at 72℃ for 5 minutes. PCR contamination was checked by inclusion of negative controls. In genotyping of the TGF-β_1 gene by RFLP, amplification products were confirmed by restriction enzyme Eco81I. The products were incubated for 2 hours at 37℃, analyzed by 3% agarose gelectrophoresis, and visualized after ethidium bromide staining.

Statistical analysis

Genotype distribution frequencies and allele frequencies were analyzed using the chi-square test. The data were expressed as mean ± SD. The relation of TGF-β_1 (C-509-T) genotypes to serum total IgE level in asthmatic children was studied by analysis of variance and the *q* test.

Results

PCR reaction products

The expected size of amplification products was 455 bp (Fig. 1). The enzyme digestion showed that the pattern for homozygotes wild type (lane 3), heterozygotes (lane 5) and homozygotes for the base exchange (lane 4) (Fig. 2).

-509C/T gene polymorphism

No significant differences were observed in genotype frequencies and T allele frequencies between the asthmatics and controls ($\chi^2 = 3.562$, $P = 0.169$; $\chi^2 = 3.607$, $P = 0.058$).

TGF-β_1 genotype distribution, allele frequency

Significant differences were seen in genotype distribution frequencies and T allele frequencies among mild asthma group, severe asthma group and normal controls ($\chi^2 = 11.185$, $P = 0.025$). A significant difference was detected for this variant when the mild asthma group alone was compared to the severe group ($\chi^2 = 6.890$, $P = 0.032$). The difference was even more significant when the genotypes of the severe asthma group and control group were compared ($\chi^2 = 9.466$, $P = 0.009$). There were significant differences in T allele frequencies between the severe asthma group and the mild asthma group ($\chi_4^2 = 7.884$, $P = 0.005$) and between the severe asthma group and the control group ($\chi_5^2 = 10.261$, $P = 0.001$). However, no significant difference was observed between the mild asthma group and control group ($\chi_6^2 = 0.337$, $P = 0.562$).

Serum total IgE level

The levels of serum total IgE in asthmatic subjects were higher than those of controls, and a significant difference

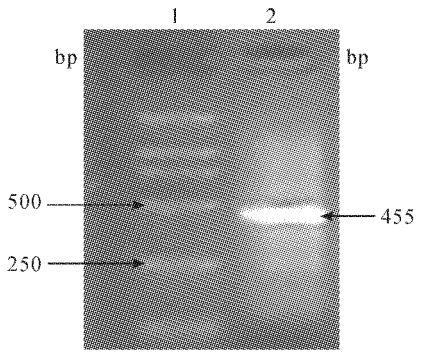

Fig. 1. PCR reaction products. Lane 1: DNA marker DL 2000; lane 2: TGF-β_1 (-509/T) PCR product.

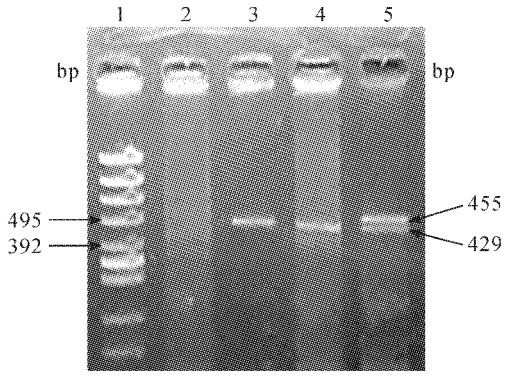

Fig. 2. PCR reaction products. 1: marker; Φ×174 Hinc Ⅱ; lane 2: negative control; lane 3: TT genotype; lane 4: CC genotype; lane 5: CT genotype.

Table 1. Genotype distribution and allele frequency of TGF-β_1 (C-509T) genetic mutation in asthmatic and normal children

Group	*n*	Genotype percentage			Chromsome	Allele frequency	
		CC(%)	CT(%)	TT(%)		C	T
Asthma	98	45(45.9)	38(38.8)	15(15.3)	196	128(0.65)	68(0.35)
Control	52	30(57.7)	19(36.5)	3(5.8)	104	79(0.76)	25(0.24)

Table 2. The association of TGF-β_1 (C-509T) genotype distribution, allele frequency with asthma severity

Group	*n*	Genotype percentage			Allele frequency	
		CC(%)	CT(%)	TT(%)	C	T
Mild	62	34(54.8)	22(35.5)	6(9.7)	90(0.73)	34(0.27)
Severe	36	11(30.5)	16(44.4)	9(25.0)	38(0.53)	34(0.47)
Control	52	30(57.7)	19(36.5)	3(5.8)	79(0.76)	25(0.24)

Table 3. The correlation of TGF-β_1 (C-509-T) genotypes and serum total IgE level in asthmatic children

Genotype	*n*	Total IgE (IU/ml)	*q* value	*P*
CC	45	180.36±43.42	$q_1=15.97$	<0.01
CT	38	210.35±54.68	$q_2=2.74$	>0.05
TT	15	390.25±138.63	$q_3=13.38$	<0.01

was noted between them. The IgE level of TT genotype asthmatics was higher than CC and CT genotype ones ($q_1=15.97$, $q_3=13.38$, $P<0.01$), but there was no significant difference between CC and CT genotypes ($q_2=2.74$, $P>0.05$).

Discussion

The pathogenesis of asthma is complicated by a variety of genetic and environment factors. As a polygenic disorder, searching a candidate gene in asthmatic molecular genetics has become a hot topic. In this chronic inflammatory disease of the airways, several cytokines forming a network participate in the activation and infiltration of eosinophils and the expression of the products. However, TGF-β_1 is very important in this network.[7-10] The human TGF-β_1 gene on chromosome 19q13[11] contains six and seven eons. The promoter for TGF-β_1 contains two major sites for the initiation of transcription and multiple regulatory motits. Polymorphisms in promoter sequence of genes result in abnormal transcriptional regulation and thereby influence the severity of the disease. The expression of TGF-β_1 is influenced by polymorphisms in the TGF-β_1 gene, and some of these polymorphisms may be associated with asthma and other diseases.[12-15] -509 bp position is one of them, and its study is very important to understand asthma.

Seven TGF-β_1 polymorphisms have been reported elsewhere.[16] Three of these allelic variations were localized in the 5′-flanking region of the TGF-β_1 gene (at positions -98C/A, 800A/G, and -509C/T), 3 were in the coding region(+869T/C, +915G/C), and 263, and an insertion in the 5′-untranslated region at position +72. These findings show that only -509 C/T variant is the most informative marker of TGF-β_1 contribution to asthma. It is associated with severe asthma[17] and elevated total serum IgE levels.[18] In our study, the TGF-β_1 gene C-509T mutation had three genotypes (CC, CT, TT) at position -509. There was a great proportion of individuals with TT genotype in the severe group compared to the mild asthmatics and the controls; however, there was no significant difference between the mild asthmatics and the controls. It is suggested that C-509T variant is associated with asthma severity. Analysis of the total serum IgE levels of different genotypes in asthmatic children revealed that the IgE level of TT genotype asthmatics was higher than that of CC and TT genotype ones; but there was no significant difference between CC and CT genotypes, suggesting that variant C-509T was related to elevated IgE levels. The result was identical to Hobbs' result, but Bockova et al[19] and Silverman[20] confirmed that C-509T polymorphism is not correlated with total serum IgE. Possibly it is related to sex, age, race, generation, and environment.

The cause of TGF-β_1-509CT variation is obscure, but three explanations may address the relation of -509T to asthma severity. First, TGF-β_1 -509 is a functional variant, located in the promoter region of TGF-β_1 and it may destroy a transcription factor binding site, thus leading to TGF-β_1 abnormal expression. Second, -509T association is characterized by the variant in linkage disequilibrium with the true functional polymorphism. Third, TGF-β_1 contribution to asthma severity may comprise the interaction of two or more polymorphisms linked to haplotype I. This phenomenon has been identified in the 2-adrenergic receptor gene, where associations can be found between bronchodilator response to agonist in asthmatics and haplotype pairs, but not individual SNPS.[21] It is possible that elevated serum IgE level may be influenced by TGF-β_1 inhibiting proliferation of mast cells and preventing IgE synthesis, while eliminating hematopoietins for eosinophils to induce apoptosis.[22]

As a polygenetic disease with complicated characteristics, the heritability of asthma is as high as 70%-80%.[23] Since TGF-β_1 gene -509C-T polymorphism is correlated with the severity of the disease, it can be a candidate gene, with which it is good to screen high risk cohort of asthma patients. Therefore, early diagnosis according to the results of research will be effective in prevention and treatment of asthma.

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