Association between the interleukin 4 gene -590C>T promoter polymorphism and asthma in Xinjiang Uighur children

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ABSTRACT. We investigated the association between the interleukin 4 gene (*IL-4*) -590C>T polymorphism and forced expiratory volume in one second (FEV_1) values, immunoglobulin E (IgE) levels, and susceptibility to asthma in Uighur children. *IL-4* -590C>T frequencies were analyzed in 38 bronchial asthmatic patients and 35 non-asthmatic controls. Polymerase chain reaction and direct sequencing were applied to determine the residue at position -590 of *IL-4*. Total serum IgE levels were detected by enzyme-linked immunosorbent assay, while lung function was examined by professionals. There were significant differences in the distribution of *IL-4* -590C>T genotypes and alleles between patient and control groups (genotypes: chi-square = 11.476, $P < 0.05$; alleles: chi-square = 14.572, $P < 0.05$). Frequencies of CC, CT, and TT genotypes were 21, 29, and 50% among patients, and 49, 37, and 14% among controls, respectively, indicating that the T allele

was significantly more frequent in the asthma group than in the control group. Total serum IgE levels were significantly higher ($P < 0.05$) and FEV_1 values were significantly lower ($F = 13.294$, $P < 0.05$) in patients than in control subjects of the same genotype. In conclusion, the *IL-4* -590C>T polymorphism is related to bronchial asthma in Uighur children, and the T allele may constitute a susceptibility factor in this group. Furthermore, this genetic variant can result in raised IgE levels and decreased FEV_1 values, suggesting that both factors are associated with bronchial asthma in Uighur children.

Key words: Interleukin 4; Polymorphisms; Children; Asthma; Uighur

INTRODUCTION

As the most common chronic childhood respiratory and allergic disease, bronchial asthma, or asthma for short, seriously affects the physical and mental health of children and imposes a heavy mental and economic burden on families and societies. This condition is therefore an active focus of research in studies of childhood chronic respiratory diseases. At present, the cause of asthma is considered to be complicated, involving a combination of genetic and environmental factors, and characterized by family aggregation and ethnic differences (Barakat-Haddad et al., 2012; Blume and Davies, 2013). With the development of molecular and genetic epidemiology, the identification of asthma susceptibility-associated genes has become a key topic in the study of its etiology internationally. Asthma is induced by the release of cytokines and mediators such as interleukin (IL)-33 and IL-4, the gene encoding which has been reported to contain a functional polymorphism. Multiple asthma-related loci have been described in *IL-4*, including single nucleotide polymorphisms associated with immunoglobulin E (IgE) levels. To date, many studies of the association between the *IL-4* gene -590C>T polymorphism and asthma have been published. Rosenwasser and Borish (1997) first reported a C>T substitution at position -590 in the *IL-4* promoter, establishing a positive correlation between the mutant T allele and IgE levels. As increased total IgE level is the main immunological feature of asthma, we can infer that the *IL-4* -590C>T polymorphism is correlated with this condition. The above study pioneered research into this matter, and the association between *IL-4* gene polymorphism and this disease has become the focus of investigations into asthma etiology. While analyses of this relationship in the Han Chinese population are common (Wang et al., 2012), few reports exist concerning its importance among Xinjiang Uighur children.

Considering the evidence supporting a pathogenic role for IL-4 in asthma, we analyzed whether the C>T substitution at position -590 in the *IL-4* promoter is associated with this disease in Uygur children, and whether this variation affects serum IgE levels and forced expiratory volume in one second (FEV_1) values. In addition, the relationship between asthma and total IgE concentrations and FEV_1 values was examined in this group.

MATERIAL AND METHODS

Subjects

A total of 38 newly diagnosed asthma patients (25 boys and 13 girls aged 4-14

years), whose families had lived for three generations in the Xinjiang Uygur Autonomous Region, were recruited from among pediatric inpatients and outpatients of the First Affiliated Hospital of Xinjiang Medical University between December 2014 and December 2013. Patients' diagnoses conformed to the Guidelines on Diagnosis and Treatment of Bronchial Asthma in Children (reversed) established by the Chinese Pediatric Society and Chinese Medical Association in October 2008. Patients were not administered adrenal corticosteroids or immunomodulators 2 weeks prior to the beginning of this study. In addition, 35 healthy non-asthmatic control individuals (24 boys and 11 girls aged 4-14 years) with no personal or family history of asthma or other allergic diseases were recruited from the same hospital over the same period. All control subjects demonstrated normal spirometric values and had no respiratory symptoms. Moreover, controls were matched by gender and ethnicity to patients. All participants were genetically unrelated. This study was conducted in accordance with the Declaration of Helsinki and with approval from the Ethics Committee of the First Affiliated Hospital of Xinjiang Medical University. Written informed consent was obtained from study subjects' guardians before participation.

Specimen collection and DNA extraction

A peripheral blood sample (4 mL) was drawn from all 73 subjects under fasting conditions in the early morning, of which 2 mL was collected in a tube containing the anticoagulant ethylenediaminetetraacetic acid for extraction of genomic DNA. The remaining 2 mL was extracted into a drying tube and allowed to stand at room temperature before centrifugation and transfer of serum into a 1.5-mL Eppendorf tube (Eppendorf, Hamburg, Germany). Both samples were then stored at -70°C until use. An EZNA Blood DNA Kit (TaKaRa, Dalian, China) was used to extract DNA from these samples, following the manufacturer protocol.

Polymerase chain reaction (PCR) amplification

PCR was performed to amplify a specific region of the extracted DNA. Primers targeting a sequence including the -590 position of *IL-4* (forward: GGA TGT GTT TAG GTT CCA TTC AA; reverse: CCT CCT GGG GAA AGA TAG AGT AA) were synthesized by Invitrogen (Carlsbad, CA, USA), and a Mastercycler Pro Gradient PCR instrument (Eppendorf) and LA *Taq* polymerase (Cat. No. DRR042A; TaKaRa) were used. Each PCR consisted of 10 μL, containing 1 μL sample DNA, 1 μL primer mix, 3 μL double-distilled H_2O, and 5 μL PrimeSTAR HS premix (TaKaRa). The PCR procedure comprised a preliminary denaturation at 95°C for 3 min, then 30 cycles, each consisting of denaturation at 95°C for 30 s, annealing at 68°C for 15 s, and extension at 72°C for 1 min, before a final extension at 72°C for 10 min. PCR products were stored at -4°C until analysis. Amplified products (4 μL) were uniformly mixed with 1 μL 6X loading buffer, and electrophoresed on a 2% agarose gel (containing 0.5 μg/mL ethidium bromide) at room temperature for 1 h at 150 V. After electrophoresis, the gel was placed in a Tanon (Shanghai, China) 1600 digital gel imaging system for visualization of an electrophoretogram (Figure 1).

Direct sequencing of PCR products

PCR products were sequenced using a 3500xL Genetic Analyzer (Applied Biosystems,

Foster City, CA, USA) and M13 primers (TaKaRa). Comparative analysis of results from the Mutation Surveyor software (SoftGenetics, State College, PA, USA) revealed three genotypes, namely wild-type (CC), heterozygous (CT), and homozygous (TT) variants.

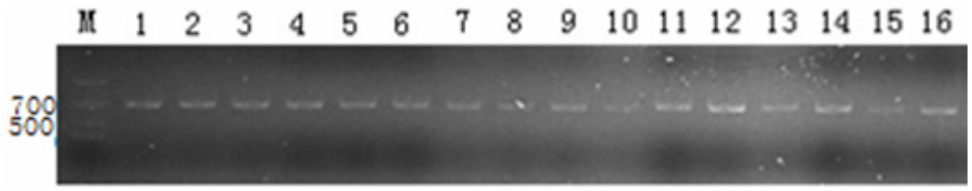

Figure 1. Electrophoretogram of *IL-4* -590C>T polymerase chain reaction products. *Lane M* = molecular marker; *lanes 1-16* = part of research object.

Tests of serum IgE levels and FEV_1 values

An enzyme-linked immunosorbent assay kit (TSZ, Framingham, MA, USA) was used to detect total serum IgE levels of asthma patients and controls by attending doctors in the allergy room of the First Affiliated Hospital of Xinjiang Medical University. FEV_1 values of children in each group were measured by associate chief doctors in the pediatric pulmonary function room using a MasterScreen CPX metabolic cart (Jaeger, Hoechberg, Germany). Multiple tests were conducted to achieve more accurate results.

Statistical analysis

General data were compared between the two groups using the *t*-test (normal distribution) or the chi-square test. Frequencies of the three genotypes and two alleles were analyzed using chi-square tests, while analysis of variance was used to compare total serum IgE levels and FEV_1 values associated with different genotypes in the same group. $P < 0.05$ indicated a statistically significant difference.

RESULTS

Comparison of genotype and allele frequencies at position -590 of the *IL-4* gene promoter in each group

Genotype distributions in the asthma and control groups were tested for Hardy-Weinberg equilibrium. Chi-square tests were used to analyze observed and expected frequencies in each group. The resulting P values were greater than 0.05, suggesting that the genotype frequencies of the study sample were in Hardy-Weinberg equilibrium within each phenotype group, and that our results are representative of the general population (Table 1).

As shown in Table 2, the distribution of *IL-4* -590 genotypes significantly differed (chi-square = 11.476, $P < 0.05$) between the asthma and control groups. Moreover, Table 3 shows that the frequency of the T allele was significantly higher in the patient group than among the controls (chi-square = 14.572, $P < 0.05$), indicating a significant correlation between this residue at position -590 and susceptibility to asthma in Uygur children.

Table 1. Hardy-Weinberg equilibrium test.

Groups	N	Genotype frequency			Allele frequency	
		CC (p^2)	CT ($2pq$)	TT (q^2)	C (p)	T (q)
Asthma group (observed frequency)	38	8 (0.21)	11 (0.29)	19 (0.50)	27 (0.36)	49 (0.64)
Asthma group (expected frequency)	38	5 (0.13)	17 (0.46)	16 (0.41)		
Control group (observed frequency)	35	17 (0.49)	13 (0.37)	5 (0.14)	47 (0.67)	23 (0.33)
Control group (expected frequency)	35	16 (0.45)	15 (0.44)	4 (0.11)		

Table 2. Distributions of *IL-4* -590 promoter polymorphism genotypes in asthma and control groups.

Groups	N	Genotype frequency			Chi-square	P
		CC	CT	TT		
Asthma group	38	8 (21%)	11 (29%)	19 (50%)	11.476	<0.05
Control group	35	17 (49%)	13 (37%)	5 (14%)		

Table 3. Distributions of *IL-4* -590 promoter polymorphism alleles in asthma and control groups.

Groups	N	Allele frequency		Chi-square	P
		C	T		
Asthma group	38	27 (36%)	49 (64%)	14.572	<0.05
Control group	35	47 (67%)	23 (33%)		

Effect of genotype on total serum IgE levels

The *t*-test was used to compare total serum IgE levels of children with the same genotype (CC, CT, or TT) in each group. Significant differences between the two groups ($P < 0.01$) were observed, in that IgE levels of asthma patients were significantly higher than those of control subjects. In addition, significant differences between the three genotypes in the asthma group were identified by analysis of variance ($F = 9.28$, $P < 0.01$). Therefore, the mutant T allele of the *IL-4* -590 promoter variant may result in raised total serum IgE levels in asthmatic children (Table 4).

Table 4. Effect of genotype on total serum immunoglobulin E (IgE) levels in asthma and control groups (data are reported as means ± standard deviation).

Groups	IgE (IU/mL)			*F*	P
	CC	CT	TT		
Asthma group	192 ± 81	311 ± 80	363 ± 82	9.28	<0.01
Control group	89 ± 52	120 ± 51	142 ± 50		

Effect of genotype on FEV_1 values

Using the *t*-test to compare FEV_1 values of children harboring the same genotype (CC, CT, or TT) in the two groups revealed a significant difference ($P < 0.01$). Furthermore, significantly different FEV_1 values were observed when comparing the three genotypes within the asthma group ($F = 13.294$, $P < 0.05$). However, no such comparison was possible for the control subjects due to the relatively low frequency of mutant genotypes in this group (Table 5). Therefore, the *IL-4* -590 T allele may lower FEV_1 values in asthmatic children.

Table 5. Effect of genotype on forced expiratory volume in one second (FEV_1) values in asthma and control groups.

Groups	FEV_1 (L)			*F*	P
	CC	CT	TT		
Asthma group	1.525 ± 0.264	1.407 ± 0.473	1.117 ± 0.225	13.294	<0.05
Control group	2.967 ± 0.129	1.933 ± 0.129	1.636 ± 0.421		

DISCUSSION

The onset of bronchial asthma depends on both genetic and environmental factors (Barakat-Haddad et al., 2012), and various airway inflammatory cells, structural cells, cellular components, and cytokines play crucial roles in its pathogenesis (Traister and Wenzel, 2012; Legath et al., 2013). IL-4, as a Th2 cytokine secreted by CD4+ T cell subsets, mast cells, and B cells, also contributes to the development of asthma. The human *IL-4* gene, including four exons and three introns, is approximately 10 kb in length and is located on chromosome 5q31-q33 (Wang and Mei, 2011). In recent years, many investigations of *IL-4* have identified single nucleotide polymorphisms at multiple loci (Tang et al., 2014; Huang et al., 2015), with the -590C>T variant having been particularly well studied. However, few reports exist regarding the association between this polymorphism and asthma in Xinjiang Uygur children. Therefore, this study aimed to analyze whether the C>T substitution at position -590 in the *IL-4* gene promoter correlates with asthma in this group, and whether this mutation affects serum IgE levels and FEV_1 values. The association between total serum IgE levels and FEV_1 values and occurrence of asthma was also assessed.

Analysis of the relationship between *IL-4* -590C>T and the prevalence of asthma in Xinjiang Uygur children showed significant differences in the distribution of the three genotypes (CT, TT, and CC) and two alleles between the study groups ($P < 0.05$). The T allele frequency among asthma patients was significantly higher than that among control subjects, indicating that the *IL-4* promoter -590C>T polymorphism correlated with childhood asthma in our study. Thus, this allele might represent a risk factor for this disease among Uighur children. Indeed, a team of Iranian scholars has found that this same *IL-4* allele confers increased susceptibility to asthma (Kamali-Sarvestani et al., 2007), and in agreement with our results, Smolnikova et al. (2013) demonstrated in a case-control study of 50 control subjects and 50 asthmatic patients that the *IL-4* -590C>T polymorphism is associated with childhood asthma. Furthermore, in a meta-analysis, Liu et al. (2012) revealed that this variation is related to susceptibility to this condition in Caucasian patients with allergic asthma.

In the present study, serum IgE levels of asthma patients harboring the CC, CT, and TT genotypes were 192 ± 81, 311 ± 80, and 363 ± 82 IU/mL, respectively, and these values were significantly different between the two study groups ($P < 0.01$). It may be concluded that the *IL-4* -590C>T polymorphism is associated with increased serum IgE level. Consistent with this, Huang et al. (2012) showed that this C>T mutation at position -590 of the *IL-4* promoter is related to increased total serum IgE in a case-control study of 122 control subjects and 100 asthmatic children from the Guangdong region of China. Furthermore, Russian researchers have shown that this substitution positively correlates with increased IgE levels in Russian asthma patients (Gervaziev et al., 2006). Serum IgE levels of control subjects with CC, CT, and TT genotypes were 89 ± 52, 120 ± 51, and 142 ± 50 IU/mL, respectively, representing significantly lower values than those of the asthma

group, and indicating that serum IgE level is associated with prevalence of asthma in Uygur children. In agreement with the above results, in a study of asthmatic children from the Guiyang area of China, Zheng et al. (2014) showed that serum IgE concentrations of asthma patients were markedly higher than those of control participants. In the present study, FEV_1 values of asthma patients harboring the CC, CT, and TT *IL-4* -590 genotypes were 1.525 ± 0.264, 1.407 ± 0.473, and 1.117 ± 0.225 L, while the corresponding values in the control group were 2.967 ± 0.129, 1.933 ± 0.129, and 1.636 ± 0.421 L, respectively. Comparing individuals of the same genotypes between the two study groups revealed a statistically significant difference ($P < 0.05$), as did a comparison of the three different genotypes within the asthma group ($P < 0.05$). It can therefore be concluded that FEV_1 value was negatively affected by the *IL-4* -590C>T polymorphism. Burchard and Silverman (1999) showed a strong correlation between the TT genotype and FEV_1 value in Caucasians in a study of the *IL-4* -590C>T polymorphism among white and African American asthmatics in the USA, consistent with our results (Winterton et al., 2001).

The prevalence of bronchial asthma in children is strongly linked to genetic factors and other aspects, such as airway hyperresponsiveness, serum IgE level, FEV_1 value, exposure to allergens, diet, and family history. Moreover, the *IL-4* gene -590C>T variant correlates with serum IgE level and FEV_1 value. Geographic, ethnic, and individual differences affect susceptibility to this disease; therefore, studies of the incidence of asthma and related risk factors in different populations are highly salient (Lin et al., 2013). In this study, we analyzed whether the *IL-4* -590C>T polymorphism was a susceptibility factor for asthma in Uighur children, and examined its correlation with serum IgE level and FEV_1 value. Our results showed that this *IL-4* sequence variation and FEV_1 values were associated with the incidence of asthma, and that the T allele might represent a susceptibility factor for this disease among Uighur children. Furthermore, this polymorphism was linked to elevated IgE levels and lowered FEV_1 values.

Certain limitations to this study should be addressed. The effective sample size was relatively small, therefore the possibility of false-positive results cannot be ruled out. In addition, the relevance of this study would have been greater had asthma severity in patients been graded. Therefore, further studies with larger sample sizes are required, incorporating deep analysis of asthma severity and family history to improve the reliability of the results obtained. Perspectives for future studies are as follows: 1) enlarging effective sample sizes of patient and healthy control groups, and grading the severity of asthma in children for further investigation of its correlation with susceptibility factors; 2) further exploring the correlation between the *IL-4* -590C>T polymorphism and asthma in Uygur and Han children in the Xinjiang area to improve the genetic characterization of Uygur children with this condition, thereby making a contribution to its prevention and treatment.

Conflicts of interest

The authors declare no conflict of interest.

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