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Association between the Polymorphisms of IL-4 Gene Promoter (-590C>T), IL-13 Coding Region (R130Q) and IL-16 Gene Promoter (-295T>C) and Allergic Asthma

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ABSTRACT

Allergic asthma is a multifactorial disease, influenced by genetic and environmental factors. Recent family-based studies have revealed evidence for linkage of human chromosomes 5q31-33, 12q15-24, 11q13 and 15q23.6 as regions likely to contain genes related to asthma. Among the candidate genes in these regions are the genes encoding for human interleukin-4, interleukin-13 and interleukin-16.

To evaluate this linkage, we examined an Iranian population of patients with asthma. A total of 30 patients with allergic asthma and 50 normal subjects were studied. Allergic asthma was confirmed using skin prick test and spirometry. DNA was extracted from blood cells and IL-4 (-590C>T), IL-13 (R130Q) and IL-16 (-295T>C) polymorphisms were determined by PCR-RFLP method.

Out of 30 patients with allergic asthma, the following genotypes for IL-4, IL-13 and IL-16 cytokines were found: IL-4 genotypes consisted of 17 (56.7%) CC, 8 (26.7%) CT and 5 (16.7%) TT; IL-13 genotypes consisted of 11 (36.7%) GG, 13 (43.3%) GA and 6 (20%) AA; IL-16 genotypes consisted of 23 (76.7%) TT and 7 (23.3%) CT. No patient showed CC genotype for IL-16. A higher proportion of case subjects with the C allele for the IL-4, G allele for the IL-13 and T allele for the IL-16 polymorphisms was found compared with the T, A and C alleles, respectively.

These results suggest an influence of genetic variability at the promoter of IL-4 gene (-590C>T) and a coding region of IL-13 gene (R130Q) on the occurrence of allergic asthma and no relationship between IL-16 promoter polymorphism (-295T>C) and this disease.

Key words: Allergic asthma; Interleukin-4; Interleukin-13; Interleukin-16; Polymorphism

INTRODUCTION

Asthma, a chronic inflammatory disorder of the airways is characterized by episodic airway narrowing, increased airway reactivity to a variety of stimuli, and pharmacologic or spontaneous reversibility. Mast cells,

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T lymphocytes, and eosinophils, contribute to inflammatory response. These cells produce multiple soluble mediators including, cytokines, leukotrienes, and bradykinins. An imbalance in proinflammatory versus inhibitory cytokines may be a fundamental part of the pathogenesis of asthma.¹ Genetic factors are major determinants of susceptibility to allergic inflammation in humans.² Atopy is characterized by elevated serum total and allergen-specific IgE concentrations, and it has been found to be a major component of asthma. Asthma and atopy are related conditions caused by a complex interaction of genetic and environmental influences.³

Allergic asthma is a multifactorial disease, predisposed by genetic and environmental factors, and it is characterized by bronchial hyperresponsiveness, presence of IgE antibodies to inhalant allergens and often also by enhanced total serum IgE levels. A switch of antibodies to IgE requires two signals from activated T cells: the expression of the ligand for CD40 and the secretion of IL-4 or IL-13. Both IL-4 and IL-13, independently of each other, are able to induce IgE antibody production.⁴ IL-16 is an immunomodulatory cytokine that acts as a chemo attractant for CD4⁺ cells and contributes to the recruitment of these cells and their activation at sites of the inflammation associated with asthma. Increased levels of IL-16 have been demonstrated in the bronchial mucosa of asthmatic lung, and the level of IL-16 production within the bronchial epithelium correlates with CD4⁺ cell infiltration.⁵ This pleiotropic cytokine has multiple effector functions with putative roles in varied T cell-mediated inflammatory diseases, such as asthma, inflammatory bowel disease and atopic dermatitis. IL-16 regulates antigen-driven T cell activation, T helper 2 cytokine production and allergic airway inflammation.⁶

Genome-wide searches suggested that asthma could be mapped to chromosomes 5q31-33, 12q15-24 and 11q13 in the Caucasian as well as Japanese populations³ and 15q26.3 as regions likely to contain genes related to asthma, elevated serum IgE levels, and bronchial hyperresponsiveness⁵. Among the genes in these regions are the genes encoding for human IL-4, IL-13 and IL-16.

In human, IL-16 production in the airways of asthmatic subjects appears to be increased compared with that seen in healthy control subjects.⁷ It has been suggested that IL-16 may play an important role in

regulating the production of cytokines which are produced in allergic states in response to antigen.^{8,9}

Since these cytokines stimuli can influence mast cell responsiveness to IgE-mediated signaling and because of the genetic variants in IL-4, IL-13 and IL-16 sequences and their gene transcription, we speculated that these sequence variants could modify asthma severity.

Thus we investigated the association of IL-4 and IL-13 common polymorphisms combined with a recently discovered common single nucleotide polymorphism (SNP) in the promoter region of IL-16 and allergic asthma. In this study, we examined a case group with asthma to find out the relationships between genetic variants in the IL-4 and IL-16 promoter regions (-590C>T and -295T>C, respectively) and a coding region of IL-13 gene (R130Q) and the risk of developing asthma or its severity.

MATERIALS AND METHODS

Study Population

This study involved 30 patients aged between 18 and 62 years, with asthma as diagnosed by the specialists in the Immunology and Allergy of the Respiratory Ward of a University Teaching Hospital (Ghaem Hospital) according to the standard criteria. Patients were recruited during January 2005 - April 2006. The written informed consent was obtained from all of participants.

All patients should have had a predicted FEV1 between 40% and 80% of predicted normal values at their first visit after at least 8 hours without inhaled β -agonist treatment.¹⁰ The FEV1 in the absence of bronchodilator medications was used as an index of asthmatic airway obstruction. Patients with heart, kidney, liver and other lung diseases were excluded from this study. Pregnant women were also excluded from the study.

All patients were asked about the history of allergic reactions or asthma symptoms while exposed to specific allergens.

Skin Prick Test showed a positive reaction in the form of a wheal equal or more than 3mm in diameter in comparison to the saline control in all of the patients.

A questionnaire was completed for each person and after taking a spirometry a blood sample was taken from each individual for DNA extraction.

Polymorphisms of IL-4 and IL-13 Gene Promoter in Allergic Asthma

An age, sex-matched control group consisting of 50 individuals without any history of asthma or allergy was selected. All control subjects passed the same procedure.

This study was approved by the ethic committee of Mashhad University of Medical Sciences and it was performed in Bu-Ali Research Institute of Mashhad University of Medical Sciences.

Data Collection

The demographic data, as well as the severity of asthma in our patients, were assessed by a standardized questionnaire including such main information:

- Age, family history.
- Past medical history, drug history, occupational exposures.
- Major asthma symptoms such as wheeze, cough, chest tightness.
- Incidence, frequency and intensity of the symptoms.
- Exacerbations frequency.
- Limitation of the everyday physical activities because of the symptoms.
- Anti-asthma treatment.

Spirometry

All asthmatic patients underwent spirometric assessment to determine their asthma severity according to the standard criteria.

FEV1, FVC, FEV1/FVC and PEFR were measured in our patients. The results were compared with the local age- and sex-matched predicted values. The patients were categorized into severity groups using predicted FEV1% (mild, FEV1>80%; moderate, FEV1 60-80% and severe, FEV1<60%). PEFR variability was also important for us (mild, PEFR variability 20%; moderate, PEFR variability 20-30% and severe, PEFR variability 30%).

Molecular Methods

Genomic DNA was extracted from 10ml of whole blood using DNA extraction kit (Biogene, Mashhad, Iran) by salting-out method. Subjects were genotyped for promoter polymorphisms of IL-4 and IL-16 genes and a coding region polymorphism of IL-13 designated R130Q using Polymerase Chain Reaction (PCR) method followed by Restriction Fragment Length Polymorphism (RFLP).

The primers for IL-4 promoter, IL-13 R130Q and IL-16 promoter genes were respectively as follows according to the published articles.^{3, 11, 12}

Forward: 5'-ACTAGGCCTCACCTGATACG-3';
Reverse: 5'-GTTGTAATGCAGTCCTCCTG-3'.

Forward: 5'-CTTCCGTGAGGACTGAATGAG
ACGGTC-3'; 5'-GCAAATAATGATGCTTTC
GAAGTTTCAGTGGA-3'.

Forward: 5'-CTCCACACTCAAAGCCTTTTG
TTCCTATGA-3'; Reverse: 5'-CCATGTCAAAAC
GGTAGCCTCAAGC-3'.

PCR was performed in a T3 Thermocycler (Biometra, Germany). Each 20µl of PCR mixture contained 100ng of genomic DNA, 0.5 unit of Taq DNA polymerase, 1X PCR reaction buffer (10mM/L Tris-HCl, 50 mM/L KCl, 1.5 mM/L MgCl₂), 0.2 mM each dNTP, 0.5µM each primer.

PCR amplification conditions for each gene were as follows:

IL-4 (-590C>T): denaturation at 95°C for 3 min followed by 35 cycles at 94°C for 1 min, 57°C for 1 min and 72°C for 1 min, and then a final extension for 5 min at 72°C. The amplified 252-bp PCR fragment was then digested by addition of 1 unit of *BsmFI* (Fermentas, Germany) and incubated for 3 hours at 65°C. *BsmFI* recognizes the restriction sequence GGGAC↓, present only in amplified DNA fragments without the polymorphic allele (-590C). Expected fragment sizes on a 3% agarose gel were 252-bp for the -590T allele, and 192-bp for the -590C allele.

IL-13 (R130Q): denaturation at 95°C for 2 min followed by 38 cycles at 94°C for 30 s, 68°C for 1 min and 72°C for 1 min, and then a final extension for 8 min at 72°C. The amplified 236-bp PCR fragment was then digested by addition of 1 unit of *BspLI* (Fermentas, Germany) and incubated for 5 hours at 37°C. *BspLI* recognizes the restriction sequence GGN↓NCC and digests 32-bp from the 3'-end of the fragment when the G nucleotide (Arg130) is present to produce a 178-bp fragment. The digested products were visualized following electrophoresis on a 3% agarose gel.

IL-16 (-295T>C): denaturation at 95°C for 3 min followed by 30 cycles at 94°C for 1 min, 59°C for 1 min and 72°C for 1 min, and then a final extension for 5 min at 72°C. The amplified 280-bp PCR fragment was then digested by addition of 1 unit of *Eam1105I* (Fermentas, Germany) and incubated for 3 hours at 37°C. *Eam1105I* recognizes the restriction GACNN↓NNGTC, present

only in amplified DNA fragments containing the -295C allele. Expected fragment sizes on a 15% polyacrylamide gel were 280-bp for the -295T allele, and 246-bp for the -295C allele. Figures 1, 2 and 3 show examples of PCR-RFLP analysis for the IL-4 (-590C>T), IL-13 (R130Q) and IL-16 (-295T>C) polymorphisms, respectively.

Statistical Analysis

Statistical analyses were performed using Fisher's exact and chi square tests. The results were considered to be significant when the P-value was less than 0.05.

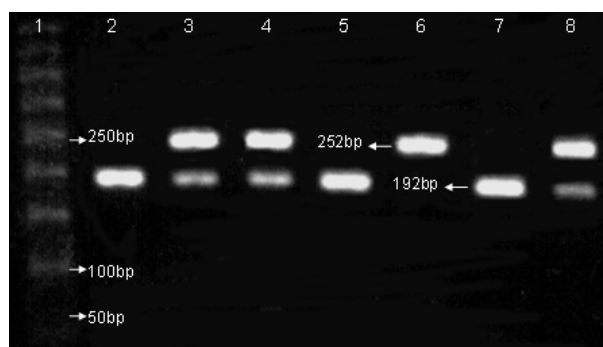


Figure 1. Analysis of the IL-4 -590 (C>T) Promoter polymorphism. Lane 1: DNA size marker (50bp); Lane 2-8: subjects with different polymorphism in -590 promoter; Lane 2, 5 and 7: Allele 1 (CC) homozygous; Lane 3, 4 and 8: (CT) heterozygous; Lane 6: Allele 2 (TT) homozygous.

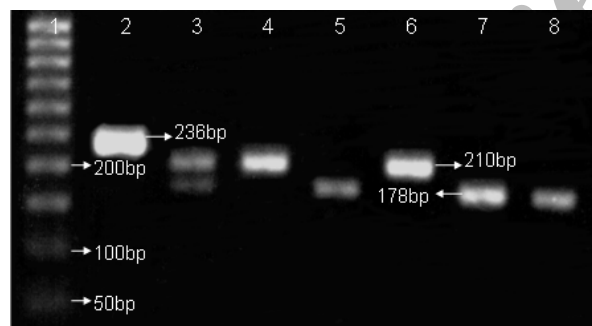


Figure 2. Analysis of the IL-13 R130Q polymorphism. Lane 1: DNA size marker (50bp); Lane 2: Uncut; Lane 3-8: subjects with different polymorphism in coding region of IL-13; Lane 5, 7 and 8: Allele 1 (GG) homozygous; Lane 3: (GA) heterozygous; Lane 4 and 6: Allele 2 (AA) homozygous

RESULTS

Subject Characteristics

Out of 30 patients with allergic asthma, we had 17(56.7%) females and 13(43.3%) males, showing a relatively more prevalence of allergic asthma among women. The mean age of our patients was 34 ± 11 years

old and the most age-group involved in our study was 4th decade of life. In our control group we had 35(70%) females and 15(30%) males with the mean age of 33 ± 9 years old. FEV1 values of >80%, 60-80% and <60% were used to categorize mild, moderate and severe disease, respectively.

Polymorphism Detection

Among the 30 patients with allergic asthma, the following genotypes for IL-4, IL-13 and IL-16 cytokines were found: IL-4 genotypes consisted of 17 (56.7%) CC, 8 (26.7%) CT and 5 (16.7%) TT; IL-13 genotypes consisted of 11 (36.7%) GG, 13 (43.3%) GA and 6 (20%) AA; IL-16 genotypes consisted of 23 (76.7%) TT and 7 (23.3%) CT. No patient showed CC genotype for IL-16. Among 50 normal subjects the following genotypes for IL-4, IL-13 and IL-16 cytokines were found: IL-4 genotypes consisted of 38(76%) CC and 12(24%) CT; no TT genotypes was found among the normal subjects for IL-4. IL-13 genotypes consisted of 34(68%) GG, 10(20%) GA and 6(12%) AA; IL-16 genotypes consisted of 32(64%) TT, 17(34%) CT and 1(2%) CC. A higher proportion of case subjects with the C allele for the IL-4, G allele for the IL-13 and T allele for the IL-16 polymorphisms were found compared with the T, A and C alleles, respectively ($P=0.004$, 0.008 , 0.22 , respectively) and the differences in genotypic distribution between the controls and patients were 0.009 , 0.022 , 0.410 , respectively.

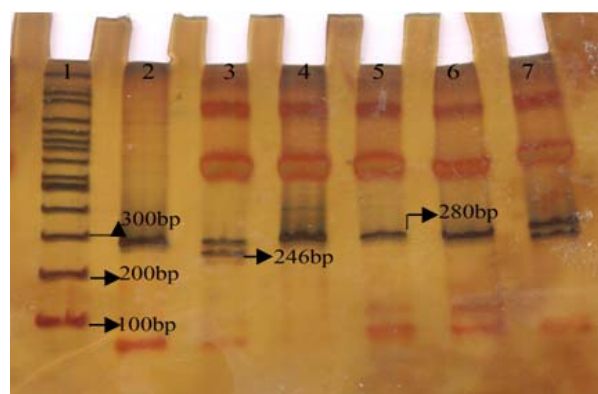


Figure 3. Analysis of the IL-16 -295 (T>C) Promoter polymorphism. Lane 1: DNA size marker (100bp); Lane 2: Uncut; Lane 3-7: subjects with different polymorphism in -295 promoter; Lane 4, 5 and 6: Allele 1 (TT) homozygous; Lane 3 and 7: (TC) heterozygous.

Association with FEV1 Values

Polymorphisms of IL-4 and IL-13 Gene Promoter in Allergic Asthma

Among our patients, the presence of the mutant IL-4 promoter, IL-13 coding region, IL-16 promoter alleles (T, A, C, respectively) were associated with a FEV1 above 80% of predicted values. The numbers of C, G and T alleles were associated with low FEV1 values in IL-4, IL-13 and IL-16 polymorphisms, respectively.

Table 1. Genotypes of IL-4, IL-13 and IL-16 cytokines in allergic asthma and normal controls.

Cytokines	Genotypes	Patients	Control
		Number (%)	Number (%)
IL-4	CC	17(56.7)	38(76)
	CT	8(26.7)	12(24)
	TT	5(16.7)	none
IL-13	GG	11(36.7)	34(68)
	GA	13(43.3)	10(20)
	AA	6(20)	6(12)
IL-16	TT	23(76.7)	32(64)
	CT	7(23.3)	17(34)
	CC	none	1(2)

An FEV1 below 60% of predicted was documented for 56.3%, 43.8%, and 68.8% of the 30 patients with CC, AG, and TT genotypes for IL-4, IL-13 and IL-16, respectively. Genotype frequencies of IL-4, IL-13 and IL-16 cytokines in allergic asthma and normal controls have been shown in table 1.

DISCUSSION

The importance of genetic factors in influencing the risk of developing allergic inflammation is well established.⁴

Our results do suggest an influence of genetic variability at the promoter of IL-4 gene (-590C>T) and a coding region of IL-13 gene (R130Q) on the occurrence of allergic asthma and no relationship between IL-16 promoter polymorphism (-295T>C) and this disease.

It has been reported that polymorphisms within the promoter region of IL-4 gene seems to correlate with enhanced IL-4 activity, secondary to modification of IL-4 gene transcription. In this sense, it has been hypothesized that the T allele may be associated with the severity of asthma.¹³ However, in our study, a trend of association of -590T IL-4 allele with mild asthma was observed.

Single Nucleotide Polymorphisms (SNPs) in coding regions constitute the majority of disease alleles in Mendelian disorders. A recent analysis suggests that common disease variants are likely to show a similar trend.² Our results show that IL-13 R130Q, a common variant encoded by IL13 +2044A has a great relationship with allergic asthma as previously shown in different studies.²

Although it has been hypothesized that the -295T>C promoter polymorphism of IL-16 may be associated with increased IL-16 gene expression, Akesson, et al. have found no association of IL-16 -295T>C polymorphism with asthma, disease severity or atopy in an Australian population.⁵ Glas et al. reported an association between the IL-16 -295T>C polymorphism and Crohn's disease. Subjects with Crohn's disease had significantly higher T-allele and TT-genotype frequencies compared with those of control subjects.¹² Burkart, et al. have demonstrated in their study that the T allele at the -295 position in the IL-16 gene promoter region is associated with reduced promoter activity relative to the less common C allele on transfection into a bronchial epithelium cell line. Their study also demonstrated an association of the TT genotype and T allele at the -295 position in the IL-16 promoter region with asthma in a white population.⁷ Another study has shown that the IL16 -295 promoter polymorphism might influence susceptibility to contact allergy.¹⁴

It is not surprising that the mean FEV1 in the entire study indicated a moderate to severe overall level of asthma disease severity. Furthermore, since a low percent of the subjects enrolled in the trial had FEV1> 80% of predicted, very few subjects met the current criteria for mild asthma; it is possible that these genetic loci may not contribute to the variation in the FEV1 in patients with mild forms of asthma. We did not find a significant association between FEV1 and genotypes. The modest size of our sample of Iranian patients may have accounted for our inability to demonstrate an association between asthma severity and genotypes at these loci in this group.

We speculate that it is more likely that each of a number of loci accounts for a small fraction of the total variance in lung function.

We noted striking differences in allele frequencies at IL-4 and IL-13 promoter loci between asthmatic patients and normal subjects. Because the overall levels of airway obstruction were not absolutely in

accordance with these differences in allele frequencies, there is additional reason to believe that these loci are ones of many that influence the severity of airway obstruction in asthma. It is quite conceivable that the knowledge of a patient's genotype at these loci could serve as an independent index of asthma severity in our Iranian patients.

We demonstrated a trend of association between -590C>T IL-4 and R130Q IL-13 polymorphisms and asthma in an Iranian population. Patients who carried both the T allele of -590C>T IL-4 and the A allele of R130Q>R IL-13 showed an increased risk of allergic asthma. It should be mentioned that our study group consisted of a limited number of asthmatic patients and these results should be confirmed in a larger population.

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