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C-590T Promoter Polymorphism of the Interleukin (IL)-4 Gene Is Associated with an Increased Susceptibility to Nasal Polyposis

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Abstract

Background: Recent studies have shown interleukin 4 (IL-4) and 5 lipoxygenase (5-LO) to play an important role in development of nasal polyposis. Investigation into the genetic factors associated with allergic and non-allergic nasal polyposis has been examined for more than fifteen years. Despite these efforts, the genetic factors underlying the development of nasal polyposis have yet to be clearly understood. The current study examined the relationship between C-590T promoter polymorphisms of the IL-4 gene and the presence of nasal polyps. Additionally, we examined the levels of 5-LO expression in nasal polyp tissue and its association with the IL-4 promoter gene polymorphisms.

Methods: A total of 320 subjects were enrolled in the study, of which 256 were healthy controls and 64 were patients with nasal polyps. The Real-Time PCR HRM-based method was used to determine the genotypes of IL-4 C-590T. The expression of 5-LO within the 64 samples of nasal polyp tissue was determined by immunohistochemical staining to examine the association of 5-LO with the IL-4 C-590T genotype.

Results: Genetic analysis showed a significant difference in the frequencies of the IL-4 polymorphisms at C-590T in patients with nasal polyps as compared with controls ($p<0.001$). No significant difference was seen in the expression of 5-LO among genotypes in patients with nasal polyps ($p=0.139$).

Conclusions: The results suggest that the inheritance of TT and CT genotypes at the IL-4 C-590T promoter gene is associated with nasal polyps however, there is no association between the expression of 5-LO in nasal polyp tissues and IL-4 C-590T genotypes in patients with nasal polyps.

Keywords: Gene polymorphism, IL-4, IL-4 C-590T, Nasal polyposis, 5-LO.

Introduction

Within the paranasal sinuses and nasal cavity, painless benign growths termed nasal polyps can develop resulting from the presence of an inflammatory reaction of the nose, paranasal sinuses, and lower airway. Nasal polyposis has been associated with several different factors including: asthma, rhinitis, recurrent infections, aspirin hypersensitivity, chronic inflammation, hormonal

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and immune disorders, pollutants, and genetics (1-3). Recent studies have shown that type 2 cytokines, particularly interleukin(IL)-4, hold a central role in the pathology of allergic diseases (4-6). Additionally, the T allele of the -590 C/T polymorphism in the promoter region of the IL-4 gene is associated with increased IL-4 gene promoter activity which has the potential role to increase the production of IL-4 (7,8). Polymorphisms in the promoter region of the IL-4 gene have been previously examined, however, the results appear to be inconclusive (9,10). The etiology of polyp development remains unclear. Some studies have reported a positive family history of nasal polyposis, while others have shown an association with various single nucleotide polymorphisms (11-15). Arachidonic acid is the precursor of powerful biological mediators that play a central role in controlling biological systems. Two major enzymatic pathways are capable of metabolizing arachidonic acid: the cyclooxygenase (COX) and the 5-lipoxygenase (5-LO) pathway (16). The 5-LO enzyme serves as a critical regulator in the synthesis of inflammatory mediators and leukotrienes (LTs) (17). Previous studies have shown cysteinyl leukotrienes (CysLts) to be upregulated by 5-LO in nasal polyp tissues (18, 19). Interleukin(IL)-4 and -13 also have been shown to have regulatory effects on the expression of cysteinyl leukotriene 1 receptor (CysLT1R). CysLT1R is expressed on monocytes and macrophages, modulating their cellular responsiveness to leukotriene D4 (LTD4) and is involved in the pathogenesis of allergic diseases (20). The current study evaluated the association between IL-4 -590 C>T gene polymorphisms and the susceptibility to nasal polyposis. Additionally, we investigated the expression of 5-LO within nasal polyp tissues and the association of the presence of this enzyme with IL-4 -590 C>T gene polymorphisms.

Materials and methods

Ethics Statement

All participants were selected from Kerman, a city in southeastern Iran. All individuals provided written informed consent prior to enrollment in the study. The research was performed from 2014 to 2015, and was approved by the Ethical Committee of the Kerman University of Medical Sciences. The approval number is K/93/106.

Selection of patients and controls

A total of 320 subjects were including in the study. Of the total participants, 256 were healthy controls from the Kerman Blood Transfusion Center and 64 were patients with nasal polyps. Patients with nasal polyps were recruited from the Department of Otorhinolaryngology at Kerman University of Medical Sciences in Kerman, Iran. The diagnosis of nasal polyps was made based on clinical history, paranasal sinus computed tomography scans, and confirmed through histopathological examination. In order to avoid including patients with allergic rhinitis, patients with a previous history of itchy eyes, nose, or throat that was accompanied by a runny nose and tearing during a specific season or following exposure to specific substances or in specific places were excluded. Prick tests were not considered in the exclusion process for patients with allergic rhinitis. Patients with a history of antrochoanal polyps, polyps associated with cystic fibrosis, or fungal sinusitis were excluded from the study. Those suffering from primary ciliary dyskinesia, chronic periodontal disease, cancer, inflammation, rheumatoid arthritis, inflammatory bowel disease, or any other inflammatory diseases were also excluded. Furthermore, patients were excluded if they had been taking any steroids (systemic or topical), anti-histamines, non-steroidal anti-inflammatory drugs (NSAIDs), or macrolide antibiotics the month prior to beginning the study. Nasal polyp tissues were removed from the middle meatus at the beginning of the surgical procedure via ppolypectomy by endoscopic approach. The expression of 5-LO in the nasal polyps of patients was determined using immunohistochemical staining. Venous blood samples were collected from patients with nasal polyps and from healthy controls to determine the genotype of IL-4 -590 C>T. The healthy controls, selected from the Kerman Blood Transfusion Center, had no history of systemic or organ specific autoimmune diseases, allergy, infectious diseases, cancer, or gastrointestinal diseases. who were taking immunosuppressive drugs or corticosteroids were excluded from the study. Demographic data showed the frequency of males to be 53.2% (number = 136) and 46.8% for

females (number= 120). The male and female frequencies for patients were 46.9% and 53.1%, respectively. The mean age for patients and controls was 38.2 ± 13.26 and 36.95 ± 11.91 years, respectively. The controls and the patients with nasal polyps were age- and sex-matched.

Real-Time PCR high-resolution-melting (HRM) analysis for IL-4 -590 C>T genotyping

Genomic DNA was extracted from whole blood using the QiaAmp DNA Mini Kit (Qiagen, Valencia, Ca, USA). The forward and reverse primers for IL-4 -590 C>T gene polymorphisms were 5'-ACTAGGCCTCACCTGATACG-3' and 5'-GTTGTAATGCAGTCCTCCTG-3',
respectively. The Real-Time PCR HRM-based method was used to detect the different genotypes. The assay was performed using SYBR Green qPCR master mix (Thermoscientific, K0221) under the following conditions: holding at 50 °C for 2 minutes, initial denaturation at 95 °C for 15 minutes followed by 40 cycles of denaturation at 95 °C for 10 seconds and annealed at 60 °C for 40 seconds. HRM ramps were generated by acquiring fluorescence data at a temperature ramp from 65 °C to 88 °C. The mean temperatures for genotypes of CC, CT, and TT were 84 °C, 81 °C, and 82 °C, respectively. The genotype was assigned according to the shape of the HRM curve as determined by Rotor-Gene software and visual inspection.

Immunohistochemical Staining

Biopsies from nasal polyps were fixed with 10% formalin in phosphate-buffered saline (PBS) solution for 24 hours. The tissues were dehydrated and embedded in paraffin through routine processing. After deparaffinization and rehydration with xylene and alcohol, the sections were microwaved in 10 mM citrate buffer (pH 6.0) for 10 minutes and then washed with 3% H2O2 in Tris-buffered-saline (TBS) for 10 minutes. Another wash with TBS was performed for 5 minutes, followed by a blocking step for 30 minutes using Blocking Solution (DAKO, Hamburg, Germany). Sections were incubated with rabbit anti-5-LO monoclonal antibody (Abcam, Cambridge, United Kingdom) diluted in a 1:250 ratio in Antibody Diluent (DAKO, Hamburg, Germany) overnight at 4 °C and then washed three times with TBS. In the next step, goat Anti-Rabbit IgG conjugated to horseradish peroxidase (Abcam, Cambridge, United Kingdom) was used as the secondary antibody to enable for the detection of the primary antibodies. The sections were then washed and incubated with 3,3'-diaminobenzidine (DAB, Sigma Fast DAB tablet, Sigma Chemical Co, St Louis, MO). Finally, the sections were washed with TBS and then counterstained with hematoxylin. Immunohistochemical staining for 5-LO was evaluated by the pathologist. Expressions of 5-LO were assessed semi-quantitatively as follows: 0 = none, 1= 25%, 2 = 25-50%, and 3 = 50%.

Statistical analysis

The genotype and allotype frequency deviations from the Hardy-Weinberg equilibrium were analysed for all individuals. Statistical analyses including, logistic regression, two-independent t-test, Chi-square, and ANOVA were all performed using SPSS software version 17.0. *P* values less than 0.05 were considered statistically significant.

Results

Positive association between IL-4 C-590T polymorphism and the development of nasal polyps

The genotype and allelotype frequencies of the IL-4 -590 C>T gene polymorphism for all individuals are summarized in Table 1. Analysis of the IL-4 -590 C>T gene polymorphism showed a significant difference in the frequencies of -590 C>T between patients and controls ($p<0.001$). Statistical analysis demonstrated that the presence of the T allele at position -590 of the IL-4 gene was associated with an increased susceptibility of nasal polyp development ($p<0.0001$). The frequencies of the CT and TT genotypes were significantly higher in patients with nasal polyps than in healthy controls ($p<0.0001$). Conversely, the frequency of the CC genotype was significantly higher in healthy controls as compared with patients with nasal polyps ($p=0.003$). Statistical analysis comparing the genotypes TT and CC, CT and CC, CT and TT, among patients and controls showed significant p values for all conditions (TT versus CC, $p <0.0001$; CT versus CC, $p<0.0001$; and CT versus TT, $p< 0.021$). Genotype distributions were in

agreement with Hardy-Weinberg equilibrium in both patient and control groups.

Table 1. Genotype and allele frequencies of IL-4 gene polymorphism at C-590T gene in patients with nasal polyps and controls.

IL-4	Genotyping	Nasal polyp		Control		p	Alleles	Nasal polyp		Control	
		%	N	%	N			%	N	%	N
-590	CC	17.2	11	89.5	229	<0.0001	C	46.1	60	94.33	483
	CT	57.8	37	9.8	25		T	53.8	70	29	5.66
C>T	TT	25	16	0.8	2						

Immunohistochemical analysis of 5-LO in nasal polyp tissues

Immunohistochemical studies revealed that the expression rates of 5-LO to be classified as mild, moderate, and severe in the submucosa of nasal polyps and in the surface of epithelial cells (Fig. 1). Expression rates of 5-LO in nasal polyps were observed to be weak (46.9%), moderate (26.6%), and severe (18.8%). A proportion of the nasal polyp tissue, 7.8%, showed no expression of 5-LO. No significant differences were found in 5-LO expression among the different genotypes of the IL-4 gene.

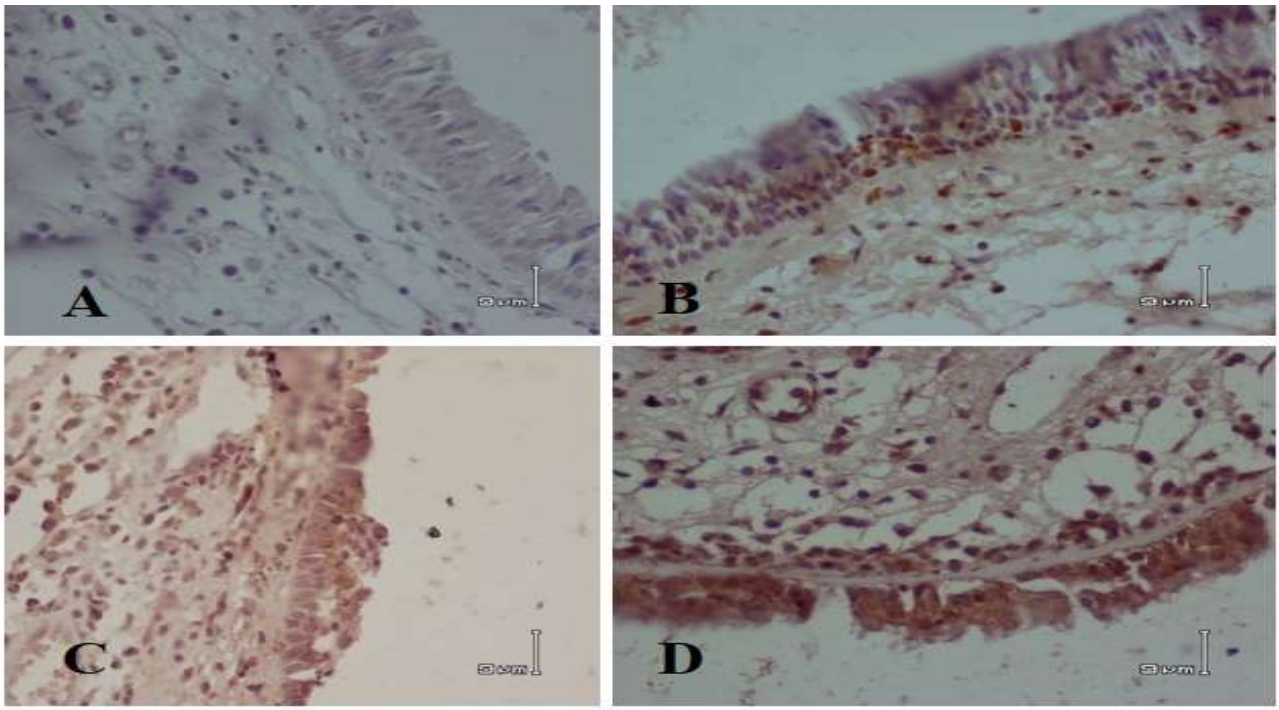

Fig. 1. Expression of 5-lipoxigenase in nasal polyps; **A:** Negative; **B:** Mild**; C:** Moderate**, D:** Severe. (Horseradish-Peroxidase-EnVision method stain; original magnification 400.)

Discussion

In our study, a significant association was found between the occurrence of nasal polyposis and IL-4 -590 C>T gene polymorphisms. Statistical analysis showed that the presence of the T allele at position -590 of the IL-4 gene is associated with increased susceptibility to nasal polyps ($p<0.0001$). Several studies have reported a significant association between the IL-4 -590 C>T gene polymorphism and allergic diseases. In a study by de Guia and Ramos (21), it was demonstrated that the -590C>T IL-4 polymorphism is a potential risk factor for atopic allergy. Additionally, a significant association between IL-4 -590 C>T and asthma has been repeatedly reported in several different population

backgrounds (22-27). The association between the IL-4 -590 C>T gene polymorphism and chronic rhino sinusitis were corroborated in a study examining the Korean population (28). A separate study examining an Iranian population found an association between IL-4 -590 C>T gene polymorphisms and allergic rhinitis (9). Another study of a Korean population showed that the T allele at IL-4 -590 was associated with protection against non-asthmatic and asthmatic nasal polyposis. These results are in disagreement with our findings in which the opposite association was found. Interestingly, the most common genotype of IL-4 -590 C>T in the control Korean population was TT, which was different from other races (10). A recent study from Ahvaz in southeastern Iran reported a lack of association between IL-4 -590 C>T and nasal polyposis (29). Discrepancies between these findings and our results may be attributed to the differences in sample size and exclusion criteria. Moreover, the study from Ahvaz included participants from all racial groups, whereas our current study selected participants exclusively from the city of Kerman. The residents in the city of Ahvaz are a mixture of Arab and Persian races, and thus may account for the differences in our findings.

5-lipoxygenase (5-LO) has a key role in the biosynthesis of LTs from arachidonic acid. A previous study showed insignificant increases in 5-LO expression within the nasal polyp tissues of patients when compared with control tissue samples (30). Park et al. (31) showed the 5-LO expression in non-allergic patients with nasal polyps to be significantly increased in individuals with the genotype CC, in comparison to those with the CT or TT genotype at IL-4 -590. Conversely, the results of the present study showed a lack of association between the expression of 5-LO and all examined IL-4 -590 gene polymorphism genotypes in patients with nasal polyps. The differences in race may have contributed to this discrepancy. Moreover, a limitation to the current study was a lack of data regarding prick tests in patients with nasal polyps. This might be another reason for the dissimilarities among results.

In summary, the current study identified a relationship between IL-4 -590 C>T polymorphisms and nasal polyposis. No association was detected between IL-4 -590 C>T polymorphism and 5-LO expression levels in nasal polyps. However, for a better conclusion regarding the role of 5-LO in development of nasal polyps, we suggest to increase the sample size for future studies.

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Conflict of interest

The authors declare that they have no competing interest.

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