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Association of interleukin 6 single nucleotide polymorphisms with allergic rhinitis

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ABSTRACT

Objectives: Allergic rhinitis (AR) is a polygenic inflammatory disorder of the nasal mucosa with an increasing prevalence worldwide. As interleukin 6 (IL-6) seems to be involved in development of allergic disorders, such as allergic rhinitis, this study was performed to evaluate the association of two promotor variants of IL-6 gene in the AR.
Methods: Ninety eight patients with AR were enrolled in this study. Genotyping was done for two polymorphisms in a promoter region of IL-6 gene (G/C at −174, *rs1800795* and G/A at −597, *rs1800797*), using a PCR sequence-specific-primers method.
Results: Patients homozygous for the G allele of *rs1800795* in IL-6 had a 3.35-fold risk of having AR than those with the C allele. AA genotype in *rs1800797* of IL-6 was associated with the increased risk of developing AR. G/G haplotype for IL-6 (*rs1800795*, *rs1800797*) was significantly higher in the patient group. In some subgroups of patients, there were significant relationships between IgE levels, eosinophil count, eosinophil percentage, nature of sensitivity and persistency of disease and these two variants.
Conclusion: We found that two promotor variants in IL-6, especially *rs1800795*, were predisposing factors for AR with a negative heterosis pattern. These SNPs could also affect the clinical parameters, the nature of sensitivity and persistency of the disease in some subgroups of the patients.

1. Introduction

Allergic rhinitis (AR) is a multifactorial inflammatory disorder of the nasal mucosa present with the clinical symptoms, such as sneezing, nasal congestion and rhinorrhea and a systemic component affecting peripheral blood, bone marrow and lungs [1]. An increasing pattern in the prevalence of the disease has been observed in the last 30–40 years, especially in the industrialized world [2–5]. It is a global health problem impairing the quality of life of 10–30% in adults and 10–46% in children. In addition, it is the first step towards the development of other conditions, such as conjunctivitis and asthma [6].

Several factors have been identified to have a role in the pathogenesis of AR. Imbalance between Th1/Th2 immune response results in selective eosinophil accumulation in the nasal mucosa and allergen-specific immunoglobulin production. The interaction between allergen-specific immunoglobulins and inhaled allergens in the upper airway contributes to underlying inflammatory process responsible for symptoms of AR [1,7]. Currently, there is no doubt that genetic predisposition has a major role in the pathogenesis of the disease [1,2,8]. These genetic factors not only affected its development, but also its severity and treatment [9,10].

Proinflammatory cytokines are emphasized as important mediators of the inflammatory response in allergic rhinitis and bronchial asthma [11], while their single nucleotide

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polymorphisms (SNPs) have been investigated in a number of diseases [12–16]. We recently showed that a number of SNPs with the genes encoding IL-1, TNF-α and IL-4 have associations with AR [17,18]. Interleukin 6 (IL-6), a multifunctional proinflammatory cytokine, theoretically could also be involved in the development of allergic disorders and chronic inflammation [19–21]. Besides inducing expression of acute-phase inflammatory response elements, such as COX-2, nuclear transcription factor (NF-κB) and C-reactive protein (CRP), it is involved in T lymphocytes stimulation and final maturation of IL-4-preactivated B cells to plasma cells [21–23]. It has been showed that local IL-6 increases nasal secretions in patients with AR [21]. Previous studies reported significant increase in IL-6 immunoreactivity in nasal biopsies of patients with AR [24–26]. In addition, previous studies found an association between IL-6 and development of allergic disorders, such as asthma [19–21] and increased nasal secretions and symptoms, such as sneezing, nasal discharge and nasal congestion in upper respiratory infections [27,28]. There was a positive correlation between IL-6 gene transcription and progressive inflammation in nasal tissue and it had a positive correlation with skin prick test results [29,30]. Functional analyses have shown that two polymorphic variants in the promoter region of the IL-6 gene, rs1800795 and rs1800797, influence the transcription of IL-6 [31–33]. The aim of this study was to evaluate the effect of these SNPs in a group of Iranian patients with AR.

2. Material and methods

2.1. Subjects

Ninety eight patients with AR referring to children medical center were included in this study as the patient group by stratified randomization regarding gender, severity and intermittency of disease. The standard allergic rhinitis criteria in the Allergic Rhinitis and its Impact on Asthma (ARIA) document was used to establish the diagnosis of AR and to classify the severity of the disease in patients [34,35]. Interview and medical records were the means of collection of demographic and clinical data. Sensitivity to allergens, including cockroach, tree pollen, grass pollen, weeds, mold, dust mite and animal dander was tested by a commercially available skin prick test kit and results were evaluated according to EAACI criteria [36]. To determine background population allele frequencies, 139 healthy volunteers were recruited as the control group [37]. The inclusion criteria were: (1) having the same ethnicity of the patients, (2) having no history of AR, (3) being asymptomatic at the time of study, and (4) having no evidence of inflammation in the nasal cavity. All the principles of the Declaration of Helsinki were applied in the study and the study was approved by the Ethical Committee of Tehran University of Medical Sciences.

2.2. Genotyping

To perform genotyping, 5 mL of peripheral blood were collected from all the participants in EDTA-treated tubes after obtaining informed consents. "Salting out" method was used to isolate genomic DNA from peripheral blood. Allele frequencies for two IL-6 promoter variants (G/C at −174, rs1800795 and G/A at −597, rs1800797) were determined, using PCR sequence-specific-primers (PCR-SSP) methods by Heidelberg cytokine gene polymorphism SSP kit (Heidelberg University, Heidelberg, Germany). PCR was carried out as explained before [17,18]. In PCR products were visualized by 2% agarose gel electrophoresis in an ultraviolet transilluminator. Then the amplified products were digested and resolved on 2% agarose gel containing ethidium bromide.

2.3. Statistics

All the statistical analyses were performed using SPSS 18 software (SPSS Inc., Chicago, IL, USA). A p value of <0.05 was considered statistically significant. Odds ratios (OR) with 95% confidence intervals (CI) for allele and genotype were calculated by comparing the frequencies among patients and controls and chi-square tests and Fisher's test were used to determine the p. One-way ANOVA test was used in order to compare numeric variables, such as IgE levels, eosinophil count and eosinophil percentage within genotypic subgroups.

3. Results

3.1. Frequencies of gene polymorphisms

The frequencies of two SNPs were compared between patient and control groups (Table 1). Homozygosity for the G allele of *rs1800795* in IL-6 was associated with 3.35-fold (95%CI: 1.88–5.98) risk of having AR comparing to CC genotype. Also, an association was found between the susceptibility to allergic rhinitis and rs1800797 in IL-6, which showed 3.84-fold (95%CI: 1.06–15.04) increase in the risk of AR in patients homozygous for A allele (Table 1).

3.2. Frequency of haplotypes

G/G haplotype for IL-6 (*rs1800795*, rs1800797) was significantly higher in the patient group (71.94% *vs.* 62.2%, respectively, $p < 0.05$, OR = 1.56, 95%CI: 1.03–2.36). C/G haplotype for IL-6 (*rs1800795*, rs1800797) was found in 3.57% of the patients and 19.8% of the controls, respectively ($p < 0.001$, OR = 0.15, 95%CI: 0.06–0.35). However, no significant difference was seen on C/A and G/A haplotypes between two groups.

Table 1
Allele and genotype frequencies of patients group and control group.

SNPs	Genotype	Controls (n = 139), N (%)	Patients (n = 98), N (%)	Odds ratio (95%CI)	p-value
rs1800795	C	101(36.3)	52(26.53)	0.63(0.42–0.96)	0.031
	G	177(63.7)	144(73.47)	1.58(1.04–2.41)	0.031
	CC	4(2.9)	12(12.2)	4.71(1.35–17.95)	0.01
	CG	93(66.9)	28(28.6)	0.2(0.11–0.36)	0.000
	GG	42(30.2)	58(59.2)	3.35(1.88–5.98)	0.000
rs1800797	A	50(18)	48(24.49)	1.48(0.92–2.37)	0.108
	G	228(82)	148(75.51)	0.68(0.42–1.08)	0.108
	AA	4(2.9)	10(10.2)	3.84(1.06–15.04)	0.037
	GA	42(30.2)	28(28.6)	0.92(0.5–1.69)	0.897
	GG	93(66.9)	60(61.2)	0.78(0.44–1.39)	0.445