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Association between IL-4 gene polymorphisms and IL-4 serum levels in patients with allergic rhinitis

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Abstract

Genetic factors play a major role in the formation of allergic rhinitis. The most studied genes for the investigation of the genetic origin of allergic rhinitis are the cytokine genes. We aimed to analyse the relation between susceptibility to allergic rhinitis and IL-4 C-590T and C+33T polymorphisms. For this aim, serum IL-4 levels and IL-4 C-590T and C+33T polymorphisms of 211 allergic rhinitis cases and 232 healthy individuals (control group) were analysed. We found that the individuals carrying IL-4 +33T allele and IL-4 -590TT and +33TT genotypes were much more predisposed to allergic rhinitis than the individuals carrying CC wild type genotypes. Our results also suggest that serum IL-4 levels of control group carrying the IL-4 -590 CT and TT genotypes and T allele and IL-4 +33 CT and TT genotypes, and T allele were significantly higher than the CC genotypes and T alleles carriers. As a result, it is possible to say that IL-4 C-590T and C+33T polymorphisms may increase the susceptibility of allergic rhinitis because of their lowering of IL-4 levels in serum.

Keywords: Allergic rhinitis, genetic susceptibility, interleukin-4, polymorphism

Introduction

Allergic rhinitis is an inflammatory nasal mucosal disease characterized by IgE-dependent Type 1 hypersensitivity reaction. Its symptom is seizure sneezing, abundant and watery nasal discharge, nasal obstruction and pruritus. These can also be accompanied by headache, impaired olfactory function, and other comorbidities (asthma, etc.). It is the most common immunological disease that affects 10-30% of the population especially in the developed societies and the incidence of the disease is increasing day by day. As a major health burden in the world the cause of allergic rhinitis is still unknown. As with other complex diseases, allergic rhinitis is caused by both genetic and environmental factors [1,2].

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Although more than 100 genetic polymorphisms have been associated with the disease, the genetic basis for allergic rhinitis is poorly understood. The most studied genes for the investigation of the genetic origin of allergic rhinitis are the cytokine genes. Since the IL-4/IL-13 pathway has a key role in the development of asthma and atopy, it has been understood that genes in this pathway are associated with asthma/atopy phenotypes in many studies [3,4]. IL-4 plays a key role in atopic immunological response by stimulating IgE synthesis from B lymphocytes and directing T lymphocytes to the Th2 subgroup [5].

IL-4 has a vital role in the development of allergic responses. Traditionally, an allergic response is thought to be composed of two main steps; sensitization and challenge with an otherwise harmless allergen such as pollen or dust that leads to the inflammatory allergic reaction. During the sensitization stage, an individual's immune system is first introduced to the allergen [4,5]. First, IL-4 must engage IL-4R on B cells and, second, the B cell must receive a contact dependent costimulatory signal through ligation of CD40. The stimulation of IgE secretion by B cells leads to binding of the immunoglobulin to the FceRI receptors on mast cells. Upon

secondary exposure to or challenge by the allergen, IgE antibodies bound to mast cells are cross-linked leading to degranulation of mast cells and release of histamine, other inflammatory molecules, and the production of arachidonic acid by-products that are also involved in allergic inflammation. This acute phase of inflammation is followed 4 hours later by a late phase response that includes the infiltration of eosinophils into the site of allergen encounter with subsequent release of inflammatory mediators leading to tissue damage and destruction [6,7]. The IL-4 required for these events is thought to be secreted by CD4+ T cells and perhaps other IL-4 producing cells such as mast cells and basophils. CD4+ T cells, mast cells, and basophils also express CD40L which is the ligand partner for CD40 expressed on B cells [8]. The role of IL-4 in induction of atopy is confirmed by analysis of cytokine expression of CD4+ T cells recovered by Broncho alveolar lavage or from peripheral blood which shows high expression of IL-4 and IL-5 with little to no expression of IFN-y in atopic individuals. Conversely, CD4+ cells from non-atopic individuals showed much lower levels of Th2 cytokine production. Furthermore, in murine models of allergic inflammation, the use of anti-IL-4 neutralizing antibodies at the point of sensitization with antigens greatly reduced IL-5 secretion and eosinophilic infiltration. There seems to be a particularly strong association to the IL-4, IL-5, IL-13 cluster. Atopy has also been weakly linked to the some HLA loci, the FceRI f5-chain locus, the TCR-a chain locus, and perhaps the IL-4R locus as well [8,9].

It has been found that IL-4 C-590T and IL-4 C+33T polymorphisms have been reported to be associated with altered promoter activity and associated with high IL-4 and IgE level in the serum [10-12]. However, the role of these polymorphisms in allergic rhinitis cases has not yet been studied in Turkish population. The aim of this study was to assess the influence of IL-4 C-590T and C+33T polymorphisms on allergic rhinitis and serum IL-4 levels.

Material and Methods

Study population

The patient group of our study consisted of 211 patients who were referred to Van Yuzuncu Yil University, Medical Faculty, Dursun Odabas Medical Center, Polyclinic of Otorhinolaryngology Department with allergic rhinitis diagnosis. Of the patients diagnosed with allergic rhinitis, 107 of them were males and 104 of them were females. The control group consisted of 232 healthy individuals who consulted to the laboratories of Yüzüncü Yıl University, Medical Faculty, Dursun Odabas Medical Center and do not have any inherited, acquired or chronic illnesses. Of the healthy individuals, 123 of them were males and 109 of them were females. Patients and healthy individuals were prospectively evaluated through January 2018–December 2018. The diagnosis of allergic rhinitis was made based on the detailed allergic disease histories of patients, ENT clinical examination data, skin test and specific IgE measurements according to the diagnostic criteria in the Allergic Rhinitis and Its Impact on Asthma (ARIA) guidelines-2016 revision [13]. Allergic rhinitis was defined as persistent or discontinuous symptoms of anterior rhinorrhoea, continuous sneezing, nasal obstruction, and itching; nasal cavity local signs including discolored and edematous mucosa and watery drainage in the common or middle nasal meatus; and positive serum antigen-specific IgE, and positive antigen SPT. All the patients were also questioned in terms of seasonal and perennial allergies. In addition to allergic rhinitis, patients with comorbid diseases such as atopic dermatitis, eczema, asthma, allergic conjunctivitis, chronic cough, laryngitis, and gastro-esophageal reflux were included in the study. Smoking habit, presence of comorbid chronic diseases (diabetes mellitus, hypertension, chronic renal failure, etc.), previous nasal surgery history, usage additional drugs, and additional pathologies (acute or chronic infection or inflammatory diseases in nasal or upper respiratory tract, nasal polyposis, nasal septal deviation, malignancies, etc.) in ENT and endoscopic nasal examinations were excluded for each participant. The study was performed in accordance with the Declaration of Helsinki and Good Clinical Practice and was approved by the Clinical Researches Ethics Committee of Van Yuzuncu Yil University (05/21.11.2017). Informed consent was obtained from all individuals or their relatives for participation in the study. 5 mL peripheral blood were taken to K2-EDTA tubes and stored at + 4°C until the analysis day from healthy individuals and untreated allergic rhinitis patients at the beginning of the study. All studies were carried out in Van Yuzuncu Yil University, Faculty of Pharmacy, Biochemistry Research Laboratory. In addition, the patient follow-up form was used for the detection of laboratory and clinical data of patients with allergic rhinitis and these forms were filled in order to refer patients' polyclinic and service files.

Method Subsections

Serum IL-4 level measurement

Serum IL-4 levels were analysed according to the manufacturer's protocol by using the enzymelinked immunosorbent assay (ELISA) kit (eBioscience; Vienna, Austria).

Determination of Genotypes

Genomic DNA isolation from whole blood samples was performed according to the Poncz method [14]. Identification of *IL-4* polymorphisms was performed using the polymerase chain reaction - restriction fragment length polymorphism (PCR-RFLP) methods using a Thermocycle PCR (Thermo Fisher Scientific, Massachusetts, USA) as previously described [14,15]. The primers were provided by PRZ BioTECH (Bilkent, Ankara, Turkey). Forward 5'-ACTAGGCCTCACCTGATACG-3' and Reverse 5'-GTTGTAATGCAGTCCTCCTG-3' primers were used to examine *IL-4* C-590T polymorphism. After the PCR amplification, RFLP analysis was performed with the restriction enzyme *FaqI* to detect the C>T change in the 252 base-pair region. The samples carrying CC genotype were identified as two bands; 192 bp and 60 bp, TT genotype were identified as single bands; 252 bp and CT genotype were identified as three bands; 252 bp, 192 bp and 60 bp [15]. For detecting IL-4 C+33T polymorphism Forward 5'-TAGAGATATCTTTGTCAGCATT-3' and Reverse 5'-AGACCCATTAATAGGTGTCG-3' primers were used. After the PCR amplification, RFLP analysis was performed with the restriction enzyme *Mnl* I to detect the C>T change in the 93 base-pair region. The samples carrying CC genotype were identified as two bands; 64 bp and 29 bp, TT genotype were identified as single bands; 93 bp and CT genotype were identified as three bands; 93 bp, 64 bp and 29 bp [16].

Statistical analysis

The distributions of the C-590T and C+33T polymorphisms of IL-4 were compared by using $\chi 2$ method and Fisher's exact test. Differences in serum *IL-4* levels between different genotypes were assessed using t-test ANOVA. Results were considered significant if $p<0.05$. Statistical analyses were performed using the SPSS statistical package (verxion 15.0 for Windows, SPSS Inc., Chicago, IL).

Results

Demographic characterization of the studied groups

In this study, we investigated whether there is a relationship between allergic rhinitis and IL-4 C-590T and C+33T polymorphisms. For this purpose, 232 healthy individuals and 211 allergic rhinitis patients were included in the study. Of the patients diagnosed with allergic rhinitis, 82 of them (38.9%) were perennial type, 96 of them (45.5%) were seasonal type, and 33 of them (15.6%) were perennial type with seasonal exacerbations. The rate of asthma prevalence was determined as 27 (28.1%), 28 (34.1%), and 9 (27.3%) in allergic rhinitis patients with seasonal type, perennial type, and perennial type with seasonal exacerbations, respectively. Considering all patients with allergic rhinitis, the rate of asthma prevalence was found to be 30.3%. The mean age of the patients was 34.53±13.1 and the age range was 6-68. The mean age of the control group was 38.96±10.93 and the age range was 13-75. The mean age of the control and allergic rhinitis groups was similar and there was no statistically significant difference between them ($p>0.05$). Among the allergic rhinitis patients in our study, 107 of them were males and 104 of them were females, while healthy individuals in the control group consisted of 125 males and 111 females. Male/female ratio was 1.03 in allergic rhinitis patients and this ratio was found as 1.13 in control group. There was no significant effect of male or female sex on allergic rhinitis ($p>0.05$). The distributions of demographic characteristics of patients with allergic rhinitis and controls are summarized in Table 1.

Comparison of serum IL-4 levels

Serum IL-4 levels were significantly higher in patients with allergic rhinitis compared to the control group ($p<0.05$). A comparison of serum IL-4 levels between the two groups has been shown in Figure 1.

Table 1. Demographic characterization of the studied groups

Characteristics		Control group (n=232)	Allergic rhinitis group (n=211	p value
Age				
	Mean age (Mean±S.D.)	38.96±10.93	34.53±13.1	p>0.05
	Age range (Min.-Max.)	13.75	6-68	p>0.05
Gender				
	Male	125	107	p>0.05
	Female	111	104	p>0.05
	Male/Female ratio	1.13	1.03	p>0.05

(S.D. = Standart deviation, Min.= Minimum, Max.= Maximum)

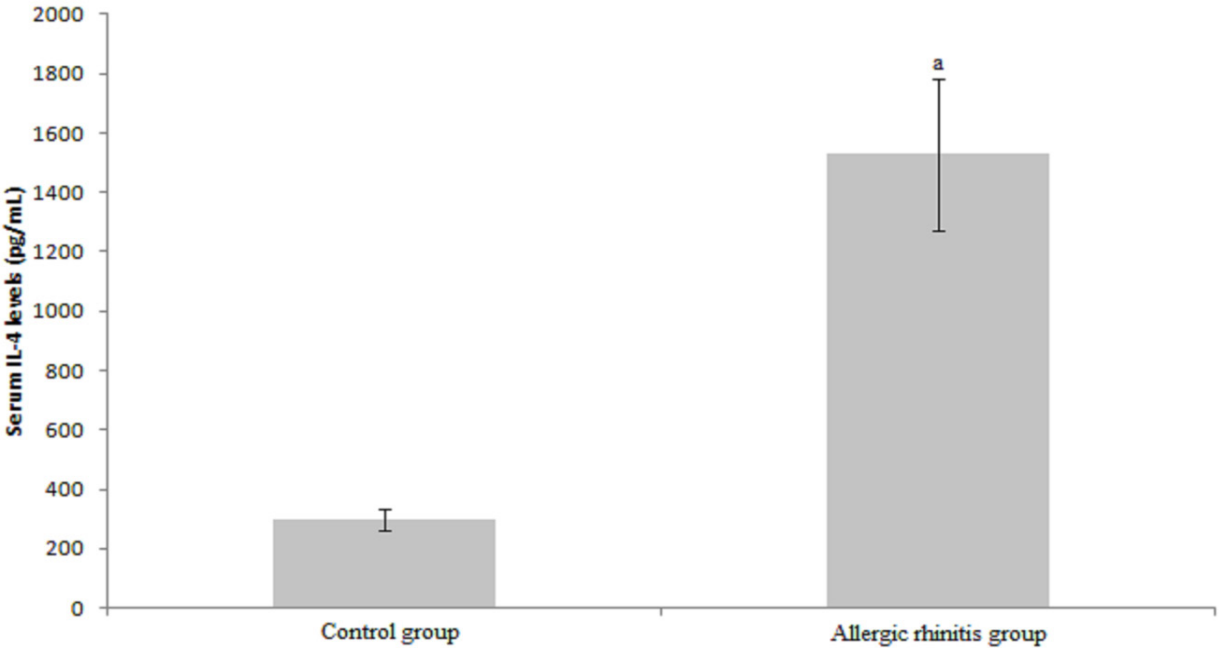

Figure 1. Comparison of serum IL-4 levels

Allele and genotype frequency of IL-4 genetic polymorphisms

The frequency of IL-4 -590CC genotype was found to be 61.21% and 46.45% in control and allergic rhinitis groups, respectively. The frequency of IL-4 -590CT genotype was found to be 24.14% and 23.7% in control and allergic rhinitis groups, respectively. The frequency of IL-4 -590TT genotype was found to be 14.65% and 29.85% in control and allergic rhinitis groups, respectively. In the light of these data, we can suggest that IL-4 -590CT genotype increases the risk of allergic rhinitis by 1.057 times [95% CI = 0.8258-1.8954], but this increase was not statistically significant (p>0.05). On the other hand, IL-4 -590TT genotype increased the allergic rhinitis risk by 1.982-fold [95% CI = 1.1244-3.4936], this increase was statistically significant (p<0.05) (Table 2).

The frequency of IL-4 -590C allele was found to be 73.28% and 58.29% in control and allergic rhinitis groups, respectively. The frequency of IL-4 -590T allele was found to be 26.72% and 41.71% in control and allergic rhinitis groups, respectively. These results show us that IL-4 -590T allele increased the allergic rhinitis risk by 1.47-fold [95% CI = 1.1-1.953], but this rise was not statistically significant (p>0.05) (Table 2).

The frequency of IL-4 +33CC genotype was found to be 57.33% and 48.34% in control and allergic rhinitis groups, respectively. The frequency of IL-4 +33CT genotype was found to be 31.9% and 33.65% in control and allergic rhinitis groups, respectively. The frequency of IL-4 +33TT genotype was found to be 10.77% and 18.01% in control and allergic rhinitis groups, respectively. In the light of these data, we can suggest that IL-4 +33CT genotype increases the risk of allergic rhinitis by 1.2937-fold [95% CI = 0.8166-2.0496], but this increase was not statistically significant (p>0.05). On the other hand, IL-4 +33TT genotype increased the allergic rhinitis risk by 2.6849-fold [95% CI = 1.6446-4.3831], this rise was statistically significant (p<0.001) (Table 2).

The frequency of IL-4 +33C allele was found to be 73.28% and 65.17% in control and allergic rhinitis groups, respectively. The frequency of IL-4 +33T allele was found to be 26.72% and 34.83% in control and allergic rhinitis groups, respectively. These results show us that IL-4 +33T allele increased the allergic rhinitis risk by 1.9617-fold [95% CI = 11.4791-2.6017], this increase was statistically significant (p<0.001) (Table 2).

Table 2. Allele and genotype frequency of IL-4 genetic polymorphisms

Polymorphisms	Genotype / Allele	Control group n (%)	Allergic rhinitis group n (%)	Odds ratio [95% CI]	p value
IL-4 C-590T					
	Genotype				
	CC	142 (61.21)	98 (46.45)	1.00 (referance)	
	CT	56 (24.14)	50 (23.7)	1.057 [0.8258-1.8954]	p=0.2906
	TT	34 (14.65)	63 (29.85)	1.9820 [1.1244-3.4936]	p=0.0180[a]
	Allele				
	C	340 (73.28)	246 (58.29)	1.00 (referance)	
	T	124 (26.72)	176 (41.71)	1.4657 [1.1-1.953]	p=0.090
IL-4 C+33T					
	Genotype				
	CC	133 (57.33)	102 (48.34)	1.00 (referance)	
	CT	74 (31.9)	71 (33.65)	1.2937 [0.8166-2.0496]	p=0.2726
	TT	25 (10.77)	38 (18.01)	2.6849 [1.6446-4.3831]	p=0.0001[a]
	Allele				
	C	340 (73.28)	275 (65.17)	1.00 (referance)	
	T	124 (26.72)	147 (34.83)	1.9617 [1.4791-2.6017]	p=0.0001[b]

[a]Shows significant differences than the CC genotype (p<0.05)
[b]Shows significant differences than the CC genotype (p<0.001)
[c]Shows significant differences than the C allele (p<0.001)
(IL-4: Interleukin-4; C: cytosine; T: thymine

Comparison of serum IL-4 levels according to the IL-4 genetic polymorphisms

Serum IL-4 levels of healthy IL-4 -590 CT and TT genotypes and T allele carries were significantly higher than CC genotype and T allele carries with allergic rhinitis, respectively ($p<0.05$). In control group, serum IL-4 levels carrying the IL-4 -590 CT and TT genotypes and T allele were significantly higher than the individuals carrying CC genotype and T allele ($p<0.05$). On the other hand, in control group, serum IL-4 levels carrying the IL-4 +33 CT and TT genotypes and T allele were significantly higher than individuals with CC genotype and T allele, respectively ($p<0.001$). In allergic rhinitis group, serum IL-4 levels carrying the IL-4 -590 TT genotype and T allele were significantly higher than the individuals carrying CC genotype and T allele ($p<0.001$). On the other hand, in allergic rhinitis group, serum IL-4 levels carrying the IL-4 +33 CT and TT genotypes and T allele were significantly higher than individuals with CC genotype and T allele, respectively ($p<0.001$) (Table 3).

Table 3. Comparison of serum IL-4 levels according to the IL-4 genetic polymorphisms

Polymorphisms	Genotype / Allele	Control group (pg/mL)	Allergic rhinitis cases (pg/mL)
IL-4 C-590T			
	Genotype		
	CC	287.63±39.84	1138.71±296.42[a]
	CT	301.02±34.95[b]	1075.37±209.13[a]
	TT	319.81±36.7[c]	916.08±193.62[a,c]
	Allel		
	C	292.93±35.01	1117.61±163.97[a]
	T	316.55±42.69[d]	969.34±206.54[a,d]
IL-4 C+33T			
	Genotype		
	CC	289.52±41.38	1464.97±306.9[1a]
	CT	302.21±35.19[b]	1203.31±263.1[a,c]
	TT	315.97±39.34[b]	1062.45±218.65[a,c]
Allele			
	C	295.61±34.61	1377.61±261.78[a]
	T	314.02±38.94[d]	1109.94±284.3[a,d]

[a]Shows significant differences than the control group ($p<0.001$)
[b]Shows significant differences than the CC genotype ($p<0.05$)
[c]Shows significant differences than the CC genotype ($p<0.001$)
[d]Shows significant differences than the C allele ($p<0.001$)
(IL-4: Interleukin-4; C: cytosine; T: thymine)

Discussion

The A hallmark feature of allergic diseases is the increased number of inflammatory cells within the airways, notably CD4+ T lymphocytes, eosinophils, basophils, and mast cells, as well as the activation of structural and resident cells, particularly the airway epithelial cells. The infiltrating inflammatory cells make their way to the target organ through site-specific endothelial adhesion molecules, as well as a wide variety of chemokines, whose expression is induced by cytokines released from these inflammatory cells. Chemokines are relevant in inflammation, not only because of their role in regulating leukocyte recruitment to allergic sites, but also because of their ability to activate inflammatory cells to release pro-inflammatory mediators at the site of inflammation where they work together to promote the Th2 inflammatory response. Mast cells and T helper cells are the main source of IL-4 promoting the differentiation of Th2 cells and IgE production. This interaction between the released mediators, cytokines and chemokines, with the resident and infiltrating inflammatory cells leads to the development of the inflammatory response in allergic diseases. The IL-4 plays a critical role in allergic diseases particularly in the late phase of the disease as it mediates important pro-inflammatory functions [8,16].

These finding are in accordance with work from previous studies evaluating the relationship between IL-4 polymorphisms and the risk of various allergic-related diseases including allergic rhinitis, asthma, and atopic dermatitis [17-19].

C-590T polymorphism (rs2243250) located at the -590. position of the promotor region of IL-4 gene has been previously shown to correlate with an increased risk of allergic diseases. This polymorphism is understood to up-regulate IL-4 gene expression and subsequently increase the levels of plasma IgE, exacerbating the symptoms of allergic rhinitis [18]. Our study showed that although CT genotype did not affect the frequency of disease occurrence, TT genotype and T allele increased the risk of disease. A separate study observed a protective effect for the CC

genotype when compared to the TC and TT genotypes of patients with allergic-related disorders [20]. Similarly, additional studies of patients with rhino conjunctivitis and hay fever showed no significant association between the IL-4 C-590T polymorphism and allergy [21,22].

A large meta-analysis evaluating several published human studies revealed that the T allele and TT genotype of the C-590T polymorphism in the IL-4 gene promoter have a positive association with allergic rhinitis risk [17]. The promoter region of the IL-4 gene contains a C-590T SNP that is understood to interact with nuclear transcription factors and regulate IL-4 expression. Specifically, the T allele has been shown to enhance the binding of nuclear transcription factors to the promoter region, ultimately upregulating the IL-4 expression [18]. Furthermore, it was found that there is a relationship between IL-4 C-590T polymorphism and IgE levels. The substitution of C with T in the -590 position of the IL-4 promoter has been observed to increase IgE levels in patients with asthma [19]. Rosenwasser et al. [23] demonstrated the association of C-590T polymorphism in IL-4 gene with increased IgE levels in asthma. Similar to our results, Lu et al. [18] indicated that allergic rhinitis patients with the TT genotype of the C-590T polymorphism have been found to have significantly higher levels of IgE and an increased risk of allergic rhinitis than those with the CT and CC genotypes in Chinese population. Contrary to previous findings, Movahedi et al. [24] reported that in allergic rhinitis patients, the CC genotype of the C-590T polymorphism within IL-4 gene was associated with increased risk of allergic rhinitis in Iranian population. Patients with the TT genotype were found to have a negative association with allergic rhinitis [24]. The inconsistencies may be explained by the variations in allele frequencies among different ethnic groups.

C33T polymorphism (rs2070874) which is located at the 33. position of the untranslated region (UTR) of IL-4 gene has been previously shown to correlate with increased risk of several allergic diseases and elevated serum IgE levels [25,26].

In our study, we found that CT and TT genotypes and T allele increased the risk of allergic rhinitis. Similarly, Suziki et al. [26] demonstrated that this polymorphism increased the risk of asthma in Japanese population. Similarly, Shamran et al. [27] found that this polymorphism increased the glioma risk in the Iraqi population. Again, the same study showed that this polymorphism increased IL-4 expression [27]. Although the exact molecular mechanism by which this increase occurs is not known, it is thought that this genetic change may cause this result by affecting gene transcription and mRNA stabilization.

It has been shown in the literature that both IL-4 C-590T and C33T polymorphisms increase serum IL-4 levels [23,27,28]. In our study, it was shown that both polymorphisms increased serum IL-4 levels, and these results were consistent with the literature. On the other hand, high levels of serum IL-4 in patients with allergic rhinitis indicate that IL-4 levels are closely related to allergic rhinitis. On the other hand, serum IL-4 levels were found to be high in various allergic diseases such as asthma and atopic dermatitis [23]. In the current study, in the control group, the presence of IL-4 -590T and 33T alleles caused a decrease in the serum IL-4 level, whereas the presence of these alleles in the allergic rhinitis group increased the serum IL-4 level. Our foresight for this situation is that IL-4 levels are controlled by different genetic and epigenetic mechanisms in the pathogenesis of allergic rhinitis besides the polymorphism of IL-4 gene.

Conclusion

In conclusion, both IL-4 polymorphism plays a critical role in the progression of allergic rhinitis. Although these polymorphisms examined in our study have been shown to increase susceptibility to allergic rhinitis, it would be more accurate to explain the etiology of this disease, which has complex features, with the interaction of both genetic and environmental factors. Since different results have been obtained in studies examining the effects of cytokine gene polymorphisms on cytokine gene expressions and serum levels, additional research is needed to further identify and evaluate genetic variants and cytokine production in allergic rhinitis. Such studies may contribute to the development of new treatment strategies for allergic rhinitis.

Conflict of interests
The authors declare that they have no competing interests.

Financial Disclosure
All authors declare no financial support.

Ethical approval
The study was conducted following ethical approval from the Clinical Researches Ethics Committee of Van Yuzuncu Yil University (05/21.11.2017).

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