

Association Between the Functional Polymorphism C-159T in the *CD14* Promoter Gene and Nasal Polyposis: Potential Role in Asthma

N Yazdani,¹ MM Amoli,² M Naraghi,¹ A Mersaghian,³ F Firouzi,³
F Sayyahpour,² Z Mokhtari¹

¹Otorhinolaryngology Research Center, Tehran University of Medical Sciences, Tehran, Iran

²Endocrinology and Metabolism Research Center, Tehran University of Medical Sciences, Tehran, Iran

³Department of Otorhinolaryngology, Tehran University of Medical Sciences, Tehran, Iran

■ Abstract

Background: Nasal polyposis (NP) is a chronic inflammatory disease that is frequently associated with allergy and asthma. Corticosteroid therapy and surgical removal of polyps are the 2 most common treatment strategies for NP. Various allergic and inflammatory mediators are thought to play a major role in the pathophysiology of this disorder. The *CD14* gene is located on chromosome 5q31-32, which is considered a critical region for several allergic and atopic diseases, including asthma. Consequently, variations in *CD14* could have functional effects on the etiology and severity of allergy and asthma. The aim of this study was to investigate the association between the polymorphism C-159T in the *CD14* gene of patients with NP and controls.

Methods: The study population comprised 106 patients with NP diagnosed based on computed tomography scan of the paranasal sinus, endoscopy, and histological examination. Findings were compared with those from 87 controls. The frequency of C-159T was determined using polymerase chain reaction-restriction fragment length polymorphism analysis. DNA was extracted using the salting out technique.

Results: A significant association was observed between C-159T and NP ($P=.04$). Patients with the CC genotype at position -159 of the *CD14* promoter region had an increased risk of asthma (OR, 3.83, 95%CI, 0.99-13.91; $P<.02$). However, we did not find an association between the distribution of C-159T and serum immunoglobulin E level.

Conclusions: A genetic variation in the *CD14* promoter might play a role in the pathogenesis of NP and in the incidence of asthma.

Key words: Nasal polyps. *CD14* polymorphism. Allergy. Asthma.

■ Resumen

Antecedentes: La poliposis nasal (PN) es una enfermedad inflamatoria crónica que se asocia frecuentemente con la alergia y el asma. Tanto el empleo de corticoides como la extirpación quirúrgica de los pólipos son las dos estrategias terapéuticas actuales más frecuentes para la PN. Existen varios mediadores inflamatorios y de la respuesta alérgica que parecen tener un papel importante en la fisiopatología de este trastorno. El gen de *CD14* se localiza en el cromosoma 5q31-32, siendo ésta una región trascendental en el estudio genético de diferentes enfermedades alérgicas, incluida el asma. Por tanto, es probable que las variaciones en el gen de *CD14* tengan efectos sobre la etiología y la gravedad de las enfermedades alérgicas y del asma. El objetivo de este estudio fue investigar la asociación entre el polimorfismo C-159T de *CD14* en pacientes con poliposis nasal en comparación con los controles.

Métodos: Se estudiaron 106 pacientes con PN diagnosticados mediante TAC de senos paranasales, endoscopia y el examen histológico, así como 87 controles. La frecuencia del polimorfismo C-159T de *CD14* se determinó por el método PCR-RFLP después de la extracción de ADN.

Resultados: Los resultados del estudio revelaron una asociación significativa entre el polimorfismo C-159T de *CD14* y PN ($p = 0,04$). Se encontró que los pacientes con genotipo CC en la posición -159 de la región promotora de *CD14* tenían un mayor riesgo de asma (OR = 3,83, IC 95%: 0.99-13.91, $p < 0,02$). Por el contrario, no se encontró ninguna asociación entre el polimorfismo C-159T de *CD14* y el nivel de IgE sérica.

Conclusiones: La variación genética en el promotor de *CD14* puede jugar un papel en la patogénesis de la PN y la incidencia de asma.

Palabras clave: Pólipos nasales. Polimorfismo de *CD14*. Alergia. Asma.

Introduction

The exact worldwide prevalence of nasal polyposis (NP) is unknown, because the results of the few epidemiological reports to date were significantly influenced by the different diagnostic methods used. The most practical approaches to the diagnosis of NP include taking a history, rhinoscopy, endoscopy, and computed tomography [1]. The incidence of NP is estimated to be about 2% to 4% [2] in the general population. NP is categorized according to its origin [3]. The polyp is composed of edematous mucous membrane with varying densities of inflammatory cells that originates around the openings of the ethmoid or maxillary sinus. NP is a chronic inflammatory disease that is associated with allergic rhinitis and several other conditions, such as asthma, aspirin intolerance, cystic fibrosis, Young syndrome, and Kartagener syndrome [1,4,5]. The 2 main treatment strategies for NP are drug therapy (corticosteroids) and surgical removal of the polyps [6].

The etiology of NP remains unclear. Inflammatory mediators may be a key element in the underlying molecular mechanisms. Although the role of allergy in the pathogenesis of NP remains open to debate, it is thought to be one of the underlying mechanisms of the disease [2,4]. Based on this hypothesis, allergic patients are more prone to polyps than the general population; consequently, positive results to allergy testing are very common in patients with NP [1].

Linkage and association studies of atopy and allergy have already identified a number of relevant chromosomal areas [7]. These findings are supported by markers within the HLA region on chromosome 6 and within the *TCR* gene complex on chromosome 14q11, which shows significant linkage with atopy [7]. A specific region in chromosome 5, 5q31-32, which contains a cluster of cytokines (interleukin [IL] 4, IL-13, IL-9, and granulocyte-macrophage colony-stimulating factor [GM-CSF]), has also been considered an important locus in regulating the allergic response [8-11]. The cluster differentiation antigen CD14, a 55-kDa glycoprotein, is also located on 5q31-32 [12]. It encodes 2 protein isoforms: a membrane molecule (mCD14) on the surface of monocytes, macrophages, and neutrophils, and a soluble form in serum [13]. CD14 is a major receptor for lipopolysaccharide (LPS), or inhaled endotoxin. LPS and other bacterial wall products can stimulate antigen-presenting cells (eg, dendritic cells) to produce interleukins through the action of soluble CD14 (sCD14) [14]. Subsequently, genetically determined higher levels of sCD14 in serum or a higher density of mCD14 could result in a stronger interleukin signal from these antigen-presenting cells, which, in turn, could be a potent signal for maturation of type 1 helper T cells (T_H1) [14].

It has been suggested that altered CD14 expression can affect the ratio of T_H1 to T_H2 cells, thereby influencing immunoglobulin (Ig) E responses and the associated inflammatory phenotype [13]. Indeed, CD14 functions may play a crucial role in switching from the T_H2 response to the T_H1 response. Activation of monocytes and macrophages through CD14 leads to the release of a complex mixture of regulatory cytokines to inhibit the production of IgE by interacting directly with T and B cells [15].

A C-to-T transition has been identified at position 159 in the promoter region of the *CD14* gene [16]. This functional single-nucleotide polymorphism, C-159T, has been associated with altered CD14 and IgE levels in patients with allergic diseases such as asthma and allergic rhinitis in various ethnic populations; however, this association has not yet been confirmed in patients with NP. Although the C-159T TT genotype is associated with higher circulating sCD14, a lower mean number of positive skin test results, and lower serum IgE levels in some populations, the CC genotype is associated with higher levels of total serum IgE and a higher number of positive skin test results [16]. Furthermore, the C allele has been associated with atopy, specifically to molds, in a Chinese population [16].

Eosinophils are induced and activated by several cytokines and chemokines. In allergic disease, blood and tissue eosinophilia are caused by upregulation of IL-5, GM-CSF, eotaxin, and regulated upon activation, normal T-cell expressed and secreted protein (RANTES) [17]. Patients with allergic NP have significantly higher tissue expression of IL-4, IL-5, GM-CSF, and IL-3 than patients with nonallergic NP. IL-5 and GM-CSF inhibit the apoptosis of eosinophils *in vitro* and *in vivo* [17]. CD14 provides essential signal molecules for the activation of the antiapoptotic pathway(s) induced by the IL-3/IL-5/GM-CSF receptor subunit in human eosinophils [17]. Thus, one possibility is that *CD14* polymorphisms in nasal polyps activate IL-5 receptors and thus prolong eosinophil survival.

Consequently, genetic variations in *CD14*, particularly polymorphisms located on the promoter region, are thought to have functional effects. While a positive correlation between NP and the results of allergy workups has been proposed [10,18-21], we analyzed C-159T to establish an allelic association between the severity of NP and IgE level in Iranian patients with NP.

Material and Methods

Sample Collection

Patients and controls were recruited from Amiralam Hospital, which is affiliated with Tehran University of Medical Sciences in Tehran, Iran. The control group comprised 87 healthy individuals with no symptoms or history of allergy or NP. The case group comprised 106 individuals with clinically and endoscopically diagnosed NP recruited between 2006 and 2008. Informed consent was obtained from all participants, and the Ethics Committee of Tehran University of Medical Sciences approved the study.

Once diagnosis was confirmed, 5 cc of peripheral blood was collected from each participant in EDTA tubes and stored at -20°C for DNA extraction. Patients then underwent surgery. If the diagnosis of NP was not verified by histopathology, the patient was excluded. The distribution of the C-159T was determined using polymerase chain reaction (PCR)-restricted fragment length polymorphism analysis with DNA extracted from peripheral blood leukocytes.

Genomic DNA was extracted from anticoagulated blood

collected in EDTA-containing tubes using the salting out method. C-159T was detected using a modification of the method described previously by Baldini et al [22]. The primers used were CD14 forward primer (5'-GTG CCA ACA GAT GAG GTT CAC-3') and CD14 reverse primer (5'-GCC TCT GAC AGT TTA TGT AAT-3') under the following reaction conditions: initial denaturation at 96°C for 5 minutes, followed by 36 cycles of 95°C for 30 seconds, 68°C for 30 seconds, 72°C for 30 seconds, and 72°C for 7 minutes as the final extension step. The 497-bp PCR product (3 µL) was digested overnight with *Ava*II (Invitrogen) (1.5 µL) in an appropriate buffer at 37°C to generate fragments of 497 bp, 353 bp, and 144 bp. The *Ava*II endonuclease-specific recognition site in the 497-bp PCR product is the GGTC sequence, which is exclusive to carriers of the CD14/-159 T allele. Therefore, a single 497-bp band characterizes the homozygous CC allele of the *CD14* gene and bands of 144 bp and 353 bp represent the homozygous TT allele. Heterozygotes exhibited all 3 bands. The digested fragments were separated by electrophoresis in 2.5% agarose gel and visualized with ethidium bromide (Figure).

Finally, serum IgE level was measured using enzyme-linked immunosorbent assay (ELISA) with a commercially available kit (Monobind Inc). Serum samples were assayed according to the manufacturer's instructions. Briefly, diluted serum was incubated in plates for 30 minutes. After washing, conjugated antibody was added; 30 minutes later the wells were washed and substrate added. Color development was recorded using the ELISA reader.

Statistical Analysis

All statistical analyses were performed using SPSS version 11.5 (SPSS Inc). The frequency of genotypes and alleles was calculated by direct counting. Hardy-Weinberg equilibrium was tested using the χ^2 test, which was also used to compare differences in genotype or allele frequencies between the groups. Furthermore, the odds ratios (OR), confidence intervals (CI), and *P* values were calculated to assess the strength of association between allele frequency and disease. A *P* value <.05 was considered to be significant.

Results

Study Population

The case group comprised 81 males and 25 females with a mean (SD) age of 41.1 (12.5) years; the control group comprised 48 males and 39 females with a mean (SD) age of 36.7 (13.3) years. No significant differences in age or sex were observed between the groups. The demographic features of the study population are summarized in Table 1.

CD14 Genotyping

The allele frequencies were 44.3% for the C allele and 55.7% for the T allele in the cases and 36.75% for the C allele and 63.25% for the T allele in the controls. Allele and genotype frequencies were in Hardy-Weinberg equilibrium. Table 2 shows the genotype frequency distribution of C-159T in both cases and controls. The results revealed a significant association between the polymorphism and NP (OR, 1.88; 95%CI, 0.99-3.56; *P*=.04).

Endoscopy revealed that 22 patients (20.8%) had polyps on the inferior turbinate, 83 patients (78.3%) had polyps on the middle turbinate, and 1 patient (0.9%) had a polyp in the narrow inlet of the ostiomeatal complex. The endoscopy findings and the Lund-Mackay score revealed no significant correlation between the polymorphism and polyp size.

Given the widespread use of systemic and topical corticosteroids in polyposis, patients were assigned to 1 of 2 groups according to length of therapy: corticosteroid-free patients (<1 month) and corticosteroid consumers (\geq 1 month). More patients had the C allele (CC and CT) than the T allele (OR, 3.43; 95%CI, 1.02-11.47; *P*<.02). The C allele was less frequent in corticosteroid users than in corticosteroid-free patients.

Nine patients (8.5%) were smokers and 97 patients (91.5%) were nonsmokers. However, as the sample was small, differences in the distribution of alleles could not be assessed.

Twenty patients (18.9%) were asthmatic and 86 patients (81.1%) were nonasthmatic. The distribution of patients with

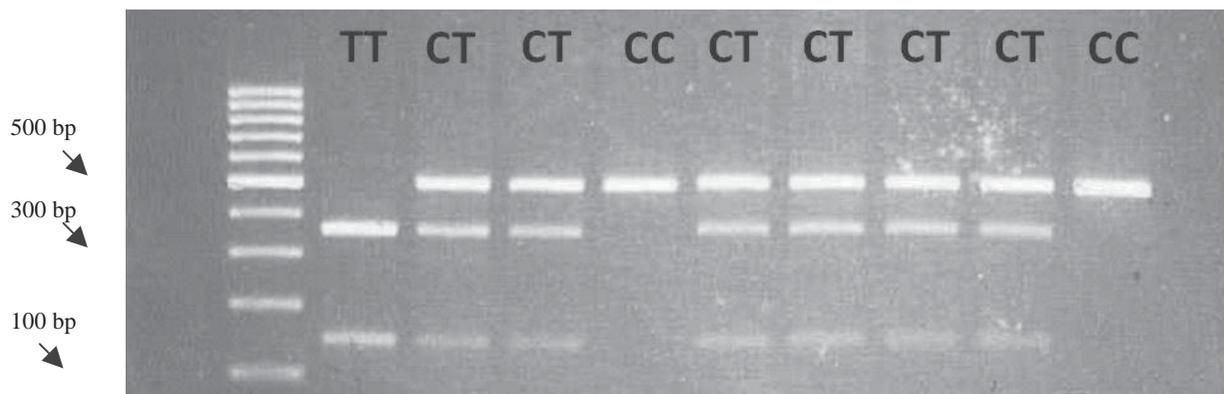


Figure. Gel photo of a polymorphism in *CD14* genotyped using the polymerase chain reaction-restricted fragment length polymorphism assay.

Table 1. Demographic Features of Cases and Controls

| | Previous Sinus Surgery | | Smoking | | Asthma | | Endoscopy: Extent of Involvement | | | Gender | | Age |
|-----------------|------------------------|-----------|---------|-----------|-----------|-----------|----------------------------------|-----------|-----------|-----------|-----------|-------------|
| | - | + | + | - | + | - | Only OMC | Up to MT | Up to IT | Female | Male | Mean (SD) |
| Cases (n=106) | 73 (75.3) | 23 (24.7) | 9 (8.5) | 97 (91.5) | 20 (18.9) | 86 (81.1) | 1 (0.9) | 83 (78.3) | 22 (20.8) | 25 (23.6) | 81 (76.4) | 41.1 (12.5) |
| Controls (n=87) | 87 (100) | 0 | 0 | 87 (100) | 0 | 87 (100) | - | - | - | 39 (44.8) | 48(55.2) | 36.7 (13.3) |

Abbreviations: IT, inferior turbinate; MT, middle turbinate; OMC, ostiomeatal complex.

Table 2. Distribution of Genotype Frequencies of the C-159T Polymorphisms in the Study Population

| | Group | | Lund-McKay Score | | Corticosteroid-Native | | Immunoglobulin E Level, IU/mL (n=91) | | |
|----|--------------|----------------|------------------|---------------|-----------------------|----------------|--------------------------------------|---------------|-------------|
| | Case (n=106) | Control (n=87) | 10-14 (n=3) | 15-24 (n=103) | Case (n=89) | Control (n=87) | <20 (n=30) | 20-200 (n=31) | >200 (n=30) |
| CC | 19 (17.9) | 15 (17.2) | 0 | 17 (16.0) | 16 (17.9) | 15 (17.2) | 12 (40.0) | 7 (22.5) | 2 (6.7) |
| CT | 56 (52.8) | 34 (39.1) | 3 (2.8) | 52 (49.1) | 51 (57.3) | 34 (39.1) | 9 (30.0) | 14 (45.2) | 18 (60.0) |
| TT | 31 (29.2) | 38 (43.7) | 0 | 34 (32.1) | 22 (24.8) | 38 (43.7) | 9 (30.0) | 10 (32.3) | 10 (33.3) |

and without asthma by genotype was as follows: CC genotype, 7 asthmatic and 12 nonasthmatic; CT genotype, 9 asthmatic and 47 nonasthmatic; TT genotype, 4 asthmatic and 27 nonasthmatic. Our results revealed a significant association between the CC genotype and asthma; in fact, asthmatic patients were 3.8 times more likely to have the CC genotype than the CT or TT genotype (OR, 3.82; 95%CI, 0.99-13.91; $P < .01$).

Finally, the statistical analysis of IgE serum levels and CD14 polymorphisms showed no significant association between the polymorphism and the plasma level of IgE in NP patients (Table 2).

Discussion

The underlying causes of NP remain unidentified, although the various molecular mechanisms proposed include the allergic response [1,2,4]. CD14 plays a major role in innate immunity and allergic responses [23] and participates in the immune response to respiratory syncytial virus [24]. Several asthma-associated markers can be found at the location of the CD14 gene [8-11]. CD14 acts as a specific receptor for LPS and other bacterial cell wall components [25-27]. In this study, we examined the C-to-T substitution in CD14 to determine the association between allelic variants and the severity of polyposis and IgE level in Iranian patients with NP.

Several population studies examine the association between polymorphisms in CD14 and atopy and asthma [8,9,18-20, 28]. Although the results seem to confirm the role of CD14 in these diseases, they are often open to debate [29,30]. We found a significant association between the CC genotype and asthma; in fact, asthmatic patients were 3.8 times more likely to have the CC genotype than the CT or TT genotype. This finding is consistent with a recently published meta-analysis in which carriers of the TT and CT genotypes were 33% less likely (OR, 0.67; 95%CI, 0.54-0.84) and 20% less likely (OR, 0.80; 95%CI, 0.66-0.95), respectively, to have atopic asthma than carriers of the CC genotype [31]. Hence, carriers of the C allele are more likely to develop asthma and NP.

IgE-mediated allergic diseases are chronic disorders with genetic and environmental components; therefore, the

CD14 gene is a promising candidate for allergic conditions. Kowal et al [32] suggested that the incidence of the T allele at nucleotide 1341 of CD14—G(1341)T polymorphism—is associated with positive skin prick test results for mite ($P=.007$) and molds ($P=.041$). Similarly, the frequency of the C allele for C–159T was significantly higher in NP patients with a positive skin prick test result for mite ($P=.046$) and molds ($P=.056$) [32].

In recent years, more than 100 candidate genes have been associated with NP. Ten of these genes are particularly promising, including *CD14*, which acts as a bacterial endotoxin receptor [18]. Previous studies indicated that patients with lower lipopolysaccharide levels who harbored CC genotypes at position –159 on *CD14* were less susceptible to progression of asthma and atopy [3,33]. To our knowledge, no previous studies have reported a significant association between the incidence of the C allele of C–159T and NP. We found NP to be significantly associated with the C-to-T substitution at position –159 of the *CD14* promoter region ($P<.05$). Moreover, the incidence of the C allele is associated with corticosteroid-free status. Nevertheless, IgE level was not associated with the *CD14* polymorphism, probably in part because of corticosteroid use.

Several studies have shown that the association between genotypes of the single nucleotide polymorphism –159 and IgE levels depends on environmental factors, such as tobacco smoke, pets, and exposure to endotoxins [16]. Similarly, the association between –159 and allergic sensitization depends on the level of exposure to endotoxins: TT homozygotes are protected at low levels of exposure and at risk at high levels [16]. These findings suggest an antagonistic interaction between environment and –159 as a determinant of allergic sensitization: the T allele could be either a protective factor or a risk factor, depending on the degree of exposure to environmental microbial products. The presence or absence of atopy in patients with polyposis should be investigated to determine the relationship between severity of polyposis (not just size) and presence of *CD14* polymorphisms. In addition, tissue eosinophilia should be evaluated in greater depth in patients with polyposis. Further studies with larger sample sizes of asthmatic patients, especially those with Samter triad, are strongly recommended.

Our results revealed that the CC and CT genotypes are significantly more frequent in NP patients. The association between serum IgE level, incidence of NP, and polymorphisms in *CD14* could not be addressed, since most patients had been receiving corticosteroids, thus rendering their serum IgE level unreliable.

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- *Manuscript received August 29, 2011; accepted for publication April 23, 2012.*
- **Zahra Mokhtari**
- Otorhinolaryngology Research Centre
Amiralam Hospital
North Saadi Ave, Tehran, Iran 1145765111
E-mail: zmokhtari@razi.tums.ac.ir
Mokhtari9002@yahoo.com