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What is This?
Lack of Association between Eotaxin-1 Gene Polymorphisms and Nasal Polyposis

Suna Ekinci, MD¹, Selim S. Erbek, MD¹, Erkan Yurtcu, PhD², and Feride I. Sahin, MD, PhD³

Abstract
Objective. To examine whether there is an association of eotaxin-1 gene polymorphisms with nasal polyposis (NP).
Study Design. Cross-sectional study.
Setting. Tertiary referral center.
Subjects and Methods. The study group included 85 patients with NP and 93 controls without sinonasal disease. Genotypes of eotaxin-1 (−384 A>G and +67 G>A) were identified by restriction fragment length polymorphism analyses after polymerase chain reaction.
Results. The −384 A>G and +67 G>A single nucleotide polymorphisms were higher in patients with NP than in controls (P = .044 and P = .019, respectively). However, their relation was statistically poor (association coefficient = 0.18). Consistent with this result, comparisons of allele frequencies for both single nucleotide polymorphisms were not significantly different (−384 A>G, P = .164; +67 G>A, P = .144).
Conclusion. In this study, eotaxin-1 −384 A>G or 67 G>A genotypes were not associated with susceptibility to NP.

Keywords
eotaxin, nasal polyposis, genotype, polymorphism

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Nasal polyposis (NP) is the presence of bilateral polyps in the nasal cavities that are endoscopically visualized in the middle meatus. Asthma, aspirin intolerance, allergies, and genetic factors are related to the disease.¹² Although the etiopathogenesis is multifactorial, the common feature is the infiltration of edematous stroma by inflammatory cells.³ Eosinophils are the predominant cells, and activation of these cells is more intense in nasal polyp tissues.¹ Therefore, research has focused on eosinophil activation and chemotaxis pathways in NP patients.
Eotaxin-1 is a CC chemokine that recruits eosinophils from the bloodstream to the inflammation area.⁴ Eotaxin was shown to take an important role in chemotraction and activation of eosinophils in nasal polyp tissue.⁵ The human eotaxin gene is located on chromosome 17q21.1. Eotaxin gene mutations have been analyzed in different diseases.⁶⁻⁸ The associations of different gene polymorphisms with NP were shown in our previous studies.⁹⁻¹⁰ Yet there is no study in the literature that investigates eotaxin gene polymorphisms in patients with NP. The aim of this study was to determine whether the eotaxin-1 gene polymorphisms in promoter (−384 A>G) or in coding (67 G>A) areas were associated with susceptibility to NP.

Materials and Methods
A prospective and controlled clinical study was designed and conducted at the Departments of Otolaryngology, Medical Biology and Genetics, and Medical Genetics, Baskent University, Ankara, Turkey. The study was performed on 85 consecutive NP patients (51 male, 34 female; mean age, 45.1 ± 12.1 years). The diagnoses were based on each patient’s medical history, nasal endoscopy, and computed tomography. Skin-prick tests were also performed on all patients. Each patient was evaluated for sensitivity to 8 common allergen extracts (ALK Abello, Madrid, Spain) of the airborne allergens present in our geographic area. A test result was considered positive when at least 1 of the induction diameters was 3 mm higher than that in the negative control. Patients with inverted papilloma, antrochoanal polyp, or cystic fibrosis were excluded. The control group consisted of 93 sex- and age-matched (57 male, 36 female; mean age, 46.2 ± 11.7 years) healthy volunteers without sinonasal disease. The Baskent University Institutional Review Board approved the study.

1Department of Otolaryngology, Baskent University, Faculty of Medicine, Ankara, Turkey
2Department of Medical Biology, Baskent University, Faculty of Medicine, Ankara, Turkey
3Department of Medical Genetics, Baskent University, Faculty of Medicine, Ankara, Turkey

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Corresponding Author:
Selim S. Erbek, MD, Department of Otolaryngology, Faculty of Medicine, Baskent University, 06490 Ankara, Turkey
Email: selimerbek@gmail.com
frequencies did not differ between the patients and controls \((P = 0.16\) for \(-384\ A>G; P = 0.14\) for \(+67\ G>A)\).

There was no association between the presence of the asthma or allergy and the eotaxin-1 genotypes \((P > .05)\).

## Discussion

Eotaxin expression is increased in many diseases due to its role in allergy and immunity.\(^{12}\) The relationship of eotaxin gene mutations with disease predisposition and clinical features in asthmatic patients have been investigated.\(^{13}\) To our knowledge, however, there is no study that investigates eotaxin gene polymorphisms in NP patients. In our study, a potential association between the eotaxin-1 gene \(-384\ A>G\) SNP in the promoter area and the \(+67\ G>A\) SNP in the coding area, and susceptibility to NP was analyzed.

The association of eotaxin-1 \(-384\ A>G\) SNP with susceptibility to diseases is controversial in the literature. Raby et al.\(^ {14}\) studied 9 polymorphisms in white, Hispanic, and African American families. The authors showed that \(-384\ A>G\) SNP was associated with asthma among African American families. In another study, there was a significant relationship between \(-384\ A>G\) SNP and serum total IgE and eotaxin levels in asthmatic patients.\(^ {15}\) Miyamatsu et al.\(^ {16}\) investigated eotaxin-1 gene polymorphisms in asthma patients. The authors could not find an association between genotypes and asthma predisposition. Moreover, Wang et al.\(^ {17}\) could not show an association between asthma and eotaxin-1 \(-384\ A>G\) genotypes. Similarly, there was no significant relationship between eotaxin-1 gene \(-384\ A>G\) and NP predisposition in our study. The genotypes of our series did not differ with regard to the presence of allergy or asthma. Ethnic variations, as in Raby’s study,\(^ {14}\) might be responsible for the above-mentioned results.

The impact of eotaxin-1 \(+67\ G>A\) SNP on diseases is also controversial in the literature. Batra et al.\(^ {15}\) found both a significant relationship between eotaxin-1 \(+67\ G>A\) genotype and asthma and a correlation between mutant genotype and plasma eotaxin level. However, other studies did not support these findings.\(^ {7,16,18}\) The present study failed to show an association between eotaxin-1 \(+67\ G>A\) genotypes and NP.

Although we could not find an association between eotaxin-1 gene \(-384\ A>G\) or \(+67\ G>A\) genotypes and susceptibility to NP in our patients, some limitations inherent to the design of our study might affect the results. First, this

#### Table 1. Primers for the Polymerase Chain Reaction

<table>
<thead>
<tr>
<th>Site</th>
<th>Primer Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>(-384\ A&gt;G)</td>
<td>Sense: 5’-CGTTTCTTGCTCTTCCCTC-3’</td>
</tr>
<tr>
<td></td>
<td>Antisense: 5’-GCAGAACGAGAAGAGGCAAC-3’</td>
</tr>
<tr>
<td>(+67\ G&gt;A)</td>
<td>Sense: 5’-GAATCTCCACACTGCTGCG-3’</td>
</tr>
<tr>
<td></td>
<td>Antisense: 5’-TCTGGAGGTGGTTACCTTAC-3’</td>
</tr>
</tbody>
</table>

Review Board and Ethics Committee approved this study, and all participants provided informed consent.

#### Genotyping

Five milliliters of venous blood was collected from each subject. Genomic DNA was extracted using the High Pure PCR Template kit (Roche Diagnostics GmbH, Mannheim, Germany). The polymerase chain reaction (PCR)–restriction fragment length polymorphism method was performed for genotyping. Two regions of the eotaxin gene, each of which contained a single nucleotide polymorphism (SNP) site, were amplified. Primers for the PCR are given in Table 1. The amplicon sizes, restriction endonuclease, and predicted fragment lengths are given in Table 2. After restriction enzyme digestion, samples were run on agarose gel, and genotypes were evaluated.

#### Statistical Evaluation

SPSS software (Statistical Package for the Social Sciences, version 13.0, SSPS Inc, Chicago, IL) was used for statistical assessments. Eotaxin-1 genotypes in patients with NP and in controls were analyzed by the likelihood ratio and the 2-proportion \(z\) test. The Fisher exact test was used to analyze the differences in eotaxin-1 genotypes with regard to the presence of allergies or asthma. A \(P\) value of \(< .05\) was considered statistically significant.

#### Results

Table 3 summarizes the clinical characteristics of the patients. Eotaxin-1 genotype distributions and allele frequencies of the patients and controls are given in Table 4. The \(-384\ A>G\) and \(+67\ G>A\) SNPs were found to be higher in NP patients than in controls \((P = .044\) and \(P = .019\), respectively). However, their relation was poor (association coefficient = 0.18). Consistent with this result, allele frequencies did not differ between the patients and controls \((P = 0.16\) for \(-384\ A>G; P = 0.14\) for \(+67\ G>A)\).

There was no association between the presence of the asthma or allergy and the eotaxin-1 genotypes \((P > .05)\).

#### Table 2. Amplicon Sizes, Restriction Endonuclease, and Predicted Fragment Lengths of the Gene Sites

<table>
<thead>
<tr>
<th>Site</th>
<th>Amplicon Size, bp</th>
<th>Restriction Endonuclease, bp</th>
<th>Predicted Fragment Lengths, bp</th>
</tr>
</thead>
<tbody>
<tr>
<td>(-384\ A&gt;G)</td>
<td>TaqI</td>
<td>204</td>
<td>(A = 204)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(G = 184 + 20)</td>
</tr>
<tr>
<td>(+67\ G&gt;A)</td>
<td>BsrI</td>
<td>247</td>
<td>(G = 247)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(A = 191 + 56)</td>
</tr>
</tbody>
</table>
Table 3. Clinical Characteristics of Patients with Nasal Polyposis

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Patients (n = 85)</th>
<th>Controls (n = 93)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age, y</td>
<td>45.15 ± 12.1</td>
<td></td>
</tr>
<tr>
<td>Sex, M/F</td>
<td>51/34</td>
<td></td>
</tr>
<tr>
<td>Allergy, n, %</td>
<td>46 (54.1%)</td>
<td></td>
</tr>
<tr>
<td>Asthma, n, %</td>
<td>29 (34.1%)</td>
<td></td>
</tr>
<tr>
<td>Polyp stage, n</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage 1</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>Stage 2</td>
<td>39</td>
<td></td>
</tr>
<tr>
<td>Stage 3</td>
<td>27</td>
<td></td>
</tr>
<tr>
<td>BT scorea (range)</td>
<td>17.4 ± 5.4 (8-24)</td>
<td></td>
</tr>
</tbody>
</table>

*Table 4. Eotaxin-I Genotypes (−384 A>G vs 67 G>A) and Allele Frequencies in Nasal Polyposis*

<table>
<thead>
<tr>
<th>Allele Frequency</th>
<th>Nasal Polyposis</th>
<th>Controls (n = 93)</th>
</tr>
</thead>
<tbody>
<tr>
<td>−384 A&gt;G</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Genotypes, n</td>
<td>33</td>
<td>44</td>
</tr>
<tr>
<td>AA</td>
<td>47</td>
<td>49</td>
</tr>
<tr>
<td>AG</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Allele frequency</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allele A, %</td>
<td>113 (66.47%)</td>
<td>137 (73.65%)</td>
</tr>
<tr>
<td>Allele G, %</td>
<td>57 (33.53%)</td>
<td>49 (26.35%)</td>
</tr>
<tr>
<td>67 G&gt;A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Genotypes, n</td>
<td>47</td>
<td>66</td>
</tr>
<tr>
<td>GG</td>
<td>37</td>
<td>23</td>
</tr>
<tr>
<td>GA</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Allele frequency</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allele G, %</td>
<td>131 (77.05%)</td>
<td>155 (83.33%)</td>
</tr>
<tr>
<td>Allele A, %</td>
<td>39 (22.95%)</td>
<td>31 (16.67%)</td>
</tr>
</tbody>
</table>

*Lund-Mackay scoring.11*

is a population-based study with a limited number of subjects, and further studies with larger groups may provide different results. Second, previous studies determined that eotaxin expression in chronic sinusitis with NP was higher when compared with chronic sinusitis without NP.19,20 Since our control group was constituted of healthy individuals without sinonasal disease, we could not assess the relationship between eotaxin-I gene −384 A>G or 67 G>A genotypes and the development of polyps in patients with chronic sinusitis. Further research should be conducted to illuminate the exact role of eotaxin genotypes on the development of NP.

**Author Contributions**

Suna Ekinci, contributed to conception and design, acquisition of data, analysis and interpretation of data, drafting the article, final approval; Selim S. Erbek, contributed to conception and design, acquisition of data, analysis and interpretation of data, drafting the article, final approval; Erkan Yurtcu, contributed to conception and design, acquisition of data, analysis and interpretation of data, drafting the article, final approval; Feride I. Sahin, contributed to conception and design, acquisition of data, analysis and interpretation of data, revising the article, final approval.

**Disclosures**

**Competing interests:** None.

**Sponsorships:** None.

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**References**


