Evaluation of autoinflammatory disease genes in nasal polyposis*

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1. Introduction

Nasal polyposis (NP) is a chronic inflammatory disease of the mucosa of the nose and the paranasal sinuses causing a stuffy nose. Genetic features and environmental factors together play a role in the development of nasal polyps (1). The role of genetic factors in the etiology of chronic rhinosinusitis and nasal polyps has been demonstrated with both clinical and experimental studies (1–3).

Autoinflammatory diseases comprise a subgroup of inflammatory diseases described recently. Cold induced autoinflammatory syndrome 1 (CIAS1, NLRP3) is the most frequent disorder in autoinflammatory diseases. Intermittent autoinflammatory diseases have also been known as periodic fever syndromes, which are characterized by recurrent episodes of painful inflammation in the abdomen, chest, or joints. These episodes are often accompanied by fever and sometimes a rash. Systemic or localized signs of inflammation accompany the fever attacks, and joint pain, headaches, and ophthalmological and dermatological symptoms are common. An increasing number of patients have been diagnosed and treated since the definition of the disease at the molecular level increased awareness in medical society.

CIAS1 gene mutations were first described in 2001 (4). It is located on the first chromosome, and a mutation in different parts of the CIAS1 gene may result in a variety of diseases, namely familial cold autoinflammatory syndrome (FCAS), Muckle–Wells syndrome (MWS), and chronic infantile neurologic cutaneous arthropathy (CINCA) (4). These 3 diseases demonstrate an autosomal dominant type of inheritance. Recent studies have demonstrated the role of this gene in the processing of interleukin (IL)-1-beta (5). In this study we only targeted the third exon of the CIAS1 gene, which is the largest among the 9 exons (56% of the coding region) and comprises almost all the mutations reported so far (90% of the mutations according to the Human Genome Mutation Database).

The aim of this study was to define the etiology of nasal polyposis and the possible associations with autoinflammatory diseases in patients with nasal polyposis (NP).

Materials and methods: The study included 30 patients diagnosed with NP and 30 healthy age-matched individuals as a control group. CIAS1 polymorphisms were assessed by DNA sequence analysis. Patients with nasal polyps and the control group were compared in terms of gene polymorphisms. Each of the 8 polymorphisms of the CIAS1 gene was analyzed separately in the patient group.

Results: The most frequently observed polymorphisms in the patient group were c.732G > A in 83%, c.663C > T in 23%, and c.1308C > A in 23% of the patients. c.732G > A polymorphism was evaluated separately. Guanine was transformed to adenine at the 732nd nucleotide position of the CIAS1 gene in the cDNA of chromosome 1.

Conclusion: The CIAS1 gene c.732G > A polymorphism was thought to be responsible for an increase in disease susceptibility. The frequency of the ‘A’ allele is higher in the patient group compared to the control group. Autoinflammatory diseases seem like a candidate to be one of these factors. This is the first report to define the role of autoinflammatory diseases among these factors.

Key words: Nasal polyps, hereditary autoinflammatory disease, polymorphism, inflammation, CIAS1

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autoinflammatory diseases, which may present with a variety of symptoms, and obtain new data on the etiology and treatment of nasal polyposis. Common features of autoinflammatory diseases are inflammation and edema. Inflammation and edema in the nasal mucosa constitute the main pathological condition in nasal polyposis, as well.

No reported studies were encountered when the literature was analyzed for the association of nasal polyposis and autoinflammatory diseases. The determination of mutations related to many diseases is getting easier with improvements in genetic testing, and thus the responsible genes in some diseases have been defined.

In light of these data, the CIAS1 gene responsible for FCAS, MWS, and CINCA was analyzed to define an association between nasal polyposis and autoinflammatory diseases.

2. Materials and methods
Patients who presented to the ENT outpatient clinic of Ankara Numune Education and Research Hospital who were diagnosed with nasal polyposis and a control group that had no nasal polyposis were included in the study. Patients were grouped homogeneously according to sex and age. All patients were older than 18 years of age. The diagnosis of nasal polyposis was made by physical examination including endoscopic examination and imaging studies (particularly by paranasal sinus tomography).

Prior to the start of the study, ethical board approval was obtained from the Evaluation Commission of the Scientific Research of Ankara Numune Education and Research Hospital. Informed consent was obtained from all patients and from the control group.

All blood samples were taken from the antecubital veins of the patients and placed into tubes containing disodium ethylenediaminetetraacetic acid (3 mg/L). Blood samples were stored at +4 °C until DNA extraction for 3 months.

Eight polymorphisms and all the mutations in the third exon of the CIAS1 gene on the short arm of the first chromosome were separately evaluated in all patients and the control group.

2.1 Genotype identification
DNA isolations were performed by using QIAamp DNA Blood Mini Kit (Qiagen Inc.) and stored at ~20 °C until the polymerase chain reaction (PCR) step.

CIAS1: To evaluate the mutations and polymorphisms in the CIAS1 gene, primer design was performed for exon number 3 and PCR amplification and Sanger sequencing were carried out for each of the samples to reveal the mutations and polymorphisms. Sanger sequencing was carried out by using BigDye 3.1 chemistry (Applied Biosystems Inc.) on an ABI 3130 capillary electrophoresis instrument (Applied Biosystems Inc.) and the electropherograms were analyzed with SeqScape 2.5 software (Applied Biosystems Inc.)

2.2 Statistical analysis
Statistical evaluations of the data obtained were performed with SPSS for Windows 15.0. Chi-square and Fisher’s exact tests were used in comparing the control and polyposis groups. In addition, odds ratio and 95% confidence intervals were given according to the alleles. The limit of statistical significance was accepted as 0.05.

3. Results
A total of 60 samples, 30 with nasal polyposis and 30 without nasal polyposis, were included in the study. There were 15 (50%) women and 15 (50%) men in the patient group, while there were 15 (50%) women and 15 (50%) men in the control group. The ages of the patients were between 18 and 68 years, with a mean age of 43.3 years. The age range of the control group was 18–64 years with a mean age of 42.7 years.

Eight polymorphisms and all the mutations in the third exon of the CIAS1 gene on the short arm of the first

<table>
<thead>
<tr>
<th>CIAS1 gene polymorphisms</th>
<th>SNP</th>
<th>Nasal polyposis</th>
<th>Control</th>
<th>Amino acid substitutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) c.663C &gt; T</td>
<td>rs7525979</td>
<td>7 (23%)</td>
<td>4 (13%)</td>
<td></td>
</tr>
<tr>
<td>2) c.732G &gt; A</td>
<td>rs3806268</td>
<td>25 (83%)</td>
<td>18 (60%)</td>
<td></td>
</tr>
<tr>
<td>3) c.786G &gt; A</td>
<td>rs4925543</td>
<td>3 (10%)</td>
<td>4 (13.3%)</td>
<td></td>
</tr>
<tr>
<td>4) c.936C &gt; T</td>
<td>rs143840033</td>
<td>1 (3%)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>5) c.1038G &gt; A</td>
<td>-</td>
<td>0</td>
<td>1 (3%)</td>
<td></td>
</tr>
<tr>
<td>6) c.1308C &gt; T</td>
<td>rs34298354</td>
<td>7 (23%)</td>
<td>5 (16%)</td>
<td></td>
</tr>
<tr>
<td>7) c.2113C &gt; A</td>
<td>rs35829419</td>
<td>4 (13%)</td>
<td>3 (10%)</td>
<td>Q705K</td>
</tr>
<tr>
<td>8) c.753C &gt; T</td>
<td>-</td>
<td>0</td>
<td>1 (3%)</td>
<td></td>
</tr>
</tbody>
</table>
higher in the patient group compared to the control group, and this difference was found to be statistically significant (OR, 2.252; CI, 1.084–4.678; P = 0.044) (Table 2).

The c.732 GA and AA genotypes were found more frequently in the nasal polyposis group than in the controls. This transformation could therefore be responsible for an increase in disease susceptibility in genotypes GA and AA.

Homozygosity of the “A” allele of c.732G > A polymorphism was observed in 10 of the NP patients (33.3%) and 5 of the control group (16.7%). Thus, homozygosity is twice as common in the patient group compared to the controls, and the difference was found to be statistically significant (OR, 4.8; CI, 1.074–21.447, P = 0.035).

Other polymorphisms in the CIAS1 gene were not different in terms of homozygosity between the groups (P < 0.05).

No statistically significant differences were found between the patients with nasal polyposis and the control group in the other 7 polymorphisms when they were evaluated separately (P > 0.05).

When the MEFV gene polymorphisms were evaluated separately, it was shown that there was no statistical significant difference between the patient and control groups (P > 0.05).

4. Discussion

Nasal polyposis is a disease that can be seen in almost all age groups. Its incidence increases with age, peaking in the fourth decade (6). The mean age of patients with nasal polyposis in our study was 43.3 years. It is more common in men. The male/female ratio is 2/1; it was 2.3/1 in this study (7).

Although the etiology of nasal polyposis is not completely revealed yet, genetic predisposition, infections, anatomic variations, allergy, regional immunological instability, and histological factors are thought to play a role in the etiology.

Nasal polyposis is also a subject of many genetic studies. Expressions of several genes were investigated by qRT-PCR in patients with allergic rhinitis either with (3 patients) or without (4 patients) nasal polyposis in a study by Fritz et al. They reported an increased incidence of the mammaglobin gene in patients with nasal polyps when they compared the results (8). However, the function of the protein that is coded by this gene it is not clear, and is thought to cause inflammation through its effect on the epithelial secretory proteins (8).

Zielinska et al. reported that the 140 A/G polymorphism of lactoferrin and the -33C/G polymorphism of osteoblast specific factor 2, which has been thought to play a role in the etiopathogenesis of nasal polyps, were seen more often in patients with nasal polyposis (9).

Tumor necrosis factor-alpha (TNF-a) is a major proinflammatory cytokine that exerts a range of important inflammatory and immunomodulatory activities in host defense. Batikhan et al. showed that TNF-a-308G > A single nucleotide polymorphism (SNP) may play a role in the etiology of nasal polyposis (10).

IL-6 orchestrates chronic inflammation and adaptive immunity by regulating T-cell differentiation and activation, inducing Th2 cytokine production. In another study on the pathophysiology of nasal polyposis and asthma, the IL-6-174G/C SNP GG genotype was found more frequently in all nasal polyps and asthma groups (11).

Cyclooxygenase-2 (COX-2) is induced during inflammation, and takes an active part in the synthesis of proinflammatory prostanooids. Mesenchymal–epithelial transition factor (MET) is a transmembrane tyrosine kinase receptor and a protooncogene. A recent study showed that the -765G/C polymorphism of COX-2 gene and the -14C/G polymorphism of MET gene may result in an increased risk of developing nasal polyps in chronic rhinosinusitis (12).

The human class II major histocompatibility complex transactivator (CIITA) encodes a major histocompatibility complex (MHC) class II transactivator and is essential for MHC class II molecules. Lack of expression of the MHC II gene is responsible for abnormal immune response and immunodeficiency. Bae et al. reported the association between CIITA variations and nasal polyposis. In that study, it was found that rs12932187 and rs11074938 may be susceptibility polymorphisms in nasal polyposis (13).

CD14 could have functional effects on the etiology of allergies and asthma. C-159T SNP has been associated with altered CD14 and IgE in patients with asthma and allergic rhinitis. In a recent article, a significant association between nasal polyp and C-159T was reported (14).

Cyclic nucleotide phosphodiesterases (PDEs) play a role in signal transduction and inflammation. Four genes encode a large number of PDE4 isoforms. PDE4D

Table 2. Genotype distribution of c.732G > A polymorphism.

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>A allele (%</th>
<th>G allele (%</th>
<th>GG (%)</th>
<th>GA (%)</th>
<th>AA (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nasal polyp</td>
<td>30</td>
<td>58.3% (35/60)</td>
<td>41.7% (25/60)</td>
<td>5 (16.6%)</td>
<td>15 (50%)</td>
<td>10 (33.3%)</td>
</tr>
<tr>
<td>Control</td>
<td>30</td>
<td>38.3% (23/60)</td>
<td>61.7% (37/60)</td>
<td>12 (40%)</td>
<td>13 (43.3%)</td>
<td>5 (16.6%)</td>
</tr>
</tbody>
</table>
(rs1588265) polymorphism was significantly higher in the nasal polyph group than in the control group (15).

In our study, 8 polymorphisms and all the mutations in the third exon of CIAS1 gene on the short arm of the first chromosome were separately evaluated in all patients and the control group. The “A” allele of CIAS1 c.732G > A polymorphism in the nasal polyposis group was greater than in the control group and the difference was found to be statistically significant. When the 8 polymorphisms in the CIAS1 gene in patients with nasal polyps were evaluated together, it was found to be increased in the patient group compared to the controls, though not statistically significant.

The CIAS1 gene is responsible for the production of proteins that play an important role in natural immunity (16). Mutations in the fourth, sixth, and especially the third exons of the CIAS1 gene have been demonstrated (16–19). However, as in many genetic disorders, environmental factors are also effective in the phenotype of the disease, in addition to genetic factors (16–20). A Swedish study reported increased activity of the CIAS1-Q705K polymorphism as demonstrated by the increased spontaneous and stimulated release of IL-1 beta and IL-18, which are responsible in chronic inflammatory diseases (21). In our study, Q705K polymorphism was detected in 4 patients and 3 individuals in the study and control groups, respectively. There were no statistically significant differences in rs35829419 (Q705K) polymorphisms between the nasal polyposis and control groups.

The etiology of nasal polyphosis has not been fully explained yet. The inflammatory process and the role of inflammatory cells and mediators have been demonstrated in many studies. Nasal polyposis and autoinflammatory diseases are thought to be related in this study, and thus polymorphisms in genes that produce autoinflammatory diseases were investigated in patients with nasal polyps and in individuals without nasal polyps as a control group.

All these data suggest that CIAS1 gene polymorphisms are not a single cause of polyp formation, but can be effective in the development of the disease. Indeed, polyp formation is a multifactorial event and is thought to occur with the presence of more than one factor. With this study, the role of autoinflammatory diseases among these factors is shown for the first time.

It is imperative to investigate relations with other autoinflammatory diseases at this point.

The significant increase in the c.732G > A polymorphism of the CIAS1 gene can lead to a treatment approach. Agents used in the treatment of autoinflammatory diseases, such as IL-1-beta receptor antagonists, IL-1-beta monoclonal anticore, and rilonacept, might be used in the treatment of nasal polyps in the future with the assistance of more detailed studies.

Acknowledgments
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References


