ORIGINAL STUDY

Possible association between -954G/C iNOS polymorphism in nasal polyposis. A case-control study in a population group of Northern Romania

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ABSTRACT

BACKGROUND. Polymorphisms for genes encoding chemosensitive signalling proteins like NOS2 might contribute to the variability in individual susceptibility to nasal polyposis. NO produced by the inducible NO synthase enzyme NOS2A is generated at high levels in certain types of inflammation, so that the role of NOS2 might also be important in nasal polyposis etiopathogeny.

MATERIAL AND METHODS. This is a cross-sectional, randomized, case-control study for the evaluation of the frequency of -954G/C NOS2A2 alleles among patients with nasal polyposis. The study included 91 cases of nasal polyposis diagnosed patients (nasal endoscopy and CT scan examination), and 117 healthy unrelated controls. NOS2 genotyping was carried out using PCR amplification of relevant gene fragment and it was followed by restriction enzyme digestion. Detection of the variant alleles was determined through analysis of resulting restriction fragment length polymorphism (RFLP) followed by gel electrophoresis.

RESULTS. Molecular analysis revealed an increased frequency of NOS2 variant allele in the study group compared to the control group (p=0.019, OR=1.991, CI=1.08-3.67). A statistically significant finding was highlighted among allergic and non-allergic patients with nasal polyposis (p=0.046, OR=0.449, CI=0.208-0.969) and a relationship between nasal polyposis patients with asthma and non-asthmatic patients (p=0.119, OR=1.825, CI=0.875-3.80).

CONCLUSION. The main finding of our study is that -954G/C polymorphism of NOS gene seems to be associated with an increased risk for nasal polyposis.

KEYWORDS: -954G/C iNOS, genetic polymorphism, nasal polyposis

INTRODUCTION

Nasal polyposis (NP) is a common pathology characterized by impaired regulation of nasal tissue growth, leading to the formation of semi translucent grapelike tumors in the upper airways. The etiology of NP remains unknown, but chronic inflammation appears to play a major pathogenic role, often involving other immune-linked statuses like allergy, aspirin intolerance and asthma.

Nitric oxide synthases (NOSs) are a family of catalyzing enzymes of nitric oxide (NO), an important signalling molecule. It has multiple biological roles, modulating vascular and airway tone, insulin secretion, neural development and angiogenesis. There are three different isoforms of nitric oxide synthase (NOSs) responsible for the synthesis on endogenous NO: inducible nitric oxide synthase (iNOS, or NOS2), highly expressed in response to pro-inflammatory stimuli; and two other constitutive forms responsible for the synthesis of specific but decreased amounts of NO, endothelial nitric oxide synthase (eNOS) and neuronal oxide synthase (nNOS).³

The inducible form of NOS (iNOS) is highlighted in an oxidative environment and regulates the immune defense mechanism by producing large amounts of NO...
MATERIAL AND METHODS

Study population
This is a cross-sectional, randomized case-control study consisting of 91 cases with nasal polyposis and 117 healthy unrelated controls. Patients with nasal polyposis were recruited between 2013 and 2015, from the ENT Department of the Emergency County Hospital, Cluj Napoca, Romania. Randomly selected controls were accrued from the Internal Medicine Department of the Emergency Hospital, Cluj. All patients included in the study were diagnosed with de novo or recurrent nasal polyposis. Associated pathology like allergic rhinosinusitis or asthma was well documented and based upon valid allergic tests (skin prick test and intradermal dilution testing) and paraclinical lung assessment by spirometry.

Selection and evaluation of patients
Nasal polyposis was confirmed in all cases after nasal endoscopic and clinical examination. All patients included in the study were diagnosed with de novo or recurrent nasal polyposis. Associated pathology like allergic rhinosinusitis or asthma was well documented and based upon valid allergic tests (skin prick test and intradermal dilution testing) and paraclinical lung assessment by spirometry.

Genotyping
DNA samples were extracted from 300μl peripheral blood, using a commercial Wizard Genomic DNA Purification Kit (Promega, Madison, USA).

The -954G/C NOS2 polymorphism was detected using a PCR-RFLP method. The PCR primers were synthesized by Eurogentec (Belgium):
- Forward primer 5’-CATATGTATGGGAATCT-GTATTTCAG-3’
- Reverse primer 5’-TCTGAACTAGTCACTT-GAGG-3’

For DNA amplification, a total amount of 100 ng of genomic DNA was amplified in a volume of 25μl reaction mixture containing reaction buffer of 1,5mM MgCl2, 20pmol of each primer, 200μm of each dNTPs and 0,5 units of Taq polymerase. Thermo cycling conditions were carried out as follows: initial denaturation at 94 °C for 1 min, 35 cycles of denaturation at 94 °C for 30 s, primer annealing at 61 °C for 45 s, primer extension at 72 °C for 75 s and then a final extension at 72 °C for 10 min. The amplification products were digested with 4 units of BsaI enzyme (Fermentas, Thermo Scientific Biosciences GmbH, Germany) for 8 hours and separated on a 2 % agarose gel (MetaPhor®, FMC Bio Products, Rockland, ME, USA), allowing detection by ethidium bromide staining. In order to validate the reliability of the results, positive and negative polymorphic controls were used for all PCR-RFLP probes. There are 3 possible genotypes, each defined by 3 distinct banding patterns: GG (437 and 136 bp fragments), GC (573, 437 and 136 bp fragments) and CC (573 bp fragment).

Statistical analysis
For statistical analysis we used SPSS 18.0 for Windows. (SPSS, Inc., Chicago, IL). Hardy-Weinberg equilibrium test and Ors were calculated to assess the relationship between -954G/C NOS2 polymorphism and nasal polyposis. For genotype-phenotype polyposis type association we used Adjusted Residual Analysis, Crosstabulation between polyposis type and genotype.
RESULTS

The characteristics of the study population are shown in Table 1.

Demographic characteristics of the studied population are presented in Table 1. The 91 cases (44 females and 47 males) ranged in age from 18 years to 77 years, with a mean (standard deviation) age of 51 (15.6) years. The 117 controls (56 females and 61 men) ranged from 18 to 81 years, with a mean (SD) age of 46 (16.1) years.

Comparative analysis between nasal polyposis patients and controls related to the distribution of the variant G alleles respectively GG/GC genotypes did not reach any positive statistical association (p=0.090, OR= 1.630, CI=0.925-2.873, respectively p=0.552, OR=0.877, CI=0.569-1.351).

Polyposis patients and the control group did not show any statistically significant difference of -954G/C polymorphism when using the recessive model (GG+GC vs. CC) association for the Fisher test (p=0.172, OR=2.35, CI= 0.67-8.30); still, the dominant model (GC+CC vs. GG) highlights statistical differences between patients and controls (p=0.019, OR=1.991, CI=1.08-3.67).

The potential association between the distribution of NOS2 genotypes did not reveal any statistically significant difference between nasal polyposis patients with no olfactory dysfunction and those with present olfactory dysfunction (p=0.158, OR=0.362, CI=0.094-1.390).

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Demographic Characteristics of Subjects and Controls</th>
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<tbody>
<tr>
<td>N</td>
<td>Age, y</td>
</tr>
<tr>
<td>Controls</td>
<td>117</td>
</tr>
<tr>
<td>Nasal Polyposis</td>
<td>91</td>
</tr>
<tr>
<td>Olfactory dysfunction</td>
<td>60</td>
</tr>
<tr>
<td>Asthma</td>
<td>27</td>
</tr>
<tr>
<td>Allergy</td>
<td>38</td>
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<tr>
<th>Table 2</th>
<th>Distribution of genotypes and allele frequencies of NOS2 polymorphism -954G/C in Nasal polyposis patients and Controls</th>
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<tbody>
<tr>
<td>954G/C</td>
<td>Polyposis</td>
</tr>
<tr>
<td></td>
<td>de novo</td>
</tr>
<tr>
<td>GG</td>
<td>5</td>
</tr>
<tr>
<td>GC</td>
<td>14</td>
</tr>
<tr>
<td>CC</td>
<td>27</td>
</tr>
<tr>
<td>C</td>
<td>68</td>
</tr>
<tr>
<td>G</td>
<td>24</td>
</tr>
</tbody>
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<tr>
<th>Table 3</th>
<th>Risk analysis for NOS2 polymorphism -954G/C in Nasal polyposis patients and Controls (Fisher test, dominant and recessive models)</th>
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</thead>
<tbody>
<tr>
<td>Genotype</td>
<td>Polyposis/Controls (N)</td>
</tr>
<tr>
<td>Dominant model</td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>0.019*</td>
</tr>
<tr>
<td>Recessive model</td>
<td></td>
</tr>
<tr>
<td>GG+GC</td>
<td>0.172</td>
</tr>
<tr>
<td>CC</td>
<td></td>
</tr>
</tbody>
</table>

* Significant association for p<0.050
We also evaluated the potential association between the distribution of NOS2 G variant allele distribution and patients with asthma and allergy. The statistical results revealed a relationship between nasal polyposis patients with asthma and non-asthmatic patients (p=0.119, OR=1.825, CI=0.875-3.80). A statistically significant finding was highlighted among allergic and non-allergic patients with nasal polyposis (p=0.046, OR=0.449. CI=0.208-0.969).

**DISCUSSIONS**

Nasal polyposis is a complex disease with a yet unknown pathogenesis, although there are several different studies that describe associations with various gene variants17-21.

In the present study we evaluated the potential implication of -954G/C NOS2 polymorphism in nasal polyposis pathogenesis and phenotype. We found an association between nasal polyposis and the investigated gene variant and also a statistically significant association between NP and specific phenotypes.

The inflammatory and allergic mechanisms are involved in the etiology of nasal polyposis, hence the frequent association of this pathology with atopy and asthma22,23. The gene for NOS2A, which is the main NO-producing enzyme in inflammation is found on chromosome 17q11.2–q12 and lies in the CC chemokine cluster region, where some studies reported a linkage with atopy and asthma24-26. Previous genetic studies for the pathogenesis of inflammation have shown the association of the genetic variants of NOS with asthma and allergy27-30. Two recent studies that have evaluated the relationship between (CCTTT)n polymorphism of NOS2A gene in nasal polyposis found that the number of repeats for this genetic variant in the promoter region could be associated with the inflammatory process of nasal polyposis31,32. To our knowledge, there is only one study evaluating 954G/C NOS2 in relationship to nasal polyposis33.

One of the findings in our study is that we highlighted an association of -954G/C NOS2 polymorphism in polyposis patients with allergy. As is already well known, atopy is one of the confusing elements in the etiopathogenesis of nasal polyposis among patients with or without asthma34,35. Increased frequency of -954G/C NOS2 variant among patients with asthma and allergic polyposis is a proof that polymorphisms of NOS gene is implied in the etiopathogenesis of nasal polyposis and associated phenotypes like asthma and atopy.

We also evaluated the potential association between -954G/C NOS2, nasal polyposis and olfactory dysfunction. Little is known about the pathogenic mechanisms leading to olfactory dysfunction in nasal polyposis; recently, it has been shown that few genetic polymorphisms could influence the outcome of the olfactory status in nasal polyposis. In our study, we found no association between -954G/C NOS2 and the olfactory dysfunction in nasal polyposis.

One of the findings of our study is that -954G/C NOS2 polymorphism is more frequent in females with nasal polyposis and olfactory dysfunction compared to males. (p= 0.0189; OR= 2.554; CI=1.192-4.650). There are few studies that have assessed gender differences in patients with olfactory dysfunction in connection with certain genetic polymorphisms. Evaluating different gene variants by gender is quite difficult because it associates a multitude of genes that interact with several sex-specific endocrine and metabolic factors; however, the result of our study would highlight the role of gene variants in evaluating the olfactory dysfunction in patients with nasal polyposis by gender.

**CONCLUSIONS**

The major finding of our study is that -954G/C polymorphism of NOS gene could be considered an independent molecular risk factor associated with an increased risk for the development of nasal polyposis, especially in allergic individuals.

**Conflicts of interests:** None

**Contribution of authors:** All authors have equally contributed to this work.

**Ethical considerations:** All subjects enrolled in the present study signed an informed consent for all ENT maneuvers, molecular genetic analysis and resulting data usage. The current study was approved by the ethics committee of “Iuliu Hatieganu” University of Medicine and Pharmacy, Cluj-Napoca, Romania, according to the Helsinki Declaration.

**REFERENCES**


