Angiotensin-Converting Enzyme Insertion/Deletion Gene Polymorphism in Chronic Rhinosinusitis with Nasal Polyps

Original Investigation

Mehmet Emre Sivrice[1], Vural Akın[2], Hasan Yasan[1], Kuyaş Hekimler Öztürk[3], Yusuf Çağdaş Kumbul[2]

[1]Süleyman Demirel University Faculty of Medicine, Department of Otorhinolaryngology and Head & Neck Surgery, Isparta, Türkiye
[2]Yüksekova State Hospital, Department of Otorhinolaryngology and Head & Neck Surgery, Hakkari, Türkiye
[3]Süleyman Demirel University Faculty of Medicine, Department of Medical Genetics, Isparta, Türkiye

Abstract

Objective: Inflammatory processes play a role in the etiopathogenesis of chronic rhinosinusitis. Many gene polymorphisms have been associated with inflammation. In this study, we aimed to examine the relationship between angiotensin-converting enzyme insertion/deletion gene polymorphism and chronic rhinosinusitis.

Methods: Fifty-two cases with nasal polyps and 139 control patients were included in the study. Angiotensin-converting enzyme insertion/deletion gene polymorphisms, genotype, and allele distributions were determined. Results were statistically compared between groups.

Results: Statistically significant differences were found between the chronic rhinosinusitis with nasal polyps group and the control group in terms of genotype and allele distribution (p=0.015, 0.003, respectively). There were no significant differences in genotype distribution in the chronic rhinosinusitis with nasal polyps group in terms of non-steroidal anti-inflammatory drug (NSAID) allergy, asthma, and NSAID-exacerbated respiratory disease (p=0.645, 0.660, 0.095, respectively).

Conclusion: We observed that the risk of chronic rhinosinusitis is higher in individuals with the deletion-deletion genotype and D allele of the angiotensin-converting enzyme insertion/deletion gene polymorphism. We believe that these results could be related to the high angiotensin-converting enzyme levels in these patients.

Keywords: Sinusitis, nasal polyps, gene polymorphism, inflammation, angiotensin-converting enzyme, genetic association studies, non-steroidal anti-inflammatory agents

ORCID IDs of the authors:
M. E. S. 0000-0002-2396-6794
V. A. 0000-0002-0050-4837
H. Y. 0000-0002-5470-6784
K. H. Ö. 0000-0002-7075-8875
Y. Ç. K. 0000-0002-0713-2933

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Corresponding Author:
Mehmet Emre Sivrice;
emresivrice@gmail.com

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Introduction

Approximately 25-30% of chronic rhinosinusitis (CRS) patients have nasal polyps, a condition that has been termed CRS with nasal polyps (CRSwNP) (1,2). Although CRSwNP has been known for over three thousand years, its etiopathogenesis has not been clarified. Its prevalence is between 1% and 4%. It is more common in males (1). It presents with symptoms that negatively affect daily life, such as nasal congestion, runny nose, and smell disorders. Its recurrence rates

are high despite the currently available medical and surgical treatments (1,2). Therefore, studies on its etiopathogenesis are important for discovering new treatment strategies.

Angiotensin-converting enzyme (ACE) is secreted from the endothelium. ACE is high in lung and brain capillaries (3). It has many different functions in the nasal mucosa as in many other tissues (4). Its main function is to convert angiotensin I to angiotensin II, a powerful vasopressor involved in fluid and electrolyte homeostasis (3). ACE has also been shown to affect inflammatory processes (5).

Genetic studies on ACE have gained popularity in recent years. The ACE gene is located on chromosome 17q23, and many polymorphisms of this gene have been described (6). The most popular polymorphism of ACE is the insertion/deletion polymorphism (I/D). In this polymorphism, ACE deletion-deletion (DD) and insertion-insertion (II) genotypes are homozygous, while ACE insertion-deletion (ID) genotypes are heterozygous. Insertion in the ACE gene reduces ACE expression. Therefore, people with the ACE DD genotype have greater ACE levels than those with the ID and II genotypes (7,8). A relationship between these polymorphisms and cardiovascular diseases such as atherosclerosis, coronary artery disease, cardiomyopathy, hypertension, preeclampsia, and some malignancies has been found (9-11). Studies about the relationship between the ACE gene and asthma and allergic rhinitis are also present (12,13). There are many studies in literature that have examined the role of various gene polymorphisms in the etiopathogenesis of nasal polyps and CRSwNP. Interleukin-1α (IL-1α), IL-1β, and tumor necrosis factor-α are popular among these gene polymorphisms (14,15). We could not identify any studies examining the CRSwNP and ACE I/D gene polymorphism in the literature. Therefore, we planned to determine the influence of ACE I/D gene polymorphism on CRSwNP.

Methods

Patients

The research protocol of this study was approved by the Süleyman Demirel University Faculty of Medicine Clinical Research Ethics Committee Presidency (no: 240, date: 04.09.2020). The study was conducted according to the international ethical standards set by the Declaration of Helsinki. The study was conducted at the Department of Otolaryngology and Medical Genetics Department of Süleyman Demirel University tertiary hospital between September 2020 and September 2021 with 181 patients. Informed consent was taken from each patient. The study group consisted of 52 patients and the control group of 139 patients. The inclusion criteria were: being older than 18 years, having been operated on for CRSwNP, preoperatively assessed with paranasal sinus computed tomography. Patients with predisposing factors such as cystic fibrosis, Kartegener's syndrome, and a history of drug use affecting the renin-angiotensin system, patients with a history of autoimmune disease, patients with systemic chronic inflammatory disease, patients operated on for antrochoanal polyps were excluded. The control group consisted of septoplasty patients over 18 who were not assessed to have chronic sinusitis in preoperative paranasal sinus computed tomography and nasal endoscopy. Patients with a history of autoimmune diseases and patients with systemic chronic inflammatory diseases were not included in the control group. In the study, age, gender, comorbidity, and drug allergy history of the patients were recorded, and ACE I/D polymorphism was analyzed.

Sampling

During preoperative routine blood tests, four cc of blood was also taken into the tubes with ethylene-diamine-tetraacetic acid for genetic analysis. These blood samples were preserved at -20 ˚C in the genetics laboratory.

Genotyping

The high pure polymerase chain reaction (PCR) template preparation kit (Roche Applied Science, Germany) provided DNA (deoxyribonucleic acid) samples in accordance with the manufacturer's protocol. The concentration and purity of the samples were adjusted by measuring with a Nanodrop 2000c spectrophotometer (Thermo Scientific, USA). The A260/A280 absorbance ratio for all DNAs was obtained in the range of 1.8-2.0. PCR was used on each DNA sample using ADE gene I/D polymorphism genotype allele-specific primers (forward: TGGAGACCACTCCCATCCTTTCT, reverse: GATGTGGCCATCACATTCGTCAGAT) and the FastStart High Fidelity PCR System dNTPack (Roche Applied Science, Germany) kit. It contains 10 pmol of F and R primers, 1.8 mM $MgCl_2$, 10 mM dNTP mix, 1.25 U Taq polymerase, and 250 ng total genomic DNA for each PCR. The PCR was performed with the following conditions: Ten minutes of pre-incubation at 94 ˚C, 35 cycles at 94 ˚C for 2 minutes, at 57 ˚C for 30 seconds, then at 72 ˚C for 1 minute, and finally at 72 ˚C for 7 minutes. After the PCR, 2% agarose gel electrophoresis was administered and stained with GelRed Nucleic Acid Gel Stain (Biotium, USA). The gel was visualized with a UV transilluminator after running. The ACE gene I and D polymorphism genotypes were stated as II genotype single band 490 bp, ID genotype double band 190 bp and 490 bp, and DD genotype single band 190 bp, and profiling was performed (Figure 1).

Statistical Analysis

For data analysis, IBM SPSS.23 (IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp.) was used. The chi-square test was used for categorical variables. Odds ratio values with 95% confidence intervals were given. The normal distribution for continuous variables

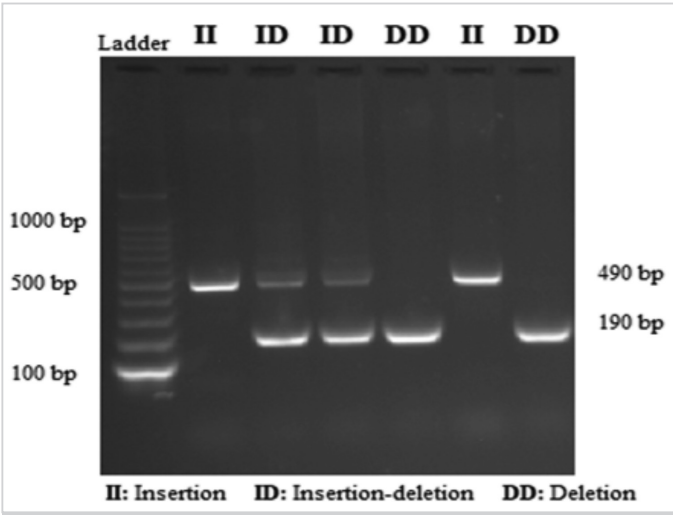

Figure 1. Gel electrophoresis of PCR-RFLP for blood samples in the ACE I/D polymorphism

PCR: Polymerase chain reaction, RFLP: Restriction fragment length polymorphism, ACE: Angiotensin-converting enzyme, I/D: Insertion/deletion

was checked with Shapiro Wilk's test of normality, and homogeneity of variance was controlled with Levene's test. Bi-level comparisons were made with the Mann-Whitney U test in patients where normal distribution was not present, and three-level comparisons were made with the Kruskal-Wallis H test. The statistical significance level was accepted as $p<0.05$.

Results

The study group had a mean age of 46.50 (±13.69) years and consisted of 12 female (23.1%) and 40 male (76.9%) patients. In the control group, there were 25 female (18.0%) and 114 male (82.0%) patients, and their mean age was 46.8 (±15.63) years. No significant difference was stated between the groups regarding gender and age (p=0.428 and 0.888, respectively). Statistically significant differences were found between the study and control groups in genotype and allele distribution (p=0.015 and 0.003, respectively; Tables 1 and 2). In the study group, no significant differences were found in genotype distribution in terms of non-steroidal anti-inflammatory drug (NSAID) allergy, presence of asthma, and presence of NSAID-exacerbated respiratory disease (NERD) (p=0.645, 0.660, and 0.095, respectively; Table 3).

Discussion

CRSwNP negatively affects patients' quality of life and reduces productivity at work. Despite the currently available medical, surgical, and combined treatments, it still has high recurrence rates (16). Systemic corticosteroids, which are effective agents in medical treatment, have the potential for serious side effects (17). Revision surgeries increase the risk of complications and the economic burden. Because of these difficulties in the treatment and control of the disease, studies are under way for the development of new treatment methods. CRS is a highly heterogeneous disease in terms of clinical presentation and pathophysiological mechanisms (18). In previous studies, it was suggested that T-helper (TH) 2 lymphocyte-mediated eosinophilic inflammation was mainly involved in the etiopathogenesis of CRSwNP; and TH1 lymphocyte-mediated neutrophilic inflammation was mainly associated with CRS without polyps (CRSsNP) (19). However, recent studies have shown that the inflammatory processes in the pathogenesis of CRS are more complex. Combined heterogeneous mechanisms involving TH1, TH2, TH17, and possibly TH22 lymphocytes have been identified (18). The key point in the development of CRSwNP is inflammation. Therefore, etiopathogenesis-based studies

Table 1. The study and control group comparison in terms of genotype and alleles

		Study Group	Control Group	p-value
		n (%)		
Genotype	II	7 (13.5)	41 (29.5)	0.015
	ID	25 (48.1)	69 (49.6)	
	DD	20 (38.5)	29 (20.9)	
Allele	I	39 (37.5)	151 (54.3)	0.003
	D	65 (62.5)	127 (45.7)	

II: Insertion-insertion, I/D: Insertion/deletion, DD: Deletion-deletion

Table 2. Odds ratio values for ACE I/D gene polymorphism genotypes

Genotype	OR	%95 Confidence interval		p-value
		Lower	Upper	
II vs ID-DD	0.372	0.155	0.893	**0.023**
II vs ID-DD	0.939	0.497	1.777	0.847
DD vs II-ID	2.371	1.186	4.738	**0.013**

ACE: Angiotensin-converting enzyme, II: Insertion-insertion, I/D: Insertion/deletion, DD: Deletion-deletion

Table 3. Comparison of genotypes in terms of NSAID allergy, asthma, and NERD of the patients in the study group

		II n=7	ID n=25	DD n=20	
		n (%)			p-value
NSAID allergy	Yes	5 (71.4)	21 (84.0)	15 (75.0)	0.645
	No	2 (28.6)	4 (16.0)	5 (25.0)	
Asthma	Yes	4 (57.1)	16 (64.0)	15 (75.0)	0.660
	No	3 (42.9)	9 (36.0)	5 (25.0)	
NERD	Yes	5 (71.4)	24 (96.0)	16 (80.0)	0.095
	No	2 (28.6)	1 (4.0)	4 (20.0)	

II: Insertion-insertion, I/D: Insertion/deletion, DD: Deletion-deletion, NERD: Non-steroidal exacerbated respiratory disease

aim to elucidate the mechanisms that trigger inflammation. In this study, we examined the I/D gene polymorphism of ACE, which was previously proven to be associated with inflammation, in patients with CRSwNP.

It has been shown that angiotensin II (a product of ACE) has a proinflammatory effect on leukocytes, endothelium, and vascular smooth muscle cells with its AT1 receptor (20-22). The pro-inflammatory effects of angiotensin II are largely mediated by increased oxidative stress and nuclear factor-kB. As a result of these mechanisms, proinflammatory cytokines like TNF-alpha, IL-1, and IL-6 are stimulated. Platelets also have angiotensin II receptors; angiotensin II binds to these receptors and releases mediators such as serotonin, norepinephrine, and histamine from the platelets (6). It is known that the cytokines and mediators mentioned above are involved in the etiopathogenesis of CRSwNP. Inflammatory pathways in which these cytokines and mediators participate support the results of our study. In our study, DD genotype and D allele were detected significantly more in CRSwNP cases than in the control group. ACE is higher in individuals with the D allele and this results in higher angiotensin II levels (7,8). Therefore, we believe that these patients may be more prone to chronic inflammation.

Recent studies have revealed that ACE II, which inactivates angiotensin II, is also highly expressed in the nasal mucosa (23). In a study by Fowler et al. (24) in which they compared CRSwNP patients and a healthy control group, it was determined that ACE II mRNA expression in non-polyp mucosal tissues was lower in CRSwNP patients. This result is supported by some previous studies (25,26). In the study of Wang et al. (25), a decrease in ACE II protein expression was observed in patients with CRSwNP and CRSsNP. A decrease in ACE II leads to an increase in angiotensin II. Therefore, the increase in these proinflammatory effects may be a predisposing factor in the formation of CRSwNP. In another study, IL-4, IL-5, and IL-13 levels (the main cytokines of TH2-mediated inflammation) were found to be higher in the nasal polyp tissue of CRSwNP patients with decreased ACE2 expression (26). According to these results, it is seen that angiotensin II increases indirectly in CRSwNP patients' tissues.

Similar results regarding ACE II mRNA expression were also found in studies about asthma and chronic rhinitis (27). In addition, Zhang et al. (13) meta-analysis of asthma and ACE I/D gene polymorphism found that the risk of asthma increased by 59% in patients with the DD genotype. We found no significant differences between the asthmatic cases in the study group and the patients without asthma in terms of genotype and alleles. This difference in our results may be related to the limited number of asthmatic patients in our study and/or ethnic differences. When we evaluated the study group patients in terms of NERD and NSAID allergy, we found no significant differences in terms of ACE I/D gene polymorphism. However, the number of cases also had to be limited to make these comparisons, so we think that these relationships should be evaluated in more comprehensive studies.

In addition to the strengths of our study, there are also some limitations. These include the exclusion of patient histories related to rhinitis subtypes, the lack of analysis of nasal mucosal inflammatory markers, the absence of classification and examination according to nasal polyp subtypes, the absence of the nasal polyp-nasal mucosa inflammatory markers and tissue ACE, and the limited number of patients for comparisons involving NERD, asthma and NSAID allergies.

Conclusion

A statistically significant relationship between CRSwNP and ACE I/D gene polymorphism was found. This result suggests that ACE gene polymorphism may play a role in the development of CRSwNP. Future studies with larger sample sizes and different populations are needed to confirm these findings and to elucidate the mechanisms by which ACE polymorphisms influence CRSwNP.

Ethics

Ethics Committee Approval: The research protocol of this study was approved by the Süleyman Demirel University Faculty of Medicine Clinical Research Ethics Committee Presidency (no: 240, date: 04.09.2020).

Informed Consent: Informed consent was taken from each patient.

Footnotes

Authorship Contributions

Surgical and Medical Practices: M.E.S., V.A., H.Y., Y.Ç.K., Concept: M.E.S., V.A., H.Y., K.H.Ö., Y.Ç.K., Design: M.E.S., V.A., H.Y., K.H.Ö., Y.Ç.K., Data Collection and/or Processing: M.E.S., V.A., Analysis and/or Interpretation: M.E.S., V.A., K.H.Ö., Literature Search: M.E.S., V.A., Writing: M.E.S., V.A., H.Y., K.H.Ö., Y.Ç.K.

Conflict of Interest: The authors declare that they have no conflict of interest.

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Main Points

- The risk of chronic rhinosinusitis is higher in individuals with the deletion-deletion (DD) genotype and D allele of the angiotensin-converting enzyme (ACE) insertion/deletion gene polymorphism.
- The II genotype has the least risk of CRS with nasal polyps (CRSwNP).
- We think that this result is related to the high amount of ACE in patients with DD genotype.
- ACE and related pathways should be studied for the treatment of CRSwNP.

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