



Role of Single Nucleotide Polymorphisms of Mammaglobin-A Gene in Nasal Polyposis: A Case Control Study

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ABSTRACT

Objective: Nasal Polyposis (NP) is a chronic inflammatory disease and genetic factors play an important role in the pathophysiology. Mammaglobin-A (MGA) gene expression was significantly higher in patients with NP and chronic rhinosinusitis compared to normal mucosa. In the present study, we investigated the relationship between single nucleotide polymorphisms (SNPs) in the MGA gene and nasal polyposis in the Turkish population.

Materials and Methods: A total of 87 patients diagnosed with NP and 60 healthy volunteers were enrolled in the study. Genotypes of MGA promoter SNPs c38C>G, c.21C >T, c55+186G>A and c.243+230A>T were determined by light SNP ASSAY after real time PCR analysis using genomic DNA samples obtained from the peripheral blood samples of all participants.

Results: A total of 87 NP patients, 51 male and 36 female, with a mean age of 38.18±9.5 years were included in the study. No significant difference was determined at all positions c38C>G, c.21C >T, c55+186G>A and c.243+230A>T in nasal polyp patients compared to controls with and without allergic rhinitis (AR).

Conclusion: MGA gene c38C> G, c.21C> T, c55 + 186G> A, and c.243 + 230A> T genotypes did not appear to be associated with susceptibility to NP with and without AR in our study population.

Keywords: Allergic rhinitis, gene expression, mammaglobin-A, nasal polyps, single-nucleotide polymorphism

INTRODUCTION

Nasal polyposis (NP) is a disease characterized by chronic inflammation of the nasal cavity and paranasal sinus mucosa (1). The polyps are soft, pink or pearl white outgrowths and benign edematous formations originating from the middle meatus and ethmoid sinus region in the nasal cavity. In studies involving a large group of patients with nasal polyposis, asthma has been reported in 20–70% of participants, and non-allergic asthma was significantly more frequently associated with polyps than allergic asthma (2,3). It has been shown that NP risk is higher in the male gender, and in patients with allergic rhinitis,

aspirin intolerance, asthma, cystic fibrosis or primary ciliary dyskinesia (1-5).

The pathogenesis and etiology have long been controversial and there is still no clear information. Heterogeneity may exist in NP. Some researchers have focused on cytokines, chemokines, growth factors and metalloproteinases to explain the characteristic histopathological features of polyps (5-9). Additionally, various epigenetic and genetic factors change the severity of chronic inflammatory diseases, affecting the emergence of the disease phenotype (10-12).

The secretoglobulin superfamily (SCGBs) gene has been identified in gene array studies on the human nasal mucosa and respiratory tract. SCGBs are protease-, heat- and pH-resistant small dimeric proteins secreted from mammals and marsupials (approximately 10 kDa in humans) (13, 14). The expression of genes encoding SCGB is regulated by cytokines (13). The SCGB2A subfamily includes SCGB2A1 (lipophilin C, mammaglobin B) and SCGB2A2 (MGA). There are many studies on the expression of mammaglobin from secretory epithelium such as the prostate and mammary glands (15, 16).

A limited number of studies have focused on the MGA gene and diseases involving the airway. Recent studies have indicated a possible role in the pathogenesis of allergic rhinitis and NP (6,13,17-19). It was suggested that nucleotide changes in the MGA gene may cause susceptibility to NP by altering the gene's transcription ability and/or protein structure by accelerating the emergence of disease phenotype along with environmental factors (18). According to the data in this study, we investigated the MGA gene polymorphism (c38C>G, c.21C >T, c55+186G>A, c.243+230A>T) in NP patients with and without AR.

MATERIALS and METHODS

This study was approved by the local ethical committee of the Institutional Review Board (number:99950669/138). All participants were informed about the study and signed a clinical trial volunteer test and consent form.

Patients

Our prospective study cohort consisted of 87 adult patients with NP who presented to the Ear Nose Throat Department. The control group was comprised of 60 healthy volunteers without a history of sinonasal disease.

All patients underwent a standardized intake assessment including history and physical examination by an otolaryngologist. Nasal endoscopic evaluation and a skin prick test were performed and paranasal sinus tomography obtained. Subjects with cystic fibrosis, inverted papilloma, antrochoanal polyp, known malignancy and proven immunodeficiency were excluded. The control group consisted of healthy volunteers who had no history of sinonasal disease and no relevant finding on nasal endoscopy. All the patients and volunteers were Turkish.

The diagnosis of NP was based on each patient's medical history, nasal endoscopy results and computed tomography. Diagnoses of asthma or the acetylsalicylic acid triad (aspirin intolerance, asthma, and nasal polyposis) were based on the patients' medical history and examinations at the department of pulmonology. Patients with a diagnosis of nasal polyps were required to have their last treatment at least 6 months ago with no acute upper respiratory tract infection in the last four weeks.

Treatment with antihistamines, intranasal corticosteroids, oral corticosteroids, and leukotriene receptor antagonists was discontinued for at least 4 weeks before undergoing skin-prick tests to common aeroallergens and nasal polyp biopsy. Skin prick tests were performed on all patients. Each patient was evaluated for sensitivity to 18 common allergen extracts (ALK Abello, Madrid, Spain) and to positive and negative control substances. A test result was considered positive for sensitivity when at least 1 of the induration diameters was 3 mm higher than that in the negative control (20).

Patients who had a clinical history of chronic persistent rhinitis and positive skin test responses were classified as nasal polyps with AR, and those patients who tested negative were classified as nasal polyps without AR (18).

Genotyping

Peripheral blood samples were drawn from all participants. Approximately 200 µl of whole blood was added to a 1.5-ml Eppendorf tube with 200 µl binding buffer and 40 µl proteinase K and incubated at 70 °C for 10 min. Then, 100 µl of isopropanol was added to the mixture and mixed well. The mixture was loaded into a High Pure Filter Tube and centrifuged for 1 min at 8000 g. Following the three washing steps, genomic DNA (gDNA) was eluted in 100 µl elution buffer. Five milliliters of venous blood was collected from each subject. The gDNA (hereafter DNA) was extracted from peripheral blood leukocytes using the High Pure Polymerase Chain Reaction (PCR) (SuperHot Master Mix, Bioron, GmbH, Germany) and template preparation kit (NucleoSpinblood DNA, Macherey-Nagel GmbH & Co. Kg, Germany) according to the manufacturer's instructions (21).

Since the SCGB2A2 gene has three exons, each exon was amplified separately for effective DNA multiplication by reducing the error rate. The primer pairs used in the PCR were designed with Primer Designer version 2.0

(Scientific and Educational Software). A ready-to-use kit was used for the purification of PCR products (nucleoFast 96 PCR, Macherey–nagelgmbh and Co. Kg, Germany). A sequence reaction was performed on purified PCR products with a ready-to-use kit (BigDye Terminator V3.1 Cycle Sequencing, Applied Biosystems, USA). Each sequence was made separately for each exon of the gene, and reverse primers were used. The obtained sequence products were purified using a sequence kit and were prepared for array analysis (ZR Sequencing Clean-up Kit D4051, Zymo Research, USA). For each individual who participated in the study, the raw sequence data obtained from the ABI PRISM 3130 genetic analyzer capillary automatic sequence equipment were aligned by transferring the data into the SEQUEncHER (version 3.1.1, gene Codes Corp., USA) and SEQMAN II module of the LASERgEnE 99 (Applied Biosystems, USA) program, and each base was controlled individually. The nucleotide changes in SCGB2A2 were investigated using the obtained data (22).

Statistical Analyses

Statistical analysis of the data was performed using SPSS (Statistical Package for Social Sciences, version 17.0, SPSS Inc., Chicago, IL, USA). Variance analysis (ANOVA) was used to determine whether there was any difference between the groups for the variables. Pearson’s X² test was

used to compare genotypes in the nasal polyp and control groups. The level of significance was set at $p < 0.05$.

RESULTS

Our patient group consisted of 87 consecutive patients; 51(58.6 %) were male and 36 (41.4 %) were female. The mean age was 38.18 ± 9.5 years with a range of 22-55 years. The controls were 60 healthy volunteers; 33 (55%) were male and 27 (45 %) were female. The mean age was 39.03 ± 9.29 years with a range of 21-55 years. There were no significant age and gender differences between the patient and the control group ($p=0.66$, $p=0.59$, respectively) (Table I). The clinical characteristics of NP patients are shown in Table II. The Mammaglobin-A genotype (c38C>G, c.21C >T, c55+186G>A and c.243+230A>T) distributions and allele frequencies of the patients and controls are shown in Table III. There was no significant difference between the patient and control groups (Table III). There was no association between the presence of asthma or allergy and any genotype in patients with NP ($p=0.136$, $p=0.101$, respectively).

DISCUSSION

MGA is a 93-amino acid secretoglobin protein that exhibited several characteristics of the normal uterus, ovary (23), thymus, testis, trachea, skeletal muscle, kidney (24),

Table I. The clinical characteristics of the study population.

Characteristic	Study group (n=87)	Control group (n=60)	p value
Age, mean, y	38.1 ± 9.5	39.03± 9.29	0.592
Sex, M/F n(%)	51(58.6)/36(41.4)	33(55.0)/27(45.0)	0.663
Total IgE Level, µg/L	11.37±10.79(0.38- 49.3)	9.05±6.33(0.38-32.9)	<0.001
Atopy n (%)	21 (24.1)	22 (36.7)	0.101
Asthma n (%)	27 (31.0)	12 (20.0)	0.136

Y: Year, M: Male, F: Female.

Table II. The clinical characteristics of NP patients.

Characteristic	NP with AR	NP without AR	p value
Age, mean, y	37.5 ± 8.8	38.3± 9.2	0.737
Sex, M/F	12/9	40/26	0.590
Atopy (n, %)	21 (24.1)	66 (75.9)	0.101
Asthma			0.136
Aspirin-tolerant	15	45	
Aspirin-intolerant	6	21	

NP: Nasal polyposis, AR: Allergic rhinitis, y: Year, M: Male, F: Female.

Table III. Frequencies of the SCGB2A2 SNP genotype and allele.

		NP with and without AR		Control Group		P	
		#	%	#	%		
c.38 C>G	CC	86	98.9	60	100.0	0.405	
	CG	1	1.1	-	-		
	GG	-	-	-	-		
	C	87	100.0	60	100.0		
	G	-	-	-	-		
c.21 C>T	CC	82	94.3	59	98.3	0.396	
	CT	3	3.4	1	1.7		
	TT	2	2.3	-	-		
	C	85	97.7	60	100.0		0.237
	T	2	2.3	-	-		
c55.187 G>A	GG	80	92.0	57	95.0	0.484	
	GA	5	5.7	3	5.0		
	AA	2	2.3	-	-		
	C	85	97.7	60	100.0		0.237
	T	2	2.3	-	-		
c243.230 A>T	AA	84	96.6	59	98.3	0.680	
	AT	2	2.3	1	1.7		
	TT	1	1.1	-	-		
	A	86	98.9	60	100.0		0.405
	T	1	1.1	-	-		

NP: Nasal polyposis, AR: Allergic rhinitis, A: Adenine, G: Guanine, C: Cytosine, T: Thymine.

sweat glands (16), salivary glands (24), and prostate (23), in addition to tumors of the gynecologic organs (15) and various types of lung carcinomas (16). In addition, there are few studies on diseases involving the Mammaglobin gene and the respiratory tract. A significant increase in MGB1 (SCGB2A2) expression has been shown in the upper respiratory tract mucosa, especially after IL-1 β , TNF- α and IFN- γ stimulation (6,13). Since NP is a disease characterized by chronic inflammation of the nasal cavity and paranasal sinus mucosa, we investigated the role of c38C>G, c.21C>T, c55+186G>A and c.243+230A>T genotypes and their functional properties in the present study. However, we failed to show any association of the genotypes with susceptibility to NP with or without AR.

After MGB (not specific) expression was detected in the nasal mucosa (6,13), the number of studies on patients with NP, chronic rhinosinusitis and asthma has increased. Liu et al. suggested that the level of mammaglobin (not specific) in the mucosa of patients with NP is higher than normal sinus mucosa tissue (6). Additionally, MGB1

expression was significantly higher in the mucosa of patients with NP and chronic rhinosinusitis compared to normal mucosa. However, the same study showed no difference between patients with and without NP among chronic rhinosinusitis patients (13). Specifically, increased MGB gene expression (not specific) has been identified in gene expression studies in patients with AR, and especially in patients with NP. According to the results, it was argued that irregular cell growth may occur with gene activation in NP (17).

Although there are supporting studies, the role of MGA in NP pathogenesis is controversial. Chusakul et al. found the expression of MGA in only one of the polyp biopsies taken from 16 patients diagnosed with AR and NP, while it was not detected in any other 15 NP patients in the control group. According to the results, they indicated that the role of MGA in NP pathogenesis is independent of the underlying AR. They also argued that differences in results may be due to differences in ethnic, geographical or tissue pattern differences (18).

According to studies in the literature, it is possible that this nucleotide change in the promoter region could alter the severity of inflammation by influencing the level of gene expression and contributing to the development of chronic infection. In line with this information, we investigated the c38C> G, c.21C> T, c55 + 186G> A, c.243 + 230A> T polymorphisms, but there was no significant relationship between NP and genotypes. Moreover, there was no relationship of gene polymorphism between AR and asthma-positive and -negative patients in both groups. Therefore, there seems to be no association between MGA gene c38C> G, c.21C> T, c55 + 186G> A, and c.243 + 230A> T polymorphisms and the risk of development of NP with or without AR in our selected population.

CONCLUSION

Genotypes of the MGA gene were not different in nasal polyp patients compared to controls with and without AR. We believe that our study provides preliminary data for further investigation of NP pathogenesis. There were no differences between groups, but the reason for this could be the fact that the studied population was limited. Alternative genetic polymorphisms should be studied in larger samples of patients to help develop future treatment strategies.

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CONFLICT OF INTEREST

We certify that we have no actual or potential conflicts of interest in relation to this article.

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