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Original Article / *Özgün Makale*

Association of TBX21 gene polymorphism with nasal polyposis

TBX21 gen polimorfizminin nazal polipozis ile ilişkisi

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ABSTRACT

Objectives: This study aims to investigate the possible relationship between nasal polyposis (NP) and transcription gene family (T-box) promoter region gene (TBX21) polymorphism.

Patients and Methods: This retrospective study included a total of 32 patients (23 males, 9 females; mean age 42.5±15.0 years; range, 18 to 83 years) who were previously operated or given medical treatment for NP (Group 1) and 50 healthy controls (21 males, 29 females; mean age 32.7±9.9 years; range, 18 to 83 years) with a negative multi-prick allergy test result without any nasal cavity pathology (Group 2) between July 2018 and September 2018. The -1993 T>C single nucleotide polymorphism (SNP) in the TBX21 promoter region was investigated in all participants.

Results: The number of TBX21 heterozygous mutation carriers was statistically significantly higher in Group 1 (p=0.001). In Group 1, allele mutation positivity with an allergy history was significantly higher than those without allergy history (p=0.028).

Conclusion: We found a higher ratio of TBX21 heterozygous mutation in the patients with NP. The -1993 T>C polymorphism of TBX21 may play a role in the etiopathogenesis of the NP.

Keywords: Allergy, nasal polyposis, T-bet, TBX21, treg.

ÖZ

Amaç: Bu çalışmada nazal polipozis (NP) ve transkripsiyon gen ailesi (T-box) promotör bölgesi gen (TBX21) polimorfizmi arasında muhtemel ilişki araştırıldı.

Hastalar ve Yöntemler: Bu retrospektif çalışmaya Temmuz 2018 - Eylül 2018 tarihleri arasında daha önce NP nedeniyle ameliyat edilen veya tıbbi tedavi verilen 32 hasta (Grup 1) (23 erkek, 9 kadın; ort. yaş 42.5±15.0 yıl; dağılım, 18-83 yıl) ve multi-prick alerji test sonucu negatif olan ve herhangi bir nazal kavite patolojisi olmayan 50 sağlıklı kontrol (Grup 2) (21 erkek, 29 kadın; ort. yaş 32.7±9.9 yıl; dağılım, 18-83 yıl) dahil edildi. Tüm katılımcılarda TBX21 promotör bölgesinde -1993 T>C tek nükleotidli polimorfizm (SNP) araştırıldı.

Bulgular: TBX21 heterozigot mutasyon taşıyıcı sayısı, Grup 1'de istatistiksel olarak anlamlı düzeyde daha yüksekti (p=0.001). Grup 1'de alerji öyküsü olmayanlara kıyasla alerji öyküsü olanlarda alel mutasyon pozitifliği anlamlı düzeyde daha yüksekti (p=0.028).

Sonuç: TBX21 heterozigot mutasyon oranı NP'li hastalarda daha yüksek bulundu. TBX21'in -1993 T>C polimorfizmi, NP'nin etiyopatogenezinde rol oynayabilir.

Anahtar sözcükler: Alerji, nazal polipozis, T-bet, TBX21, treg.

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Nasal polyposis (NP) is an inflammatory disease of the nose and paranasal sinus mucosa.[1] The etiology of NP is multifactorial, and many theories have been proposed regarding the pathogenesis of NP, although none of them have been proven, yet.[2]

The first step of the pathogenetic reaction which progresses to NP is a mucosal stimulus formed by a triggering agent (i.e., bacteria, virus, fungus, or allergen). Also, many other cytokines and inflammatory proteins are produced by the migration of inflammatory cells to the mucosa and the expression of adhesion molecules.[3]

The promoter region gene (TBX21) as a member of transcription gene family (T-box) is located at 17q21.32 chromosome and encodes the transcription factor. Szabo et al.[4] first explained the T-box transcription factor (T-bet) having a role in naïve CD4 T-helper (Th) cell transformation for progression through Th1 response. The other transcription factors for naïve CD4 Th cell transformation are GATA binding protein 3 (GATA-3) for Th2 response, retinoic acid-related orphan receptor (ROR-γt) for Th17 response, and Forkhead box P3 (Foxp3) for regulatory T cell (Treg) response.[5,6]

The literature data demonstrate that T-bet and GATA3 levels increase and Foxp3 levels decrease in NP, while the ROR-γt level shows mild changes. In addition, the inadequacy of Treg response and the imbalance at Th1/Th2 reactions are the key drivers of development of various autoimmune and allergic diseases.[3,7] In their study, Akahoshi et al.[8] showed that TBX21 gene polymorphism had an effect as increased T-bet expression in aspirin-induced asthma and NP and might cause inappropriate Th1 and antigen-specific Th2 responses in the airway, leading to severe airway inflammation.

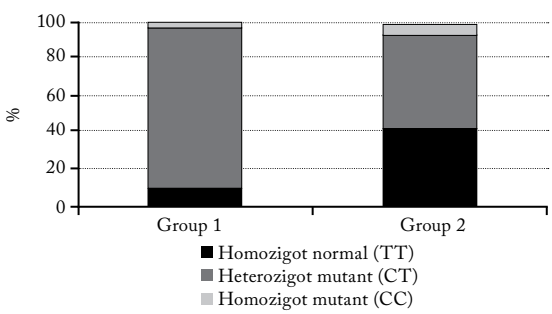

Figure 1. Distribution of allele mutation in nasal polyposis patients and healthy controls.

The TBX21 gene polymorphism affects the CD4 Th cell transformation, and induced cytokines which provide Th1 proliferation may have a role in chronic inflammation. In the present study, we aimed to investigate the possible relationship between NP and TBX21 gene polymorphisms.

PATIENTS AND METHODS

This retrospective study included a total of 32 patients (23 males, 9 females; mean age 42.5±15.0 years; range, 18 to 83 years) who were previously operated or given medical treatment for NP (Group 1) and 50 healthy controls (21 males, 29 females; mean age 32.7±9.9 years; range, 18 to 83 years) with a negative multi-prick allergy test result without any nasal cavity pathology (Group 2) between July 2018 and September 2018.

Those who had a history of nasal tumor, another chronic inflammatory disease, chronic sinusitis, additional systemic disease, and those who refused genetic analysis were excluded from the study. Healthy participants from the allergy outpatient clinic who had apositive multi-prick skin test result were also excluded. Allergic examination of all participants was carried out by the multi-prick skin test. A written informed consent was obtained from each patient. The study protocol was approved by the Marmara University School of Medicine Ethics Committee. The study was conducted in accordance with the principles of the Declaration of Helsinki.

The diagnosis of NP was made based on nasal examination, anterior rhinoscopy, nasal endoscopic examination, and computed tomography (CT) of the paranasal sinus. The 4-mm 0 degrees endoscopes were used for endoscopic examination and scores were assigned according to Lanza and Kennedy.[9,10]

The -1993 T>C single nucleotide polymorphism (SNP) (rs4794067) in the TBX 21 promoter region was investigated at Marmara University School of Medicine Department of Medical Genetics in all patients. Two milliliters of blood samples were collected in sterile ethylene diamine tetra-acetic acid (EDTA) tubes for deoxyribonucleic acid (DNA) isolation and stored at 4°C until analysis. The DNA isolation was performed using 200 mL blood samples proteinase K and the column elution. Following polymerase chain reaction (PCR) amplification of TBX21 gene, the resulting product was cut with restriction enzyme (HhaI) using the restriction fragment length polymorphism (RFLP) method.

The PCR cycle program was implemented: three min denaturation at 94°C, 36 cycles of 30 sec denaturation at 94°C, 30 sec matching at 56°C, two min synthesis

Table 1
Allergy test results in nasal polyposis patients

			Group 1	
			n	%
Allergy test		Negative (-)	14	43.8
		Positive (+)	18	56.2
Allergic factors	Mite	-	16	50.0
		+	14	43.8
		+++	2	6.3
	Grass	-	26	81.3
		+	5	15.6
		++	1	3.1
	Animal epidermal	-	26	81.3
		+	5	15.6
		++	1	3.1
	Fungus	-	30	93.8
		+	2	6.3
	Weed	-	25	78.1
		+	7	21.9
	Cockroach	-	27	84.4
		+	3	9.4
		++	2	6.3
	Tree	-	28	87.5
		+	4	12.5

at 72°C, and seven min elongation at 72°C. Ten µL of amplicons were checked for appropriate band quality, and 1 µL of HhaI enzyme, 2 µL of enzyme buffer, and 18 µL of distilled water were mixed to obtain 31 µL volume. The samples were incubated at 37°C overnight and the resulting products were analyzed with 4% agarose gel.

The results of the -1993 T>C polymorphism of TBX21 promoter region were recorded as homozygous normal (TT), heterozygous mutant (CT), and homozygous mutant (CC).

Statistical analysis

Statistical analysis was performed using the SPSS version 15.0 software (SPSS Inc., Chicago, IL, USA). Descriptive data were expressed in mean ± standard deviation (SD), median (min-max), or number and frequency. For normally distributed numerical variables, the independent Student's t-test was used to compare the groups, while the Mann-Whitney U test was used for non-normally distributed variables. The ratios were compared using the chi-square test. A p value of <0.05 was considered statistically significant.

RESULTS

Of all participants included in the study, 38 (46.3%) were females and 44 (53.7%) were males. The results of allergy test and the level of allergy factors of Group 1 are shown in Table 1. In Group 1, 56.2% had positive results for allergy (Table 1).

Table 2
Treatment-related parameters in nasal polyposis patients

	n	%	Mean±SD	Median	Min-Max
Postoperative follow-up time (month)	32		11.71±8.61	9.5	2-30
Lanza Kennedy Endoscopic Scores	32		7.81±2.23	8	4-11
Treatment					
Operation	24	75			
Medically	8	25			

SD: Standard deviation; Min: Minimum; Max: Maximum.

Table 3
Distribution of allele mutation TBX21

Allele mutation	n	%
Homozygote normal	24	29.3
Heterozygote mutant	54	65.9
Homozygote mutant	4	4.9

TBX21: Promoter region gene.

Table 4
Assessment of TBX21 allele mutation according to groups

	Group 1		Group 2		
Allele mutation	n	%	n	%	*p*
Homozygote normal	3	9.4	21	42	
Heterozygote mutant	28	87.5	26	52	0.001
Homozygote mutant	1	3.1	3	6	

TBX21: Promoter region gene.

The mean Lanza and Kennedy endoscopic score of the patients was 7.81±2.23. A total of 75% of the patients were operated and the mean postoperative follow-up time was 11.7±8.61 (range, 2 to 30) months (Table 2).

The TBX21 allele mutations in patient and control groups were detected as homozygous normal (TT) in 29.3%, heterozygous mutant (CT) in 65.9%, and homozygous mutant (CC) in 4.9% (Table 3).

Table 5
TBX21 allele mutation according to allergy test in nasal polyposis patients

	Allergy test				
	Negative		Positive		
Allele mutation	n	%	n	%	*p**
Homozygote normal	3	21.4	0	0	
Heterozygote mutant	10	71.4	18	100	0.028
Homozygote mutant	1	7.1	0	0	

TBX21: Promoter region gene; Chi-square test; * p<0.05.

The number of heterozygous mutation carriers was higher in Group 1, while homozygous normal state was more frequent in Group 2 (Figure 1). The allele mutation ratio of Group 1 was significantly higher, compared to Group 2 (p=0.001) (Table 4).

In Group 1, allele mutation positivity with an allergy history was significantly higher than those without allergy history (p=0.028) (Table 5). On the other hand, there was no statistically significant correlation between allele mutation ratios, compared to each allergic agent separately (Table 6).

Table 6
TBX21 allele mutation according to allergy factors in nasal polyposis patients

		Allele mutation				
	Negative/positive	Yes	No	Yes	No	
Allergic factors		n	%	n	%	*p*
Mite	-	12	75.0	4	25.0	0.145
	+	14	100	0	0.0	
	+++	2	100	0	0.0	
Animal epidermal	-	22	84.6	4	15.4	1.000
	+	5	100	0	0.0	
	++	1	100	0	0.0	
Fungus	-	26	86.7	4	13.3	1.000
	+	2	100	0	0.0	
Weed	-	21	84.0	4	16.0	0.552
	+	7	100	0	0.0	
Cockroach	-	23	85.2	4	14.8	1.000
	+	3	100	0	0.0	
	++	2	100	0	0.0	
Tree	-	24	85.7	4	14.3	1.000
	+	4	100	0	0.0	
Grass	-	22	84.6	4	15.4	1.000
	+	5	100	0	0.0	
	+	1	100	0	0.0	

DISCUSSION

The incidence of NP varies between 1 and 4%, and its prevalence is higher in males, compared to females.

The prevalence of allergy in patients with NP is higher than the general population.[11] It varies between 10 and 96.5% in patients with NP.[12-14] In our study, the multi-prick skin test results were positive in 56.2% of the patients with NP. Also, the allele mutation ratio of the patient group with allergy was significantly higher than those without allergy findings (p=0.028).

The factors which play a critical role in the development of chronic inflammation and edema in NP still remain unclear.[15] Inflammatory cytokines produced by T lymphocytes and macrophages induce inflammatory responses from the beginning to the end of the cascade.[16] Increased inflammatory cytokines in NP are common conspicuous factors.[15] In Western populations, NP is often characterized by type 2 inflammation with elevated levels of Th2-specific cytokines. In addition, in Asian populations, NP may be characterized by Th1/Th17 mixed inflammatory pattern.[17,18]

According to Yoshikawa et al.,[19] Th1 and Th2-specific cellular infiltration of NP tissues significantly increases in chronic rhinosinusitis and, also, following a viral infection. Migration of Th1 cells through nasal polyps may be induced by C-X-C motif chemokine 10 (CXCL10) which is produced by fibroblasts. As a result, excessive Th1 type response in NP patients is effective.

In another study, Baba et al.[20] reported that messenger ribonucleic acid (mRNA) levels of FoxP3 and T-bet in non-eosinophilic NP increased, compared to eosinophilic NP. Also, they reported that increases in Th1 and Treg cells might occur at non-eosinophilic NP. The balance between Th1 and Th2 is controlled by Treg cells which play a key role in the expression and regulation of T-cell subtypes in NP.[20] Treg dysfunction and Th1/Th2 network imbalance results in the development of various autoimmune and allergic diseases.[21] Besides, T-bet induces the production of interferon-gamma (IFN-γ) from Th1 cells, while suppressing interleukin (IL) 4 and IL-5. The IFN-γ secreted from Th1 cells upregulates the intercellular adhesion molecule-1 (ICAM-1) and has a negative effect on Th2 response.[6]

The TBX21 gene mutations and sequence changes are associated with asthma pathogenesis. In a study, Finotto et al.[22] found that deletions in the TBX21 gene were associated with eosinophil and lymphocyte infiltration and exhibition of signs of airway remodeling in mouse bronchi. In addition, Akahoshi et al.[8] suggested that transcriptional activity increased at the promoter region of TBX21 gene might cause aspirin-induced asthma and NP. In the present study, we found that the ratio of TBX21 gene allele mutations in NP patients were significantly higher (p=0.001).

In conclusion, we investigated whether TBX21gene promoter polymorphism -1993 T>C SNP (rs4794067) played a possible role in the pathogenesis of NP. We found a higher ratio of TBX21 heterozygous mutation in the patient group and there was a statistically significant difference in the -1993 T>C SNP (rs4794067) allele frequency between the NP patients and healthy controls. Also, there was a significantly higher ratio of TBX21 heterozygous -1993 T>C SNP mutation in NP patients with allergy. Based on these findings, we suggest that this promoter polymorphism may play a predisposing role in NP. Nonetheless, further studies are still needed to clarify the contribution of TBX21 gene polymorphism in the NP pathogenesis.

Declaration of conflicting interests

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