

# FOXP3 Promoter polymorphism (-3499 A/G) is not associated with osteoarthritis in a Turkish population

## Türk popülasyonunda *FOXP3* geni promotor polimorfizmi (A-3499G) osteoartit ile ilişkili değildir

Nilgun Cekin<sup>1</sup>, Ergun Pinarbasi<sup>1</sup>, Gonca Donmez<sup>2</sup>, Aslihan Esra Bildirici<sup>1</sup>, Zekeriya Oztemur<sup>3</sup>, Serdal Arslan<sup>1</sup>, Okay Bulut<sup>3</sup>

<sup>1</sup>Cumhuriyet University, Faculty of Medicine, Department of Medical Biology, Sivas

<sup>2</sup>Omer Halis Demir University, Faculty of Medicine, Department of Medical Biology, Nigde

<sup>3</sup>Cumhuriyet University, Faculty of Medicine, Department of Orthopedics and Traumatology, Sivas

**Corresponding author:** Ergun Pinarbasi, Cumhuriyet University, Faculty of Medicine, Department of Medical Biology, 58140, Sivas, Turkey.

**E-mail:** epinar@cumhuriyet.edu.tr

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### SUMMARY

**Objective:** In this study we aimed to find out whether *FOXP3* promoter region -3499A/G polymorphism was associated with osteoarthritis in a Turkish population.

**Method:** The study group consists of 50 patients in 3rd stage and 100 patients in 4th stage osteoarthritis and control group consists of 150 healthy individuals. *FOXP3* genotypes were examined by PCR-RFLP method.

**Results:** Our results show that there is no statistically significant association between osteoarthritis and *FOXP3* -3499A/G polymorphism. The wild type AA genotype was 63%, polymorphic AG was 31% and GG was 6% in the control group, while they were 56%, 37% and 7% in the study group respectively.

**Conclusions:** Osteoarthritis was seen higher in women than that of men in our study which is compatible with the results of previous results.

**Keywords:** *FOXP3*, Osteoarthritis, promoter, polymorphism

### ÖZET

**Amaç:** Bu çalışmada *FOXP3* geni promotor bölgesi -3499A/G polimorfizmi ile osteoartrit riski arasındaki ilişki bir Türk popülasyonunda araştırılmıştır.

**Yöntem:** Çalışma grubu, 3. evre 50 ve 4. evre 100 OA hastasından, kontrol grubu ise 150 sağlıklı bireyden oluşmuştur. *FOXP3* genotipleri PCR-RFLP yöntemleri kullanılarak elde edilmiştir.

**Bulgular:** Sonuçlarımız istatistiksel olarak OA riski ve *FOXP3* geni promotor bölgesi -3499A/G polimorfizmi arasında anlamlı bir ilişki olmadığını göstermiştir. Kontrol grubunda doğal tip AA genotipi %63, polimorfik AG %31 ve GG genotipi oranı %6 bulunurken çalışma grubunda bu oranlar sırasıyla %56, %37 ve %7 olarak bulunmuştur.

**Sonuç:** Kadınlarda erkeklere oranla daha sık gözlenmiştir ve bu gözlem, önceki çalışmalarda elde edilen sonuçlar ile uyumludur.

**Anahtar sözcükler:** *FOXP3*, Osteoartrit, promotor, polimorfizm

## INTRODUCTION

Osteoarthritis (OA) is one of degenerative chronic joint diseases that pathogenesis has not been clarified exactly. Over the age of 60, 10% of men and 18% of women are affected by the disease. OA causes joint destruction in cartilaginous joint and proliferative changes in surrounding bone and soft tissues by holding synovial joints. It is thought that genetic, environmental, mechanical and endocrine factors contribute to etiology. In addition these, the OA risk is increase by advanced age, diabetes, overweight, joint surgeries and traumas<sup>1-5</sup>. Joint swelling, joint anomalies and pain in the individuals with the disease, decrease the quality of life of the patients<sup>6</sup>.

Epidemiological and genetic studies have shown that genetic factors play an important role in OA. The main strategies used to investigate the role of genetics in OA are familial cluster surveys, twin studies, linkage analysis and genetic association studies<sup>7</sup>. A large number of genomic regions in 2, 4, 6, 7, 11, 16, 19 and X chromosomes have been linked with OA susceptibility<sup>7</sup>.

Although previous epidemiological studies suggest that polymorphisms in different genes contribute to the development of OA by individually or in combination, there is no general opinion about this issue yet. It is unknown that which genetic changes, which alignment and whether single or not have contributed to the onset or development of OA<sup>8</sup>. It is suggested that inflammation which seen in synovial membranes in OA, triggers inflammation-promoting mediators<sup>9</sup>. In study of rheumatoid arthritis (RA), one of the autoimmune diseases, it has been determined that variations in the *FOXP3* (transcription factor forkhead box P3) promoter region linked with inflammation increase the risk of the disease<sup>10</sup>. Other studies suggested that polymorphic variations in members of the Toll-like receptor family are expressed in immune system cells, increase OA risk<sup>10,11</sup>. *FOXP3* is a member of the "forkhead winged-helix transcription factor" family. The human *FOXP3* gene is 1296 bp in length and consists of 11 exons. *FOXP3* encodes a protein containing 431 amino acids, is located on the short arm of chromosome X (Xp11.23) and undergoes X-chromosome inactivation<sup>12, 13</sup>. The *FOXP3* gene encodes a transcription factor that is thought to be crucial for the development and function of regulatory T (Treg) cells<sup>14</sup>. *FOXP3* regulate development and function of a subset of CD4<sup>+</sup> T cells that express Treg cells CD25 (IL-2 receptor  $\alpha$  chain). Treg suppresses the proliferation of autoreactive lymphocytes both in the thymus and around the thymus by manner of cell-cell

interaction. Thus *FOXP3* contributes to natural tolerance to its own antigens by mediating development of Tregs<sup>15</sup>.

There are five SNPs (single nucleotide polymorphism) in the promoter region of *FOXP3*: -924A/G (rs2232365), -1383C/T (rs2232364), -2383C/T (rs3761549), -3279C/A (rs3761548) and -3499A/G (rs3761547) [16]. In our case-control study, we investigated whether A/G polymorphism in the *FOXP3* promoter -3499 region is a risk factor for OA.

## MATERIAL AND METHODS

### Patients

Patient and control group consisted of 300 (150 patient and 150 healthy) individuals who were applied to the Cumhuriyet University, Medical Faculty, Department of Orthopedics and Traumatology. While the patient group consisted of 150 patients who were diagnosed with OA, 150 individuals from the control group who are not diagnosed with OA, have no autoimmune family history and no chronic disease. The study was approved by Cumhuriyet University Ethical Committee (Decision number: 2015-06/02). Before the samples were collected, approval form was taken from the patients and the control group.

### Genotyping

From each individual, 10 ml blood samples were taken and their DNA was isolated by salting-out method<sup>17</sup>. The *FOXP3* promoter region was amplified by polymerase chain reaction (PCR) to include the region -3499. Primer sequences were forward (5'-CTCTGGCTCTCCATGCATGT-3') and reverse (5'-TGCAGGGCTTCAAGTTGACAG-3'). The method for PCR included an initial denaturing at 94°C for 4 min, followed by 30 cycles at 94°C for 1 min, 56°C for 30 s, 72°C for 30 s with a final extension at 72°C for 7 min. The resulting 158 bp amplicons were incubated with PvuII restriction endonuclease enzyme at 37°C for 1 hour and then separated on agarose gel. For the AG genotype 158+123+35 bp fragments, for the GG genotype 123+35 bp fragments and for wild type AA genotype 158 bp fragment were analyzed<sup>15</sup>.

### Statistical Analysis

The data were analyzed using the SPSS 23.0 (SPSS, Chicago, IL, USA) program. Chi square and Fisher's exact tests were used to compare the data. OR and 95% confidence intervals for obtained results were given.  $p < 0.05$  was considered significant in all tests.

## RESULTS

Patient and control group characteristics in this study are given in Table 1. According to Table 1, while age distribution of patients and control group

is statistically insignificant, in terms of gender, OA is occurred statistically higher in female. While %33 of the patients are in third grade of OA, %67 of the patients are in fourth grade.

**Table 1:** OA patients and control group demographic data

	Control (n=150)	Patient (n=150)	p value
Age	61±11.98	63±10.77	>0.05 (0.93)
Gender			
Female	81 (54%)	110 (73%)	<0.05 (0.00051)
Male	69 (46%)	40 (26.7%)	
Stage of Disease			
Grade 3		50 (33.3%)	
Grade 4		100 (66.7%)	

When we investigated genotypes of patient and control group whether or not FOXP3 -3499 A/G polymorphism, our results show that wild type AA is found almost equal value in patient and control group. Wild type was seen in 56% of patients group and 63% of control group. AG heterozygote is found 56% and 47% in patient and control groups

respectively, and GG homozygote polymorphic genotype is found 10% and 8% in patient and control groups, respectively. When we evaluate these data statistically, significant differences with two groups are not found. Genotypes distribution, p value and risk ratio and confidence interval were shown in Table 2.

**Table 2:** FOXP3 promotor region -3499 A/G genotype data according to OA patient and control group distribution

Genotype	Control (n=150)	Patient (n=150)	p value	OR (95%CI)
AA	95 (63%)	84 (56%)		
AG	47 (31%)	56 (37%)	0.22	1.34 (0.82-2.19)
GG	8 (6%)	10 (7%)	0.49	1.42 (0.52-3.99)
<b>Genotype</b>				
AA	95 (63%)	84 (56%)		
AA+AG	55 (37%)	66 (44%)	0.19	1.35 (0.85-2.16)
<b>Allele</b>				
A	237 (79%)	224 (74%)	0.21	1.27 (0.87-1.87)
G	63 (21%)	76 (26%)		

When we investigated association between disease stage and FOXP3 promotor region polymorphism, we observed that 100 of total 150 patients are in 4th grade, 50 patients are in 3rd grade of OA. There is

no statistically significant difference between disease stage and the interested polymorphism (Table 3). Genotype distribution of the patient and control group is similar (p>0.05).

**Table 3:** *FOXP3* promoter region -3499 A/G genotype and distribution of allele frequency in OA disease stage

Genotype	Control n=150 (%)	OA Stage 3 n=50 (%)	p value	OR (95%CI)	OA Stage 4 n=100 (%)	p value	OR (95%CI)
AA	95 (63)	30 (60)			62 (62)		
AG	47 (31)	17 (34)	0.69	1.14 (0.56-2.28)	31 (31)	0.96	1.01 (0.57-1.76)
GG	8 (6)	3 (7)	0.78	1.18 (0.24-4.63)	7 (7)	0.59	1.38 (0.44-3.99)
<b>Genotype</b>							
AA	95 (63)	30 (60)			62 (62)		
AG+GG	55 (37)	20 (40)	0.67	1.15 (0.59-2.22)	38 (38)	0.83	1.05 (0.62-1.78)
<b>Allele</b>							
A	237 (79)	77 (77)	0.66	1.12 (0.64-1.92)	155 (78)	0.68	1.09 (0.70-1.68)
G	63 (21)	23 (23)			45 (22)		

## DISCUSSION

Studies investigated OA-polymorphism association are showed that fifty different genes are associated with OA. However, the gene variations in OA undetermined in all studies, so debate about this issue is continued. While COL6A4P1 polymorphism (collagen type VI, alpha 4 pseudogene 1) increases OA risk in Asian population, the same risk is not seen in Caucasian populations<sup>18</sup>. Such as COL11A1, VEGF, GDF5 and IL8 gene polymorphisms in different population studies are described as risk factors. However OA linked genetic polymorphism studies don't make a consensus, researchers concentrate on different gene studies<sup>18-22</sup>. One of these genes is *FOXP3*. Polymorphisms at promoter region are introduced that can be important for rheumatoid arthritis (RA). Recent studies were reported that association with *FOXP3* promoter region polymorphisms and various diseases especially autoimmune diseases<sup>23</sup>. Nevertheless OA isn't autoimmune disease like RA, recent studies about OA supported that Treg cells aggregate at joints like RA. Polymorphisms described in *FOXP3* promoter cause deficient of Treg cells and in this way immun reactions improve<sup>9, 11, 23, 24, 25</sup>. In another study that compared with OA and RA, Treg cells equally transfers to synovial membrane and synovial fluid in joints both of OA and RA and Treg aggregation is not spesific to arthritis with inflammation<sup>26</sup>.

Based on literature, *FOXP3* promoter -3499 A/G polymorphism that is suggested as risk factor is investigated whether is associated with OA or not. Our results show that there is no association between 3rd and 4th grade OA and *FOXP3* -3499 A/G polymorphism (Table 3).

When we analyzed demographic data, female patients are seen statistically high prevalence compared to male patients with OA and this result is compatible with many studies. While control

group and patient group distribution was investigated according to *FOXP3* promoter -3499 A/G polymorphism, it is similar to other populations. Wild type AA genotype is 65%, heterozygote AG genotype is 28%, homozygote GG genotype is 4-7% in Asian population. In Caucasian population AA is 60%, AG is 35% and GG is 3-5% [15, 27, 28]. In our study we found that AA is 63%, AG is 31% and GG is 6% (Table 3).

In this study first time, *FOXP3* promoter region -3499 A/G polymorphism is investigated in Turkish OA patients. Our results show that there is no association between disease risk and interested polymorphism. In different disease there is an association with four different SNPs in *FOXP3* promoter region. To study these SNPs in OA may present clear evidence.

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