



## The association between cyclooxygenase-2 (COX-2/PTGS2) gene polymorphism and osteoarthritis

Siklooksijenaz-2 (COX-2/PTGS2) gen polimorfizmi ile osteoartrit arasındaki ilişki

Volkan G. Güler,<sup>1</sup> Serap Yalın, PhD.,<sup>1</sup> Mehmet Berköz,<sup>2</sup> Özlem Bölgen Çimen, M.D.,<sup>3</sup>  
Erдің Yalın, PhD.,<sup>4</sup> Pelin Erođlu,<sup>1</sup> Arzu Kanık, PhD.<sup>5</sup>

Departments of <sup>1</sup>Biochemistry, <sup>2</sup>Pharmaceutical Technology, Mersin University Pharmacy Faculty, Mersin, Turkey;  
Departments of <sup>3</sup>Physical Medicine and Rehabilitation, <sup>5</sup>Biostatistics, Medical Faculty of Mersin University, Mersin, Turkey;  
<sup>4</sup>Departments of Biochemistry, Medical Faculty of Çukurova University, Adana, Turkey

**Objectives:** This study aims to investigate the relationship between the risk for the development of osteoarthritis and Cyclooxygenase-2 (COX 2) -765G>C gen polymorphism.

**Patients and methods:** We included a total of 100 osteoarthritis patients (18 males, 82 females; mean age 60.4±8.4 years; range 41 to 81 years) who were treated in the Physical Therapy Clinic and 100 healthy subjects without a history of arthritis (40 males, 60 females; mean age 30.9±7.5 years; range 16 to 48 years) in our study between September 2006 and May 2008. The frequency of -765G>C gene polymorphism in the COX-2 promoter region was investigated in the osteoarthritis patients and the control group without a history of arthritis using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. The data were analysed with chi-square and logistic regression analysis.

**Results:** The frequencies of -765G>C polymorphism for GG, GC and CC genotypes were found to be 54%, 35%, and 11% in the control group and 48%, 34% and 18% in the osteoarthritis group, respectively.

**Conclusion:** Based on the data obtained, it can be stated that there is no significant relation between COX-2 -765G>C polymorphism and osteoarthritis disease. Furthermore, this study presents the first results of COX-2 promoter variant in Turkish patients with osteoarthritis.

**Key words:** COX-2; osteoarthritis; polymorphism.

**Amaç:** Çalışmada osteoartrit hastalığının gelişme riski ile Cyclooxygenase-2 (COX 2) -765G>C gen polimorfizmi arasındaki ilişki araştırıldı.

**Hastalar ve yöntemler:** Eylül 2006 - Mayıs 2008 tarihleri arasında Fizik Tedavi Kliniğinde tedavi edilen osteoartritli 100 hasta (18 erkek, 82 kadın; ort. yaş 60.4±8.4 yıl; dağılım 41-81 yıl) ve artrit öyküsü bulunmayan 100 sağlıklı gönüllü (40 erkek, 60 kadın; ort. yaş 30.9±7.5 yıl; dağılım 16-48 yıl) çalışmaya alındı. Cyclooxygenase-2 promoter bölgesindeki -765G>C gen bölgesinin-polimorfizminin sıklığı osteoartritli hastalarda ve artrit öyküsü bulunmayan kontrol grubunda polimeraz zincir reaksiyonu-restriksiyon parça uzunluk polimorfizmi (PCR-RFLP) yöntemi kullanılarak araştırıldı. Veriler ki-kare ve lojistik regresyon analizi ile analiz edildi.

**Bulgular:** GG, GC ve CC genotiplerine ilişkin -765G>C polimorfizmi sıklığının kontrol grubunda sırasıyla %54, %35 ve %11, osteoartrit grubunda ise sırasıyla %48, %34 ve %18 olduğu tespit edildi.

**Sonuç:** Elde edilen verilere göre, COX-2 -765G>C polimorfizmi ile osteoartrit hastalığı arasında anlamlı bir ilişki bulunmadığı söylenebilir. Ayrıca bu çalışmada osteoartritli Türk hastalardaki COX-2 promoter varyantı konusunda ilk bulgular sunulmaktadır.

**Anahtar sözcükler:** COX-2; osteoartrit; polimorfizm.

Osteoarthritis can be defined as a degenerative joint disease. Clinically, osteoarthritis is characterized by joint pain, tenderness, limitation of movement, crepitus, occasional effusion and variable degrees of local inflammation, but without systemic effects.<sup>[1]</sup> Constitutional

and environmental risk factors for the development of osteoarthritis such as age, obesity, hormonal status, bone density, physical activity, mechanical factors, past history of trauma and genetic susceptibilities may also contribute to osteoarthritis progression. It has

been reported that multiple genes may influence the development of osteoarthritis.<sup>[2-4]</sup> Various chromosomal regions have shown genetic linkage with osteoarthritis. Some genes, such as collagen genes (COL1A1, COL2A1, COL9A1, COL11A2), interleukin-1 (IL-1), interleukin-4 receptor (IL-4R), asporin (ASPN), metalloprotease (ADAM12), calmodulin-1 (CALM1), estrogen receptor (ER) and cyclooxygenase-2 (COX-2) genes may play a role in osteoarthritis pathogenesis.<sup>[2,5,6]</sup>

Cyclooxygenase is the multifunctional enzyme that catalyzes conversion of arachidonic acid to prostaglandin H<sub>2</sub> (PGH<sub>2</sub>) and is thus also called prostaglandin-endoperoxide synthase (PTGS).<sup>[7,8]</sup> There are three isoforms of COX, designated as COX-1, COX-2 and COX-3.<sup>[8,9]</sup> Cyclooxygenase-2 (PTGS2) plays an important role in the tissue destruction of bone and therefore, COX-2-dependent-prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) synthesis is considered to be an important mediator of tissue destruction in inflammatory bone diseases.<sup>[10,11]</sup>

There are many gene polymorphisms that may affect the expression of COX-2. The most well-known and identified COX-2 gene polymorphism is COX-2 -765G>C polymorphism.<sup>[12]</sup> Polymorphic -765C allele is functional and reveals a significantly lower promoter activity (30%) when compared with the -765G allele.<sup>[12,13]</sup> Furthermore, -765C allele reduces the COX-2 gene expression and consequently the inflammatory response.<sup>[14]</sup>

Although osteoarthritis is known to have non-inflammatory pathology, there is a growing body of evidence indicating that there is a significant inflammatory component to disease pathogenesis.<sup>[7,15]</sup> The precise mechanism by which the -765G>C polymorphism of COX-2 gene may affect the susceptibility of osteoarthritis is unclear. We hypothesized that COX-2 -765G>C polymorphism might have a possible role as a genetic risk factor on osteoarthritis by influencing prostaglandins (PGs) production in inflamed cells. Thus, it affects susceptibility to osteoarthritis. In the present study, we investigated the relation between osteoarthritis and -765G>C gene polymorphism.

## PATIENTS AND METHODS

### Patients

A total of 100 osteoarthritis patients (18 males, 82 females; mean age 60.4±8.4 years; range 41 to 81 years) and 100 healthy subjects (40 males, 60 females; mean age 30.9±7.5 years; range 16 to 48 years) admitted the outpatient clinic of Mersin University, Faculty of Medicine, Department of Physical Medicine and Rehabilitation between September 2006 and May 2008 were included in our study. Patients diagnosed with primary osteoarthritis according to the criteria of the

American College of Rheumatology and had radiographic changes of at least grade III or IV were included in the study.<sup>[16]</sup> Patients with osteoarthritis secondary to other conditions such as inflammation, sepsis, metabolic abnormalities and trauma were excluded. No suggestive of skeletal dysplasia or developmental dysplasia case was included.

The control subject pool was composed of patients who were visiting the hospital for a health examination, but had no blood relationship with the osteoarthritis patients. The control group never had any signs or symptoms of osteoarthritis, other arthritis or joint diseases (pain, swelling, tenderness, or restriction of movement) at any site based on their medical history and a thorough examination conducted by an experienced physiatrist. For this aim, we used a questionnaire to obtain demographic and other information such as stage, type, onset age and family history. Both patient and control groups in this study were of Turkish ethnicity and were free from systemic, chronic, autoimmune, allergic and anti-inflammatory diseases.

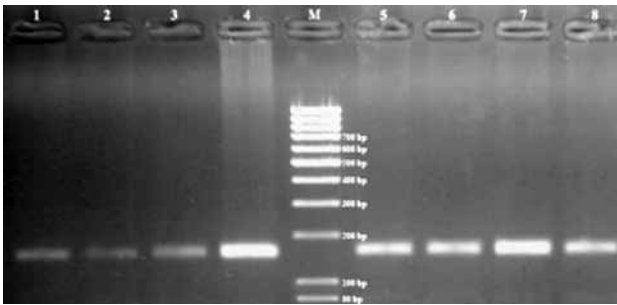
After obtaining written consent of the subjects, blood samples were collected from both patients and healthy volunteers for this study. The research protocol was designed and performed according to the principles of the Helsinki Declaration upon approval of the Ethics Review Board of Mersin University.

### Deoxyribonucleic acid (DNA) isolation

Blood samples from osteoarthritis patients and control group were collected by Vacutainer® tubes (Becton, Dickinson and Company, Franklin Lakes, NJ USA) and were transferred to EDTA tubes. DNA was extracted from the peripheral blood leucocytes by standard phenol/chloroform extraction techniques and precipitation with ethanol.<sup>[17]</sup>

### COX-2 G-765C genotyping

Genotyping of COX-2 gene was assayed with the polymerase chain reaction (PCR) restriction fragment length polymorphism based methods as described by Pereira et al.<sup>[18]</sup> The polymerase chain reaction reactions were conducted in a reaction volume of 50 µL with 20 ng genomic DNA, 10× PCR buffer, 200 µM dNTPs, 10 pmol of each primer and 1 unit of Taq polymerase (Fermantas-EP0402). The PCR primers used were 5'-ATTCTGGCCATCGCCGCTTC-3' as a forward primer and 5'-CTCCTTGTTTCTTGGAAGAGACG-3' as a reverse primer. Amplification was carried out as follows: 35 cycles consisting of 1 min of denaturation at 94 °C, 1 min of annealing at 57 °C and 1 min of extension at 72 °C with an initial denaturation step of 10 min at 95 °C and a final extension of 10 min at 72 °C



**Figure 1.** Polymerase chain reaction product of cyclooxygenase-2 G-765C gene region on 2% agarose gel electrophoresis. Lines 1-8 show amplified deoxyribonucleic acid samples and M shows 100 bp deoxyribonucleic acid marker.

in a thermocycler (Techne, TC-312, UK). The resultant PCR products showed single fragment at 157 bp (Figure 1). 10  $\mu$ L of 157-bp product were then digested with 10 units of Bsh1236I (FnuDII) restriction enzyme (Fermentas - ER0922) at 37 °C for 3 h. Digestion products were visualized on a 3% agarose gel containing ethidium bromide. Wild-type genotype (GG) produced double band at 134 and 23 bp, heterozygotes (GC) produced three bands at 157, 134 and 23 bp and homozygote polymorphic genotype (CC) produced only one band at 157 bp (Figure 2).

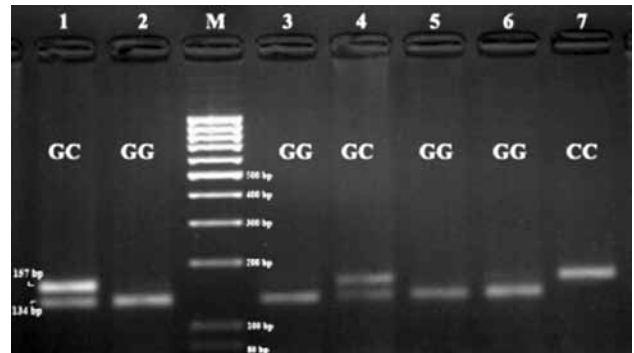
### Statistical analysis

For this case-control association study, we used Pearson's chi-square-test to determine the significance of differences in allelic and genotypic frequencies between osteoarthritis patients and control subjects.  $P < 0.05$  was considered statistically significant. Odds ratios (ORs) with 95% confidence intervals (CIs) were also calculated. Allele and genotype proportions were tested for Hardy-Weinberg equilibrium. All statistical analyses were performed with SPSS version 15.0 software (SPSS Inc., Chicago, IL, USA).

## RESULTS

At first we categorized our osteoarthritis cases as lumbar spondylosis, gonarthrosis and cervical spondylosis. Among all patients, 59 patients with gonarthrosis were in the age range of 48 and 78 with a mean age  $60.11 \pm 7.86$  whereas 27 patients with lumbar spondylosis were between the ages of 41 and 81 with a mean age  $62.37 \pm 10.07$ . On the other hand, 14 patients with cervical spondylosis were between the ages of 43 and 69 with a mean age  $57.64 \pm 6.82$ . There was no association between patient age and subtype of osteoarthritis.

The frequencies of GG, GC and CC genotypes in the control group were 54%, 35% and 11% whereas the osteoarthritis group had 48%, 34% and 18% frequencies, respectively. Furthermore, GG, GC and CC geno-



**Figure 2.** Polymerase chain reaction product of cyclooxygenase-2 G-765C gene region on 3% agarose gel electrophoresis after restriction by Bsh1236I enzyme. Lines 1 and 4 show GC genotype (157 and 134 bp), lines 2, 3, 5 and 6 show GG genotype (134 bp), line 7 shows CC genotype (157 bp) and M shows 100 bp deoxyribonucleic acid marker.

type frequencies were 44%, 36% and 20% in gonarthrosis, 48%, 33% and 19% in lumbar spondylosis and 64%, 29% and 7% in cervical spondylosis, respectively. There was no statistically significant difference between osteoarthritis frequencies and COX-2 -765GC heterozygote and COX-2 -765CC homozygote polymorphic genotypes (Table I).

Allele frequencies of G and C were 71.5% and 28.5% in the control group whereas they were 65% and 35% in the osteoarthritis group. Furthermore, G and C allele frequencies were 61.9% and 38.1% in gonarthrosis, 64.8% and 35.2% in lumbar spondylosis and 78.6% and 21.4% in cervical spondylosis cases, respectively. Harmonious to the genotype frequency, the polymorphic C allele did not statistically correlate with osteoarthritis frequencies (Table II).

## DISCUSSION

In the current study, the association between the osteoarthritis and COX-2 -765G>C gene polymorphism was examined and according to our results, no significant relationship between COX-2 -765G>C polymorphism and osteoarthritis was found.

Osteoarthritis is the most common form of arthritis. It is the foremost cause of disability in the elderly population, affecting approximately 10% of those over the age of 60 years.<sup>[19]</sup> Several reports suggest that genetic influences contribute considerably to the development of osteoarthritis. However, the relevance of the genetic component varies among subgroups of patients, and as yet it is not clear which genes are involved. Although some candidate genes are detected that may have a role in osteoarthritis regeneration, a few of them have been certainly associated or linked with osteoarthritis.<sup>[20]</sup> Polymorphism at the ER $\alpha$  gene locus

TABLE I

Genotype frequency and odds ratio of COX-2 G-765C polymorphism in control, osteoarthritis, gonarthrosis, lumbar spondylosis and cervical spondylosis cases

Genotypes	Control (n=100)		Osteoarthritis (n=100)		Gonarthrosis (n=59)		Lumbar spondylosis (n=27)		Cervical spondylosis (n=14)	
	n	%	n	%	n	%	n	%	n	%
GG	54	54	48	48	26	44	13	48	9	64
GC	35	35	34	34 <sup>a</sup>	21	36 <sup>b</sup>	9	33 <sup>c</sup>	4	29 <sup>d</sup>
CC	11	11	18	18 <sup>e</sup>	12	20 <sup>f</sup>	5	19 <sup>g</sup>	1	7 <sup>h</sup>

<sup>a</sup>: Odds ratio [95% CI]= 1.1 [0.59-2.01] (p=0.776); <sup>b</sup>: Odds ratio [95% CI]= 1.25 [0.61-2.55] (p=0.546); <sup>c</sup>: Odds ratio [95% CI]= 1.07 [0.41-2.76] (p=0.892); <sup>d</sup>: Odds ratio [95% CI]= 0.69 [0.2-2.4] (p=0.555); <sup>e</sup>: Odds ratio [95% CI]= 1.84 [0.79-4.29] (p=0.157); <sup>f</sup>: Odds ratio [95% CI]= 2.27 [0.88-5.82] (p=0.089); <sup>g</sup>: Odds ratio [95% CI]= 1.89 [0.56-6.38] (p=0.307); <sup>h</sup>: Odds ratio [95% CI]= 0.55 [0.06-4.76] (p=0.583).

appeared to be associated significantly with primary generalized osteoarthritis and knee osteoarthritis.<sup>[21]</sup> Interleukin-1, IL4R, and CALM1 gene polymorphisms are associated with hip osteoarthritis. ADAM12 and ASPN gene polymorphisms increase the risk of knee osteoarthritis.<sup>[2,20]</sup>

Expression of COX-2 is highly induced by pro-inflammatory agents, tumor promoters, oncogenes, growth factors, and mitogens. Overexpression of the COX-2 protein plays an important role in many pathophysiological states, including inflammation, cancer, angiogenesis, Alzheimer's disease, and several forms of arthritis.<sup>[8]</sup> It is believed that COX-2 is responsible for PG synthesis at sites of inflammation. Prostaglandins are potent, multifunctional regulators of bone metabolism which have both stimulatory and inhibitory effects. Skeletal tissue is an abundant source of PG production, so that endogenous PGs are likely to play important roles in skeletal physiology and pathophysiology.<sup>[8]</sup>

There is a significant inflammatory component to osteoarthritis pathogenesis although osteoarthritis is known to be a noninflammatory pathology. The exact role of PGs in the pathogenesis of osteoarthritis and thus, the consequences of inhibiting their synthesis have not been fully elucidated.<sup>[10]</sup> Cyclooxygenase-2 induction has been observed in both human osteoarthritis-affected cartilages as well as in synovial tissue taken from the patients afflicted with rheumatoid arthritis.<sup>[7,10,15]</sup>

Many single nucleotide polymorphisms have been identified in the COX-2 gene. Some of these polymorphisms can alter the expression or the function of COX-2.<sup>[22]</sup> A functional G>C polymorphism at a putative Sp1 binding site in the COX-2 promoter in the promoter region, 765 bases upstream of the transcription start site is the most known and studied COX-2 variant among other COX-2 polymorphisms. -765G>C polymorphism could change the transcription factors binding to this region.<sup>[22]</sup>

The -765C allele has been shown to be associated with a 30% reduction in promoter activity in vitro when compared to the wild-type -765G allele.<sup>[13]</sup> Cyclooxygenase-2 gene expression is lower and consequently, the COX-2 dependent PGE2 production is reduced.<sup>[12,13]</sup> The association of functional outcome in the -765G>C polymorphism and a variety of diseases has been evaluated.<sup>[23,24]</sup> Some studies reported that the -765C allele may be protective in cardiovascular diseases (myocardial infarction, stroke and outcome of coronary artery bypass surgery).<sup>[13]</sup> -765C allele led to susceptibility in bronchial asthma of females and sarcoidosis.<sup>[13,25,26]</sup> The COX-2 -765 genetic polymorphism seems to be protective or is associated with susceptibility in some diseases and is consistent with COX-2 having pro-, anti-inflammatory and anti-fibrotic functions.<sup>[2,27,28]</sup> The -765C allele reduces the COX-2 gene expression and consequently, the inflammatory

TABLE II

Allele frequency and odds ratio of cyclooxygenase-2 G-765C polymorphism in control, osteoarthritis, gonarthrosis, lumbar spondylosis and cervical spondylosis cases

Alleles	Control (n=100)		Osteoarthritis (n=200)		Gonarthrosis (n=118)		Lumbar spondylosis (n=54)		Cervical spondylosis (n=28)	
	n	%	n	%	n	%	n	%	n	%
G	143	71.5	130	65	73	61.9	35	64.8	22	78.6
C	57	28.5	70	35 <sup>a</sup>	45	38.1 <sup>b</sup>	19	35.2 <sup>c</sup>	6	21.4 <sup>d</sup>

<sup>a</sup>: Odds ratio [95% CI]= 1.07 [0.69-1.64] (p=0.767); <sup>b</sup>: Odds ratio [95% CI]= 1.55 [0.96-2.5] (p=0.075); <sup>c</sup>: Odds ratio [95% CI]= 1.36 [0.72-2.58] (p=0.341); <sup>d</sup>: Odds ratio [95% CI]= 0.68 [0.26-1.78] (p=0.433).

response.<sup>[27,28]</sup> Based on these findings in the literature, we hypothesized that COX-2 G-765C polymorphism could show a protective effect against osteoarthritis. However, in the current study, we have not found a significant difference between patient and control groups for -765C allele frequency. Not only gonarthrosis, but also lumbar spondylosis and cervical spondylosis frequencies were unrelated with COX-2 -765G>C polymorphism. However, Schneider et al.<sup>[29]</sup> reported that -765G>C promoter variant of the COX-2 gene is associated with a lower risk for end-stage hip and knee osteoarthritis.

In conclusion, our data suggest that the polymorphism studied in the COX-2 gene is not to be associated with susceptibility to osteoarthritis in the Turkish population. Further studies in patients with osteoarthritis from different ethnic populations will be necessary to confirm these findings.

#### Declaration of conflicting interests

The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

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