

The Relationship Between Primary Knee Osteoarthritis (OA) and MMP-1 and MMP-3 Gene Polymorphisms in a Turkish Population: A Population-Based Case-Control Study

Türk Toplumundaki Primer Diz Osteoartriti (OA) ile MMP-1 ve MMP3 Gen Polimorfizmleri Arasındaki İlişki: Topluma Dayalı Bir Olgu Kontrol Çalışması

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Abstract

The development of matrix metalloproteinases (MMPs) plays a key role in the normal physiology of connective tissue during morphogenesis and wound healing. The irregular synthesis and activity of MMPs can be related with osteoarthritis (OA) which is a major cause of disability in elderly population. We have determined the relationship between occurrence of knee OA and MMP-1 single nucleotide polymorphism (SNP) rs5854 (A/G) and MMP-3 SNP rs679620 (C/T) in 100 primary knee OA patients and 83 healthy controls in Turkey, using the ABI 7500 real-time PCR system. Genotypic distributions and allelic frequencies were compared between the patient and control groups considering age, gender and body mass index (BMI). We found no significant difference between the two groups in genotype distribution and allelic frequency of MMP-1 rs5854 and MMP-3 rs679620. The A- and G-allele frequencies were 31% and 69% in OA patients, respectively, and 27% and 73% in the control group. Stratification analysis revealed that there was no difference in the association between presence of OA in rs679620 (MMP-3) CT heterozygotes and CC homozygotes in either gender. The GG, GA and AA genotypes of rs5854 (MMP-1) were not significantly associated with the risk of knee OA, even after further stratification analysis according to gender. We conclude that MMP-1 G/A gene AND MMP-3 C/T polymorphisms do not have a role in the development of knee OA in a Turkish population.

Keywords: Gene, Metalloproteinases, Osteoarthritis, Polymorphism

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Introduction

Knee osteoarthritis (OA) is a dynamic disease process of joints caused by an imbalance between formation and removal events in the joint cartilage and subchondral bone (1). It is a chronic disease characterized by capsular fibrosis, softening and disintegration in the articular cartilage, increased osteoblastic activity and vascular congestion in the subchondral bone, and cartilage and bone growth at the edge of the joints. The pathogenesis of OA is triggered by various biochemical and mechanical

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Özet

Matris metalloproteinazların gelişimi, yara iyileşmesi ve morfogenez sırasında konnektif dokunun normal fizyolojisinde anahtar bir rol oynar. Metalloproteinaz (MMP)'lerin aktivitesi ve düzensiz sentezi yaşlı nüfusta sakatlıkların ana bir nedeni olan osteoartrit ile ilişkili olabilir. Türkiye'de 100 primer diz osteoartrit (OA) hastası ve 83 sağlıklı kontrol grubunda diz OA oluşumu ile MMP-1 tek nükleotid polimorfizm (TNP) rs5854 (A/G) ve MMP-3 TNP rs679620 (C/T) arasındaki ilişkiyi ABI 7500 real-time PCR sistemi kullanılarak tanımladık. Genotipik dağılım ve allelik sıklıkları hasta ve kontrol gruplarının yaş, cinsiyet ve vücut kitle indeksleri (VKİ) düşünülerek karşılaştırıldı. İki grup arasında MMP-1 rs5854 ve MMP-3 rs679620 genotipik dağılım ve allelik sıklıklarında anlamlı fark saptanmadı. A ve G allel sıklıkları OA hastalarında sırasıyla %31 ve %69, kontrol grubunda %27 ve %73 olarak bulunmuştur. Stratifikasyon analizinde cinsiyette de rs679620 (MMP-3) CT heterozigot and CC homozigotları ile OA in varlığı arasında ilişki saptanmamıştır. rs5854 (MMP-1) ün GG, GA ve AA genotipleri ile diz OA in riski arasında cinsiyete göre stratifiye edildikten sonra bile önemli ilişki bulunamamıştır. MMP-1 G/A gen ve MMP-3 C/T polimorfizmleri ile Türk halkındaki diz OA in gelişimi arasında bir role sahip olmadığı sonucuna vardık.

Anahtar Kelimeler: Gen, Metalloproteinazlar, Osteoartrit, Polimorfizm

factors (1,2).

Recent reports have indicated that several candidate genes and splice sites are associated with primary OA susceptibility (3). Many genes involved in development of cartilage and bone have been proposed to as having a role in formation and progression of OA (3,4). Studies on MMP genes in various populations, and some primary gene polymorphisms have been shown to increase susceptibility to OA (5-7).

Matrix metalloproteinases (MMPs) are members of the zinc-dependent endopeptidase family that can proteolyze at least one component of extracellular matrix. The activity of MMPs must be balanced by their specific endogenous tissue inhibitors (TIMPs) (8). MMP overexpression occurs during physiological and pathological tissue remodeling, and are influenced by factors that can affect gene transcription (9). Under these

conditions, production of MMPs may exceed that of TIMP (10). An increased MMP activity results in collapse of the matrix and several pathophysiological effects (11). MMP gene expression has been shown to be influenced by SNPs on MMP promoters (10,11). MMP-1 is an interstitial collagenase in cartilage and joints. MMP-3 (Stromelysin-1) breaks up the non-helical parts of proteoglycans, laminin, fibronectin, and collagen-4. Their genes are located at 11q22.2-22.3 (6,7,10,11).

Studies on different populations have reported a relationship between MMP-1 rs5854 gene polymorphism and primary OA (5,6,12). MMP-3 rs679620 gene polymorphism has been associated with primary OA in a Taiwanese population (7), but MMP3 (-1171 5A/6A) gene polymorphism has not been associated with primary in an Egyptian population (12). We have assessed the genetic relationship primary knee OA and MMP1 rs5854 and MMP3 rs679620 gene polymorphism. This study was performed to identify the genotype frequency of this polymorphism in Turkish patients.

Materials and Methods

Patient Selection

We selected 100 patients primary knee OA and 83 control subjects who had no evidence of arthritis or joint disease. All of the participants were over 50 years of age. Age, gender and body mass index (BMI) of patients and control subjects were recorded.

The diagnosis of knee OA was based on the criteria of the American College of Rheumatology that is primary OA with any symptoms and radiographic signs of OA according to the Kellgren-Lawrence (KL) grading system (13). Radiographic findings of OA were classified as KL grade 2, 3, or 4.

Blood samples were taken from patients and controls after obtaining informed written consent. The research protocol was designed and conducted according to the principles of the Helsinki Declaration and following approval from the Muğla Sıtkı Kocman University Faculty of Medicine Ethics Committee.

Genotypic Analysis of MMP-1 and MMP-3 Gene Polymorphism

Blood was drawn into EDTA (ethylenediamine tetraacetic acid) tubes with a 2 cc syringe. The samples were stored at -20°C until the DNA purification process which used (purelink® Genomic DNA Mini Kit, Invitrogen, Carlsbad, CA 92008 USA), and the target DNA polymorphisms and SNPs (ID rs5854, rs679620) were studied using the ABI 7500 Real Time System (Applied Biosystems, Foster City, CA) with TaqMan probes.

The mix for each PCR reaction contained 5µl of TaqMan Genotyping Master Mix, 0.25 µl of TaqMan genotyping assay (40×), and 2.75 µl of DNase-free, RNase-free water. The PCR protocol was briefly consisted of an initial step of 94°C for 10 min, followed by 40 cycles of 95°C for 15 sec for denaturation, 60°C for 1 min for annealing. Homozygous mutant, heterozygous, and homozygous wild-type genotypes were identified according to software-based allele discrimination.

Statistical Analysis

All statistical analyzes were performed using the Statistical Package for Social Science (SPSS) 20.0 program. Compliance of the genetic distribution with the Hardy-Weinberg equilibrium was analyzed with a χ^2 - goodness of fit test. Genotypic and allelic distribution differences between patients and controls were evaluated with the χ^2 test. Values of $p < 0.05$ were considered statistically significant.

Results

The demographic characteristics of the two groups are shown in Table 1. There were no significant differences between the groups in terms of sex and age. However, the BMI of the OA group was significantly higher than that of the controls ($p < 0.001$, one-sided t-test).

Table 2 presents the genotype distributions and allele frequencies of MMP1 (rs5854) polymorphisms in the two groups. The genotype frequencies for all polymorphisms did not significantly differ from those expected under the Hardy-Weinberg equilibrium ($p = 0.706$ and $p = 0.501$) and were similar between the two groups (Table 2).

Table 2 presents the genotype and allele frequencies of the MMP-3 (rs679620) polymorphism in the two groups. CC was the most frequent genotype in both groups, and genotype frequency fell within the Hardy-Weinberg equilibrium. There was no significant difference between the groups with respect to genotype distribution ($p = 0.23$). The C allele frequency was 65.5% and that of the T allele was 34.5% in OA patients, and 57.2% and 42.8% in the control group, respectively. There was no association between the allele frequencies of MMP-3 gene CT polymorphism and the clinical characteristics of the two groups ($p > 0.05$) or between male and female ($p > 0.05$).

Discussion

The first study regarding the relationship between OA and its genetic components was conducted in 1940. However, a few more comprehensive studies, including twin shift, sibling

risk and segregation, began to be realized in the middle of the 1990s in North America and Europe (3,5). These studies indicated that heredity accounts

for 40-80% of OA pathophysiology, indicating that OA is a complex, multifactorial disease that is inherited by multiple genes (5).

Table 1. Characteristics of OA patients and control individuals

Clinical characteristics	Controls (n=83)	OA Patients (n=100)	P-value
Age (years)	64.95±8.95 (range 51-82)	65.57±7.64 (range 52-78)	0.611
Female/Male	57/26 (69/31%)	76/24 (75/25%)	0.322
BMI (kg/m ²)	24.65±3.34 (range 19.2-32.7)	26.28±3.18 (range 19.4-32.4)	0.001
K-L score			
1	-	0	
2	-	43 (43.6%)	
3	-	34 (33.7%)	
4	-	23 (22.8%)	

Table 2. Genotype and allele distributions of MMP1 (rs5854) and MMP3 (rs679620) gene polymorphisms

	Genotype Allele	Patient	Control	χ^2	P-value
		n (%)	n (%)		
rs5854	GG	47 (0.470)	44 (0.530)	0.697	0.706
	GA	44 (0.440)	33 (0.398)		
	AA	9 (0.090)	6 (0.072)		
	G	138 (0.690)	121 (0.729)		
rs679620	A	62 (0.310)	45 (0.271)	0.664	0.501
	CC	41 (0.410)	22 (0.278)		
	CT	49 (0.490)	47 (0.595)		
	TT	10 (0.100)	10 (0.127)		
	C	131 (0.655)	91 (0.576)		
	T	69 (0.345)	67 (0.424)		

The synthesis and activity of MMPs are tightly regulated by the level of gene expression in a tissue specific manner. An understanding of the factors influencing the production of MMP may allow better identification of patients at risk of developing destructive disease. The MMP-1 SNP rs5854 G/A and 1G/2G and MMP-3 rs679620 C/T and 5A/6A polymorphisms contribute to the development of rheumatoid arthritis (11,14,15). According to studies, some functional polymorphisms have been identified in OA, and these involve MMP-1 G/A and MMP-3 C/T polymorphisms (16).

In this case-control study, we found no significant differences in MMP-1 rs5854 and MMP-3 rs679620 gene polymorphism genotype and allele distributions between the patients and the control group ($p>0.05$). It is interesting that Barlas *et al.* found that MMP-1 promoter polymorphisms, 1G/1G or 1G/2G genotypes at position -1607 had a greater risk for knee OA susceptibility in a Turkish population (6). Also that Lepetosos *et al.* stated that the MMP-1 -1607 1G/2G (rs1799750) gene may be a risk factor for knee OA in a Greek population (5); however, and Abd-Allah *et al.* stated that MMP1(-1607 1G/2G) haplotypes in an Egyptian population may represent a genetic marker for OA, and for predicting the activity and severity of disease (12). The explanation for these conflicting

results remains unclear, but may be attributed to differences in disease advancement, populations, or assays applied.

The MMP-3 rs679620 G/A gene encode an enzyme that degrades fibronectin, laminin, collagen, proteoglycans and cartilage. Since the MMP-3 protein stromelysin is produced in the knee joints, the cartilage superficial zone and synovium of OA patients (15,16), Yan *et al.* found higher levels of stromelysin in stage IV OA than in stage II OA (18). Honsawek *et al.* did not report any correlation with knee OA in a Taiwanese population (7); however, in contrast to our findings, Abd-Allah *et al.* found an important relationship between OA susceptibility and MMP-3 (-1171 5A/6A) polymorphism in an Egyptian population (12).

There are some limitations to our current study. First, the group sizes are small; our findings need to be verified in larger groups and among different populations in Turkey. Second, we examined only two polymorphisms, and therefore, we could have missed an association of another specific polymorphism. Further, the BMIs of our study population were not homogeneous, and the study was conducted on a single joint.

In conclusion, MMP-1 SNP rs5854 G/A and MMP- 3 rs679620 C/T polymorphisms are not

associated with development of knee OA in a Turkish population.

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