

FokI polymorphism in the vitamin D receptor gene in patients with hip osteoarthritis: A case-control study

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ABSTRACT

Objectives: This study aimed to examine whether there is an association between hip osteoarthritis and vitamin D receptor gene FokI polymorphisms.

Patients and methods: In this case-control study, a total of 162 volunteers (43 males, 119 females; mean age: 62.4±9.3; range, 50 to 80 years) were included between March 2011 and March 2012. The patient group included 80 individuals with a diagnosis of coxarthrosis. Eighty-two individuals with normal hip, low back, and sacroiliac joint examination were included in the control group. The American College of Rheumatology hip osteoarthritis classification criteria were used in the diagnosis of coxarthrosis. Analysis of FokI polymorphisms was performed with restriction fragment length polymorphisms.

Results: When the genotype and allele distributions of vitamin D receptor FokI polymorphisms were examined, no statistical difference was found in both groups.

Conclusion: No significant association between the hip osteoarthritis and FokI polymorphisms could be obtained.

Keywords: FokI, osteoarthritis, polymorphisms, vitamin D receptor gene.

Osteoarthritis is described by the erosion of the cartilage, bone hypertrophy, and subchondral sclerosis at the margins of the joint, and some biochemical and morphological changes in the cartilage, synovial membrane, and joint capsule.^[1] Osteoarthritis includes a complex etiology, and the processes that cause its development have been tried to be clarified by genetic and epigenetic studies.^[2] *In vitro* vitamin D has been found to have a positive effect on the synthesis of proteoglycans in the articular cartilage structure. This finding suggests that vitamin D affects joint cartilage metabolism. Vitamin D has biological effects on bone, muscle, and cartilage functions. Osteoarthritis affects the joint cartilage, bone, and

muscles around the joint; therefore, supplementing vitamin D deficiency can have a positive effect on joint structures. Vitamin D insufficiency may have a place in the etiology of osteoarthritis as it causes endothelial cell dysfunction, inflammation, and vascular smooth muscle cell proliferation.^[3] A 2015 study found an increased prevalence of radiographic coxarthrosis and gonarthrosis in a significant percentage of patients with vitamin D insufficiency.^[4] The VDR (vitamin D receptor) is found in a wide variety of tissues, including chondrocytes.^[5] Some VDR gene polymorphisms are involved in vitamin D₃ (cholecalciferol) uptake and regulate the metabolism and function of vitamin D.^[6] Therefore, abnormalities

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in the VDR gene have been defined as possible risk factors predisposing to the development of osteoarthritis.^[7]

The VDR, which plays a vital role in bone mineral density (BMD), is 100 kb long, and the VDR gene, is localized in the 12q13-14 region. Four different polymorphisms of VDR were defined using the restriction fragment length polymorphisms technique, which are FokI, ApaI, TaqI, and BsmI. The most investigated gene polymorphisms are ApaI, TaqI, and BsmI.^[8] The genetic contribution to the pathogenesis of osteoarthritis is difficult to analyze due to the high prevalence of osteoarthritis in the general population and its widespread clinical heterogeneity. Nevertheless, some genetic variations have been described in inherited forms of osteoarthritis. However, the effect of the genetic factors varies in different localizations. For example, some researchers have shown that VDR gene polymorphisms are associated with gonarthrosis,^[9] hand osteoarthritis,^[10] and lumbar spinal osteophyte,^[11] but its association with hip osteoarthritis has not been fully determined. It is beyond doubt that the demonstration of these genetic associations will likely contribute to the identification of the determinants that are to be taken into consideration in the diagnosis and treatment of osteoarthritis and to the discovery of the genetic variations that may predispose to disease progression. In view of the foregoing, our aim in this study was to determine whether the FokI polymorphism has an effect on the development of hip osteoarthritis.

PATIENTS AND METHODS

The case-control study was conducted with 162 participants (43 males, 119 females; mean age: 62.4±9.3 years; range, 50 to 80 years) at the Department of Physical Medicine and Rehabilitation of Bülent Ecevit University Faculty of Medicine between March 2011 and March 2012. The patient group included 80 individuals with a diagnosis of coxarthrosis. Individuals with normal hip, low back, and sacroiliac joint examinations were included in the control group. Two-way comparative hip radiographs of the patients with hip pain were taken, and the radiographs of the patients were evaluated by comparing them with atlas index graphics in accordance with the radiological staging determined by the Osteoarthritis Research Society International. The diagnosis of coxarthrosis was made using the American College of Rheumatology hip osteoarthritis classification criteria. Patients with

Stage 2-4 osteoarthritis according to the Kellgren and Lawrence classification were included. Patients who had secondary coxarthrosis or inflammatory joint disease were excluded from the study. The weight and height of all participants were measured, and the body mass index (BMI) was calculated.

Genetic evaluation

Two milliliter blood samples were taken from all participants using complete blood count tubes, and the deoxyribonucleic acid (DNA) was obtained using the E.Z.N.A® Blood DNA Isolation Kit (cat. no. D3392-02). Single nucleotide polymorphism in the VDR gene creates a cut-off site for the FokI enzyme. The polymorphism site was investigated with the following primers: FOK-F (5'- AGC TGG CCC TGG CAC TGA CTC TGC TCT-3') and FOK-R (5'-ATG GAA ACA CCT TGC TTC TTC TCC CTC-3 '). Polymerase chain reactions were performed using 50 pmol of each primer, 2 mmol/L MgCl₂, 10 mmol/L Tris-HCL, 50 mmol/L KCl, two units of Taq DNA polymerase, 0.2 mmol/μL deoxynucleotide triphosphate mix, and 4 μL DNA samples for a final volume of 25 μL. Polymerase chain reaction cycles were as follows: an initial denaturation at 94°C for 4 min, followed by 35 cycles of 30 sec denaturation at 94°C, 30 sec annealing at 58°C, and 1 min extension at 72°C. A 265 bp PCR product was left for enzyme (FokI) digestion for 3 h, and subsequently, the fragments were analyzed. The undigested product was named F (265 bp), and the digested product was named f (196/69 bp) (Figure 1). A 265 bp product was found in FF genotype (wildtype/homozygous normal), two products of 196/69 bp in ff genotype (homozygous mutant), and 265, 196/69 in Ff genotype (heterozygous).

Statistical analysis

The SPSS version 13.0 software (SPSS Inc., Chicago, IL, USA) was used to analyze the study data. Variables

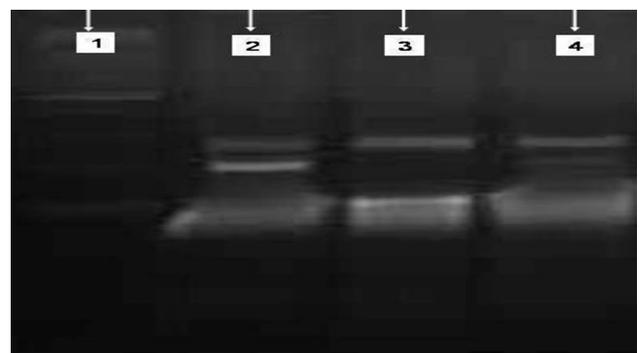


Figure 1. Vitamin D receptor FokI gene polymorphism 3. 1: DNA marker; 2: ff genotype; 3: FF genotype; 4: Ff genotype.

TABLE 1 Age, sex, and BMI distribution by groups							
Demographic variables	Patient group (n=80)			Control group (n=82)			p
	n	%	Mean±SD	n	%	Mean±SD	
Age (year)			62.8±10.2			61.9±9.0	0.717
Sex							
Male	21	26.2		22	26.8		
Female	59	73.8		60	73.2		
Body mass index (kg/m ²)			27.05±3.36			26.88±2.78	0.933
SD: Standard deviation.							

with continuous values were expressed as mean, standard deviation, minimum, and maximum, whereas the variables with categorical values were expressed as frequency and percentage. The odds ratio was used as the measure of effect size. The conformity of continuous variables to normal distribution was tested using the Shapiro-Wilk test. In the study, continuous variables that provided the parametric test assumption in comparisons between groups were tested with Student's t-test. Pearson's chi-square test was used in the group comparisons of categorical variables. A *p* value of <0.05 was considered statistically significant.

RESULTS

There was no significant difference between the groups in sex, age, and BMI (*p*=0.933, *p*=0.717, and

p=0.469, respectively; Table 1). The proportions of FF homozygote, Ff heterozygote, and ff homozygote in the study were found to be 51% (n=83), 44% (n=71), and 5% (n=8), respectively. We found homozygous FF genotype in 53.8% of the patient group and 48.8% of the control group. Minor ff homozygous genotype was observed in 2.5% of the patient group and 7.3% of the control group. Heterozygous Ff genotype was detected in 43.7% of the coxarthrosis patients and 43.9% of the control group. In the VDR genotype distribution analysis, no statistically significant difference was observed between the groups. The analysis of the allele distribution revealed 121 F and 39 f alleles in the patient group and 116 F and 48 f alleles in the control group. The comparison of the VDR allele distribution between the groups did not reveal a statistically

TABLE 2 Vitamin D receptor genotype distribution						
Polymorphism	Patient group		Control group		p	OR (95% CI)
	n	%	n	%		
FF	43	53.8	40	48.8	0.334	0.904 (0.480-1.704)
Ff	35	43.7	36	43.9		
ff	2	2.5	6	7.3		
Total	80	100	82	100		
OR: Odds ratio; CI: Confidence interval.						

TABLE 3 Vitamin D receptor allele distribution						
Allele	Patient group		Control group		p	OR (95% CI)
	n	%	n	%		
F	121	75.7	116	70.8	0.320	0.779 (0.476-1.276)
f	39	24.3	48	29.2		
Total	160	100	164	100		
OR: Odds ratio; CI: Confidence interval.						

significant difference. Tables 2 and 3 demonstrate the FokI genotype and alleles of controls and cases, including 95% confidence intervals and adjusted and raw odds ratios.

DISCUSSION

Studies have suggested that VDR gene polymorphisms may also play a role in the emergence of diseases such as prostate and breast cancer, osteoarthritis, diabetes, psoriasis, coronary artery disease, and primary hyperparathyroidism.^[9,12-15] Osteoarthritis is the most common joint disease. Vitamin D has an important place in musculoskeletal function and calcium homeostasis. This is achieved by the binding of vitamin D to the VDR.^[16] Four different polymorphisms of VDR have been defined using the restriction fragment length polymorphisms technique, which are ApaI, TaqI, BsmI, and FokI.^[8] A strong linkage disequilibrium (LD) has been determined between the alleles of ApaI, TaqI, and BsmI polymorphisms in the 3'UTR region of the VDR gene as they are near each other.^[8] However, LD was not determined between the FokI polymorphism and other polymorphisms. In addition, the LD area surrounding the FokI polymorphism was found to be very small.^[8] In the VDR gene, the FokI polymorphism is located at a different site from the TaqI, BsmI, and ApaI polymorphisms. The FokI polymorphism is located in the protein-coding region of the VDR gene. Therefore, it has been shown to cause serious structural changes in the VDR protein.^[17] FokI in exon 2 delays translation initiation. As a result of the T-C change in the start codon, ATG turns into ACG and translation starts from the second ATG. As a result, the 424 amino acid long (F allele) VDR protein is synthesized. In the absence of T-C exchange, translation starts from the first ATG, and a VDR protein three amino acids longer (427 amino acids, f allele) is synthesized.^[18] The association between BMD and VDR gene polymorphism has been demonstrated.^[19] It has been shown that the VDR protein is elongated by three amino acids in the f allele of the FokI polymorphism and that it is associated with BMD.^[20] In a meta-analysis conducted in 2021, the VDR FokI genotype was found to be associated with an increased risk of osteoporosis in Asian women; however, a similar association was not observed in Caucasian women.^[21] In a study conducted on 174 postmenopausal Korean women in 2000, BMD was found to be significantly lower in those with the ff genotype in the FokI

polymorphism.^[22] Many cross-sectional studies have shown that increased BMD is associated with osteoarthritis.^[23-25] In this context, studies have been conducted to investigate whether the VDR gene polymorphism, which was previously found to be associated with BMD, is associated with the risk of developing osteoarthritis.

In a meta-analysis published in 2009, to reveal the association between the most evaluated polymorphisms of the VDR (ApaI, TaqI, and BsmI) and osteoarthritis, polymorphisms of the VDR gene were investigated in patients with hip, hand, knee, and lumbar osteoarthritis. When the results were examined, no significant association was found between osteoarthritis types and VDR gene polymorphisms.^[16] In parallel with the findings of this study, Huang et al.^[26] did not find any association between any VDR gene polymorphism they studied and any osteoarthritis type in the study they conducted with 270 patients and 161 control subjects in Japan.

Two updated meta-analyses were published in 2014 on the association between VDR gene polymorphisms and osteoarthritis. The first of these studies was carried out by Liu et al.^[27] to identify whether TaqI, ApaI, and BsmI polymorphisms are associated with osteoarthritis susceptibility. The study, which included eight studies comprising 3,650 participants, concluded that the TaqI, ApaI and BsmI polymorphisms may not be important predictors of osteoarthritis. Similarly, Zhu et al.,^[28] investigating 13 studies, determined no significant difference between BsmI and TaqI polymorphisms and osteoarthritis. They also found a statistically significant difference between the FokI polymorphism and osteoarthritis, although this result was obtained from only two studies. A moderate but statistically significant association was found between ApaI polymorphisms and the susceptibility to osteoarthritis. A meta-analysis published in 2021, which included a total of 18 studies, revealed that TaqI and BsmI polymorphisms are associated with osteoarthritis susceptibility in the spine.^[7] In addition, a statistically significant difference between the FokI polymorphism and the knee osteoarthritis susceptibility was determined in only one of the studies out of the 18 studies included in the meta-analysis. Contrary to the meta-analysis conducted in 2014,^[28] it has been stated that the ApaI polymorphism is not an important genetic risk factor for osteoarthritis.^[7] In the mentioned

meta-analyses, no significant difference was found between the three polymorphisms of the VDR, ApaI, TaqI, and BsmI, and peripheral osteoarthritis, whereas a significant relationship between the FokI polymorphism and peripheral joint osteoarthritis was demonstrated in a limited number of studies. This highlights the investigation of the association between osteoarthritis and FokI polymorphism and shows that more studies are needed to clarify this association.

As noticed in the meta-analysis studies, mostly ApaI, BsmI, and TaqI polymorphisms were studied. An association between VDR polymorphisms and osteoarthritis was not found. However, a limited number of articles have found an association between the FokI polymorphism and osteoarthritis.^[29-31] In this study, we examined whether this association exists between hip osteoarthritis and the FokI polymorphism.

In comparison, the findings of this study did not reveal any statistical difference between the FokI polymorphism and hip osteoarthritis. Nevertheless, this result cannot be interpreted as that hip osteoarthritis does not have a genetic component since it is not possible for a single gene to be responsible for a disease such as osteoarthritis with multifactorial etiology and many pathogenetic processes.

The main limitation of this study is the evaluation of only the FokI polymorphism in the VDR gene. Unfortunately, other known polymorphisms of the VDR gene (ApaI, BsmI, and TaqI) could not be investigated due to the necessity to include a large number of patients with high costs. Examination of FokI and other polymorphisms in groups containing more patients will be useful in determining the association between coxarthrosis and VDR gene polymorphisms.

In conclusion, no statistically significant association between hip osteoarthritis and the FokI polymorphism could be obtained. Further studies may produce more definitive results on the difference between different VDR gene polymorphisms and hip osteoarthritis.

Ethics Committee Approval: The study protocol was approved by the Bülent Ecevit University Faculty of Medicine Ethics Committee (date/no: 23-06-2009/2009-08). The study was conducted in accordance with the principles of the Declaration of Helsinki.

Patient Consent for Publication: A written informed consent was obtained from each patient.

Data Sharing Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

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