

An Investigation Into the Relationship Between TaqI and ApaI Polymorphisms of the Vitamin D Receptor Gene and the Development of Osteoarthritis

Osteoartrit Gelişimi ve Vitamin D Reseptör Geni TaqI ve ApaI Polimorfizmleri Arasındaki İlişki Üzerine Bir Araştırma

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Objectives: This study aims to determine whether Vitamin D receptor (VDR) gene TaqI and ApaI polymorphisms are involved in the development of osteoarthritis in the Turkish population.

Patients and methods: In this study, genomic DNA was isolated from 140 subjects (95 patients with osteoarthritis and 45 healthy controls). The DNA was amplified with specific primers by polymerase chain reaction and the restriction fragment length polymorphism (RFLP) technique was used to analyze the VDR gene TaqI and ApaI polymorphism genotypes. Polymerase chain reaction-RFLP products were assessed with and ultraviolet transilluminator by being exposed to agarose gel electrophoresis.

Results: In terms of the genotype distributions and the allele frequencies of VDR gene TaqI and ApaI polymorphisms, there was no statistically significant difference ($p>0.05$) between the osteoarthritis patients and the controls. In addition, there was also no statistically significant difference between the genotypes and clinical characteristics of the patients or controls.

Conclusion: In conclusion, the results of the present study suggest that VDR gene TaqI and ApaI polymorphisms are not involved in the development of osteoarthritis in the studied Turkish population.

Key words: ApaI; osteoarthritis; polymorphism; TaqI; vitamin D receptor.

Amaç: Bu çalışmada vitamin D reseptör (VDR) geni TaqI ve ApaI polimorfizmlerinin Türk toplumunda osteoartrit gelişiminde rol oynayıp oynamadığının belirlenmesi amaçlandı.

Hastalar ve yöntemler: Çalışmada 140 kişiden (95 osteoartriteli hasta ve 45 sağlıklı kontrol) genomik DNA izole edildi. DNA spesifik primerler ile polimeraz zincir reaksiyonu yöntemi ile amplifiye edildi, VDR geni TaqI ve ApaI polimorfizmi genotiplerinin analizi için ise restriksiyon parça uzunluk polimorfizm (RFLP) tekniği kullanıldı. Polimeraz zincir reaksiyonu-RFLP ürünleri agaroz jel elektroforezine tabi tutularak ultraviyole translüminatör ile değerlendirildi.

Bulgular: Vitamin D reseptör geni TaqI and ApaI polimorfizmleri genotip dağılımları ve alel frekansları açısından osteoartrit hastaları ile kontrol grubu arasında istatistiksel olarak anlamlı bir fark yoktu ($p>0.05$). Buna ek olarak, hem hasta hem de kontrol grubuna ait genotipler ve klinik özellikler arasında istatistiksel olarak anlamlı bir fark yoktu.

Sonuç: Sonuç olarak, bu çalışmanın sonuçları üzerinde çalışılan Türk toplumunda VDR geni TaqI and ApaI polimorfizmlerinin osteoartrit gelişiminde rol oynamadığına işaret etmektedir.

Anahtar sözcükler: ApaI; osteoartrit; polimorfizm; TaqI; vitamin D reseptörü.

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Osteoarthritis (OA) is the most common form of arthritis and a leading cause of musculoskeletal disability in middle-aged and elderly people.^[1] Pathologically, OA is characterized by the focal degeneration of the smooth articular cartilage in any part of the synovial joints of the body, and the most frequently affected joint sites are the spine, hands, knees, and hips.^[2] The most frequent clinical findings in OA are pain associated with loading and limited range of motion in the joints at later stages.^[3] Because there is no effective therapy to reverse or slow down the degenerative process, OA has a high socio-economic burden.^[4] Several factors play a role in the variability of OA risk, including age, sex, genetics, ethnicity, behavioral influences, obesity, and occupation.^[5,6] Genetic factors account for 40 to 85% of OA development.^[5] The identification of disease-susceptibility genes would help us not only to understand the pathogenesis of OA better but also to gain insight into new therapeutic targets.^[7] Until now, some candidate genes have been proposed for OA, including the vitamin D receptor (VDR) gene. The VDR gene is an important regulator of calcium metabolism and bone cell function. Genetic variants of the VDR gene locus on chromosome 12 are associated with bone density, which was found to increase in patients with OA.^[8] Nonetheless, it is still uncertain whether genetic variants of the VDR gene (TaqI and ApaI polymorphisms) are involved in the development of OA.^[9-11] Since gene pools, lifestyle, and gene-environment interactions vary between populations, the risk should not be the same with respect to genotypes in every population.^[12] The aim of the present study was to determine if the polymorphisms of the VDR

gene, TaqI and ApaI, are involved in the development of osteoarthritis in the Turkish population.

PATIENTS AND METHODS

Study population

This study included 95 unrelated OA patients and 45 healthy controls without OA who were recruited from the Department of Orthopedics and Traumatology in the Private Muş Şifa Hospital, Muş, Turkey. Informed consent was obtained from each patient in accordance with the study protocol, and the ethics committee of Medical Faculty, Eskişehir Osmangazi University, Eskişehir approved this study. Inclusion criteria were as follows: any symptom and/or sign of OA, positive findings in the radiographs according to Kellgren-Lawrence grading, and no evidence of arthritis due to other diseases. Clinical information was obtained by patient history, physical examination, and radiographic findings. In addition, demographic characteristics (age, gender), body mass index (BMI), and clinical features of the disease severity assessed by Kellgren-Lawrence grading^[13] and Lequesnes' functional index^[14] were recorded. The Kellgren-Lawrence grade represents the severity of the disease on radiographs whereas Lequesne's functional index represents the functional or symptomatic status of the patients. The radiographic findings of OA were classified as Kellgren-Lawrence grades 1, 2, 3, or 4. The functional or symptomatic status of the patients was classified as mild (Lequesne's functional index IB10) or severe (Lequesne's functional index IC10). Control subjects were consecutively selected among people without a personal and familial history of OA. The clinical characteristics of the patients with OA and controls are summarized in Table 1.

Table 1. Clinical characteristics of osteoarthritis patients and controls

Clinical characteristics	Patients (n=95)			Controls (n=45)		
	n	Mean±SD	Min.-Max.	n	Mean±SD	Min.-Max.
Gender						
Male	28	–	–	13	–	–
Female	67	–	–	32	–	–
Average age (years)		52.4±1.48			45.9±2.3	
BMI (kg/m ²)		28	(26-30)		26	(27-27)
LO/GO	28/67			–	–	–
Pain severity mild/severe	27/68			–	–	–
Grade						
1	6			–		
2	17			–		
3	36			–		
4	36			–		

Min.: Minimum; Max.: Maximum; BMI: Body Mass Index; LO: Lombar osteoarthritis; GO: Gonad rose osteoarthritis.

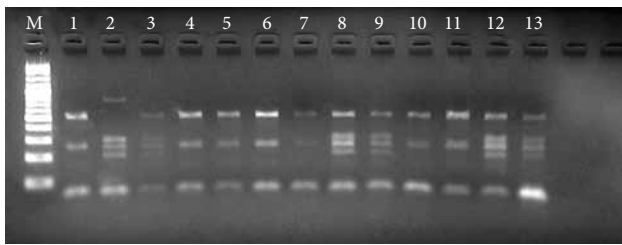


Figure 1. Gel image of VDR gene TaqI polymorphism PCR-RFLP products. M: Molecular size marker (100 bp, Vivantis) Rows 1, 4, 5, 6, 7, 10, 11: TT homozygosity produces fragments of 495 and 245 bp; Row 2: tt homozygosity produces fragments of 290, 245, and 210 bp; Rows 3, 8, 9, 12, 13: Tt heterozygosity produces fragments of 495, 290, 245, and 210 bp.

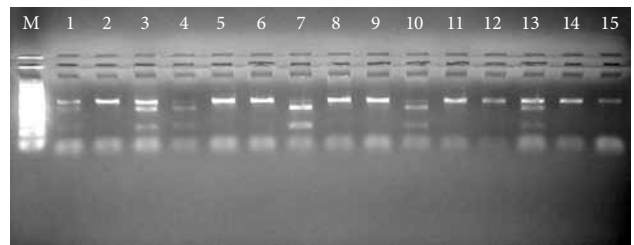


Figure 2. Gel image of VDR gene ApaI polymorphism PCR-RFLP products. M: Molecular size marker (100 bp, Vivantis) Rows 2, 5, 6, 8, 9, 11, 12, 14, 15: AA homozygosity produces fragment of 740 bp; Rows 4, 7, 10: aa homozygosity produces fragments of 530 and 210 bp; Rows 1, 3, 13: Aa heterozygosity produces fragment of 740, 530, and 210 bp.

Deoxyribonucleic acid (DNA) isolation

DNA was extracted from venous blood according to a kit procedure (Vivantis, Malesia) and stored at -20°C until analysis.

Genotype determination of ApaI and TaqI polymorphisms of VDR gene

The DNA was amplified by a polymerase chain reaction (PCR) in a thermal cycler (Nyx Technik, Inc., Amplitronyx 4, CA, USA). Allele-specific primers used in the PCR were as follows: forward, 5'-CAG AGC ATG GAC AGG GAG CAA-3', and reverse, 5'-GCA ACT CCT CAT GGC TGA GGT CTC-3'. A DNA sample of 5 μl was amplified for 35 cycles with denaturation at 94°C for 30 seconds, annealing at 70°C for 30 seconds, and extension at 72°C for 30 seconds using a 50 μl PCR mixture that contained 10 pmol of each primer, a 10x PCR buffer, 2 mM dNTPs, and a 5U Taq polymerase. After confirmation of an amplified fragment of the expected size (740 bp) by electrophoresis on 2% agarose gel, the PCR products were digested with 5 units of restriction enzyme TaqI (Vivantis, Malesia) at 65°C for one hour for TaqI polymorphism genotype determination and 5 units of restriction enzyme ApaI (Vivantis, Malesia) at 37°C for one hour for ApaI polymorphism genotype determination. Digested PCR products were separated

by electrophoresis on 2% agarose gel containing 4 μl ethidium bromide. They were then visualized using a UV transilluminator (Nyxtechnik, Inc., CA, USA) and photographed with a CCD camera (Cleaver, UK), (Figures 1 and 2).

Statistical analysis

Statistical analysis was performed using Statistical Package for Social Sciences (SPSS Inc., Chicago, Illinois, USA) software package version 15.0. The Shapiro-Wilk normality test was applied for all variables. Because the variables did not distribute normally, they were reanalyzed using the Mann-Whitney U and Kruskal-Wallis tests. Categorical variables were compared by Pearson's chi-square and the Continuity Correction chi-square tests. Hardy-Weinberg equilibrium was assessed by chi-square analysis. The values were expressed as means \pm standard error (SE) and median (25-75%) quartiles. Variables were given as "n" and "%". *P* values of less than 0.05 were considered statistically significant.

RESULTS

The genotype distributions of the VDR gene TaqI and ApaI polymorphism were in agreement with the Hardy-Weinberg expectations in the patient and control groups ($p>0.05$). The distribution of VDR gene

Table 2. Distribution of vitamin D receptor gene TaqI polymorphism genotypes and allele frequencies between osteoarthritis patients and controls

	Distribution of genotypes			Allele frequencies			
	TT	Tt	tt	T allele		t allele	
	n	n	n	n	%	n	%
Patients	46	36	13	128	67.4	62	32.6
Controls	25	16	4	66	73.3	24	26.7
<i>P</i>	>0.05			>0.05			

Table 3. Distribution of vitamin D receptor gene ApaI polymorphism genotypes and allele frequencies between osteoarthritis patients and controls

	Distribution of genotypes			Allele frequencies			
	AA	Aa	aa	A allele		a allele	
	n	n	n	n	%	n	%
Patients	42	41	12	125	65.8	65	34.2
Controls	21	19	5	61	67.7	29	32.3
<i>P</i>	>0.05			>0.05			

TaqI polymorphism genotypes in OA patients and control subjects are shown in Table 2. There was no statistically significant difference between groups with respect to genotype distribution and allele frequencies of the VDR gene TaqI polymorphism ($p>0.05$). The distribution of VDR gene ApaI polymorphism genotypes and allele frequencies in patient and control groups are shown in Table 3. There was also no statistically significant difference between the groups with respect to genotype distribution of the VDR gene ApaI polymorphism ($p>0.05$). Moreover, there was no correlation between VDR gene TaqI and ApaI polymorphism genotypes and the clinical characteristics of the patients and the controls.

DISCUSSION

The present study analyzed the distribution of the VDR gene TaqI and ApaI polymorphisms in OA patients in Turkey in order to assess their possible roles in the development of OA. The results of the present study indicated that the percentage of allele frequencies and the distribution of genotypes of the VDR gene TaqI and ApaI polymorphisms were not significantly different when analyzing the OA patients and the controls. Moreover, there was no association between VDR gene TaqI and ApaI polymorphism genotypes and the clinical characteristics of the patients and the controls. To our knowledge, there are no studies examining the relationship between OA and VDR gene TaqI and ApaI polymorphisms in a Turkish study population. Therefore, the present study is the first to do this. In accordance with our results on allele frequencies and genotype distributions of the VDR gene TaqI and ApaI polymorphisms in patients with OA, several authors had also previously reported that allele frequencies and genotype distributions of the VDR gene TaqI and ApaI polymorphisms were not significantly different in patients with other diseases, such as diabetes mellitus,^[15,16] osteoporosis,^[17] chronic periodontitis,^[18] and urolithiasis.^[19] This was also true for postmenopausal women^[20] compared with

control subjects in the Turkish population. Similarly, an arthritis study in a Turkish study population suggested that polymorphisms of VDR do not play a role in rheumatoid arthritis.^[21] However, in a study of sporadic prostate cancer in the Turkish population,^[22] the VDR gene ApaI polymorphism was found to be associated with the disease. Although, VDR gene polymorphisms were discovered to be associated with hand OA in Finnish women^[10] and with radiographic OA in Netherlands,^[8] Huang et al.^[11] did not find any association between VDR gene polymorphisms and hand, hip or knee OA in a Japanese population. This finding is consistent with our results. Moreover, a meta-analysis also revealed no association between OA and VDR gene TaqI and ApaI polymorphisms in 10 relevant studies.^[9] As a result, we suggest that the genotype distribution of VDR gene TaqI and ApaI polymorphisms in the studied Turkish population are similar to most other populations with OA. The present study also did not show such a predisposition in this Turkish study population with OA.

However, genetic polymorphism studies in larger populations should yield more significant results. Also, it should not be suggested that the results of this study should be used as a litmus test for the whole Turkish population, but rather it renders an opinion. As previously mentioned, gene pools, lifestyle, and gene-environment interactions vary between populations, so the risk of OA development cannot be assumed to be similar with respect to genotypes for all people. Further studies with larger and even more varied populations as well as studies performed using other genetic polymorphisms which might be considered to be risk factors for OA should provide important validation for these results.

Declaration of conflicting interests

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