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Original Article

Relationship of the Vitamin D Receptor and Collagen Iα1 Gene Polymorphisms with Low Bone Mineral Density and Vertebral Fractures in Postmenopausal Turkish Women

Postmenopozal Türk kadınlarında D Vitamini Reseptörü Geni ve Kollajen Iα1 Geni Polimorfizmlerinin Düşük Kemik Mineral Yoğunluğu ve Vertebral Kırıklar ile İlişkisi

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Objectives: This study aims to investigate *(i)* the frequencies of the vitamin D receptor (VDR) gene and collagen Iα1 (COL Iα1) gene polymorphisms and *(ii)* whether there is a relation between the Bsm1 polymorphisms in the VDR gene, and the Sp1 polymorphisms in the COL Iα1 gene and low bone mineral density (BMD), and vertebral fractures in postmenopausal Turkish women.

Patients and methods: A hundred postmenopausal Turkish women (mean age 63.4±8.7 years; range 48 to 86 years) who were admitted to our polyclinic for the diagnosis or treatment of osteoporosis were included in this study. The study population was divided into three groups according to the T score value based on BMD measurements. Patients with a T score of >-1.0 formed the control group (n=30). In addition, patients with a T score of <-1.0–>-2.5 formed the osteopenic group (n=30), and those with a T score of <-2.5 formed the osteoporotic group (n=40). The Bsm1 (B/b) polymorphism in the VDR gene and the Sp1 (S/s) polymorphism in the COL Iα1 gene were detected by two parallel polymerase chain reactions and subsequent hybridization. Bone mineral density of the lumbar spine and femur were measured by dual-energy X-ray absorptiometry.

Results: The genotype frequencies of the Bsm I (B/b) polymorphism in the VDR gene and the Sp1 (S/s) polymorphism in the COL Iα1 gene were not statistically different among the three study groups. Additionally, no significant difference was found between the patients with vertebral fracture and the patients without fracture in terms of the BB, Bb, and bb genotypes of the VDR gene or in terms of the SS, Ss, and ss genotypes of the COL Iα1 gene.

Conclusion: Our findings showed that the Bsm1 polymorphism of the VDR gene and the Sp1 polymorphism of the COL Iα1 gene were not associated with low BMD or vertebral fractures in postmenopausal Turkish women.

Key words: Bone mineral density; collagen Iα1; postmenopausal women; Turkish; vitamin D receptor.

Amaç: Bu çalışmada postmenopozal Türk kadınlarında *(i)* D vitamini reseptörü (VDR) geni ve kollajen Iα1 (COL Iα1) geni polimorfizmlerinin sıklığını ve *(ii)* VDR genindeki BsmI polimorfizmi ve COL Iα1 genindeki Sp1 polimorfizmleri ile düşük kemik mineral yoğunluğu (KMY) ve vertebral kırıklar arasında bir ilişki olup olmadığının araştırılması amaçlandı.

Hastalar ve yöntemler: Osteoporoz tanı ve tedavisi nedeniyle polikliniğimize başvuran postmenopozal 100 Türk kadın (ort. yaş 63.4±8.7 yıl; dağılım 48-86 yıl) çalışmaya dahil edildi. Çalışma grubu KMY ölçümü temelinde T skoru değerine göre üç gruba ayrıldı. Buna ek olarak T skoru >-1.0 olanlar kontrol grubunu (n=30) oluşturdu. BT skoru <-1.0–>-2.5 olanlar osteopenik grubu (n=30) ve T skoru <-2.5 olanlar (n=40) osteoporotik grubu oluşturdu. D vitamini reseptörü genindeki Bsm1 (B/b) ve COL Iα1 genindeki Sp1 (S/s) polimorfizmleri iki paralel polimeraz zincir reaksiyonu ve ardından hibridizasyon ile belirlendi. Lomber omurga ve femur kemik mineral yoğunlukları çift enerjili X ışını absorbsiyometrisi yöntemi ile ölçüldü.

Bulgular: D vitamini reseptörü genindeki Bsm1 (B/b) polimorfizmi genotip frekansları ve COL Iα1 genindeki Sp1 (S/s) polimorfizmi genotip frekansları üç çalışma grubu arasında istatistiksel olarak farklı değildi. Ayrıca VDR genindeki BB, Bb, bb genotipleri ve COL Iα1 genindeki SS, Ss, ss genotipleri açısından vertebral kırığı olan hastalar ile kırığı olmayan hastalar arasında anlamlı farklılık bulunmadı.

Sonuç: Bulgularımız postmenopozal Türk kadınlarında VDR genindeki Bsm1 polimorfizmi ile COL Iα1 genindeki Sp1 polimorfizmlerinin düşük kemik yoğunluğu ya da vertebral kırıklar ile ilişkili olmadığını göstermiştir.

Anahtar sözcükler: Kemik mineral yoğunluğu; kollajen Iα1; postmenopozal kadın; Türk; D vitamini.

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Bone mineral density (BMD) is commonly used in the diagnosis of bone disorders. Low BMD is an important predictor of fracture risk and osteoporosis.[1] Osteoporosis is a systemic skeletal disorder characterized by low BMD and microarchitectural deterioration of bone tissue. Consequently, this leads to an increased risk of fracture.[2,3] Osteoporosis is a significant health problem and, at some point, decreases the quality of life. Many factors influence the risk of osteoporosis, such as diet, physical activity, medication use, and coexisting diseases. However, one of the most important clinical risk factors is a positive family history, which emphasizes the importance of genetics in the pathogenesis of osteoporosis.[4] The genetic study of osteoporosis has been based mainly on research into candidate genes relevant to bone metabolism.[5]

The vitamin D receptor (VDR) gene has been declared as one of the candidate genes for genetic control of bone mass.[6] The VDR mediates 1α,25 (OH)2D3 action by modulating the transcription of target genes.[7] Polymorphisms at the VDR locus, identified by the restriction endonuclease enzyme Bsm I, were originally thought to explain a large percentage of genetic variation in BMD population studies.[8] However, recent meta-analysis has suggested that the VDR gene polymorphisms (Bsm I, Apa I, Taq I haplotypes) have no effect on either BMD or fracture risk.[9]

The collagen Iα1 (COL Iα1) gene, which encodes the alpha I chain of type 1 collagen, is one of the most commonly studied candidate genes for susceptibility to osteoporosis.[10] Guanine (G)-thymidine (T) polymorphisms, which affect the binding site for the transcription factor specificity protein 1 (Sp1) in the first intron of the COL Iα1 gene, has been associated with low bone density and increased occurrence of osteoporotic fracture.[11] It has been suggested that heterozygotes at the polymorphic Sp1 site (Ss) have significantly lower bone mineral density than SS homozygotes and ss homozygotes. Consequently, the Sp1 polymorphism of the COL Iα1 gene might contribute to peak bone mass.[12]

Some of the studies have stated that VDR and COL Iα1 genes are associated with BMD and fracture risk.[13-15] On the other hand, some other studies have shown no relationship between VDR, COL Iα1 genes, and BMD or fracture risk.[16,17] Further work is required to determine whether genetic factors do indeed contribute significantly to the regulation of bone loss. Moreover, the importance of these polymorphisms within various ethnic populations, including the Turkish population, is not clear yet. There has been one study[18] from the Turkish population that investigated VDR genotypes in postmenopausal women with osteoporosis. Another Turkish study group.[19] Evaluated the effects of hormone replacement therapy (HRT) on BMD in postmenopausal women who had osteoporosis both with and without the COL Iα1 Sp1 binding site polymorphism. To our knowledge, there has not been any study evaluating both of these polymorphisms in Turkish postmenopausal women with regards to osteoporosis or vertebral fractures.

Our study is a preliminary study investigating the frequencies of Bsm I polymorphisms in the VDR gene and Sp1 polymorphisms in the COL Iα1 gene while also evaluating the associations between VDR, COL Iα1 polymorphisms and BMD and bone fractures in postmenopausal Turkish women with osteopenia or osteoporosis.

PATIENTS AND METHODS

Study subjects

The study included 100 postmenopausal Turkish women, aged 48-86 years (mean age 63.4±8.7 years), who were referred to the Department of Physical Medicine and Rehabilitation Clinic for the measurement of BMD. A detailed medical history was obtained from all of the women. Each patient was examined clinically, and routine biochemical tests were performed on all patients in order to exclude any underlying secondary causes of osteoporosis (systemic and metabolic bone disease). Patients who had diseases capable of influencing calcium and phosphorus metabolism, such as hyperparathyroidism, renal failure, liver diseases, hyperthyroidism, hyper/hypocortisolism, diabetes, or other chronic illnesses, were excluded.

Height and weight were measured at the time of BMD measurement. The body mass index (BMI) was calculated as weight (in kilograms) divided by height (in meters) squared. Study participants consented to participate, and the local ethics committee approved the study.

Bone mineral density measurement

Bone mineral density at the lumbar spine and femoral neck was measured by dual-energy X-ray absorptiometry (DXA) using a Hologic DXA system (Discovery QDR, W S/N 81754) and was reported as grams per square centimeter. Based on the BMD

measurement, which was calculated using the WHO (World Health Organization, 1994) criteria,[20] the study population was divided into three groups according to the T score value: >-1.0 as the "control", <-1.0–>-2.5 as osteopenic, and <-2.5 as osteoporotic. For comparison of genotype frequencies, we selected an age-matched subgroup of women (30 women; mean age 62.4±8.7 years) as the control group.

Fracture assessment

Lateral lumbar and thoracic spine radiographies were taken for all of the individuals; however, eight were excluded when evaluating VDR, Bsm I, and COL Iα1 Sp1 polymorphisms in terms of vertebral fracture risk because these radiographies were not available. The lateral lumbar and thoracic spine radiographies were evaluated in terms of vertebral fracture with the method defined by Genant et al.[21] in which torso heights of the vertebras between T4-L4 are evaluated by visual determination. We excluded vertebral fractures that occurred because of major trauma and vertebral deformities due to causes other than osteoporosis.

Analysis of the VDR gene, Bsm I, and COL Iα1 gene Sp1 polymorphisms

The blood samples gathered for gene polymorphism analyses were stored at +4 °C for deoxyribonucleic acid (DNA) isolation. The DNA was isolated from whole blood within the first 24 hours. Genomic DNA was extracted from samples of peripheral venous blood using a commercially available kit (Invisorb® Spin Blood Mini kit, Invitek, Germany). The DNA was kept at -80 °C until the analysis.

In order to analyze the genetic polymorphisms in the genes for the human COL Iα1 and VDR, the "Genetics Risk Factors for Osteoporosis: Collagen type Iα1 S/s and Vitamin D receptor B/b alleles, Reverse hybridization kit for the detection of the most important genetic risk factors for osteoporosis" was used (GenID® GmbH, D - 72479 Straßberg, Germany; Cat. No: RDB2055 12 Tests).

This kit detects the Sp1 (Ss) polymorphism in the COL Iα1 gene and the Bsm I (B/b) polymorphism in the VDR gene by two parallel polymerase chain reactions (PCR) and subsequent hybridization. Using the DNA isolated from whole blood, two PCRs were first carried out. In this way, one fragment of the COL Iα1 and one fragment of the VDR gene were amplified with specific biotin-labeled primers. The characterization of the amplified gene fragments was carried out in a hybridization reaction with sequence-specific oligonucleotide probes (SSOP) which were immobilized on nitrocellulose strips (reverse hybridization). The nitrocellulose strips had gene probes for the wild type and mutated alleles of both gene loci as well as various control zones. During hybridization, the denatured amplified DNA, mixed from both PCRs with PN-VDR and PN-COLIA, binded to the gene probes attached to the strips. A highly specific washing procedure ensured that the hybrids would only survive if the probe's sequence was 100% complementary to that of the amplified DNA. Streptavidin-coupled alkaline phosphatase binded to the hybrids of the gene probe and biotin-labeled amplified DNA. This complex then was detected by a color reaction of BCIP/NBT at the alkaline phosphatase. The band pattern was analyzed using the supplied template.

The washing and incubation steps were carried out on a horizontal shaker at 70-80 rpm in order to get optimized results. The washing procedures were very carefully carried out, and smooth, blunt plastic forceps were used in order to move the strips.

For the evaluation of the results, we used the kit-specific evaluation sheet on which the reaction zones were marked (Figure 1). After drying, the strip was laid with the bottom end line onto the template of the evaluation sheet. A reaction zone was identified if a marked position on the evaluation sheet corresponded exactly to that reaction zone on the strip. Each strip had a conjugate, specificity, and two sensitivity control zones. The conjugate and sensitivity control zones had to be completely developed during the test. If those control zones were not developed, there would be a false negative result, and, in that case, the test was repeated. If the specificity zone was developed, there was an incorrect positive result. If that occurred, the test was also repeated. Probe signals were only interpreted as positive if they were at least as intensive as the respective sensitivity control.

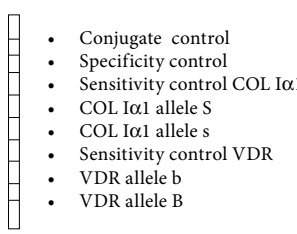

Figure 1. Control zones and gene probes on the nitrocellulose strip. VDR: vitamin D receptor.

Statistical analyses

Statistical analyses were performed using "SPSS 15.0 version for Windows" (SPSS Inc., Chicago, Illinois, USA). The chi-square test was used to confirm that the VDR gene Bsm I and COL Iα1 gene Sp1 polymorphisms were in Hardy-Weinberg equilibrium. Differences between genotype groups were examined using one-way analysis of variance (ANOVA), Student's t-test, and the chi-square test. Metric variables were given as mean±SD. Differences in mean or genotype frequencies were considered statistically significant for p values <0.05.

RESULTS

The comparisons of demographic and clinical properties separated by the groups of patients according to their VDR, Bsm I, and COL Iα1 Sp1 genotypes are shown in Table 1. The genotype frequencies in our total population for the VDR gene Bsm I site polymorphism were 13% BB, 58% Bb, and 29% bb. For the COL Iα1 gene Sp1 site polymorphism, they were 64% SS, 32% Ss, and 4% ss. The distribution of both VDR and COL Iα1 genotypes was consistent with the expected frequency by the Hardy-Weinberg equilibrium law (p>0.05). The chi-square test was used to compare the frequency of the genotypes. There were no statistically significant differences in BMD or age at menopause among the VDR and COL Iα1 genotypes (Table 1). We observed no association between VDR and COL Iα1 genotypes and BMD.

The VDR, Bsm I, and COL Iα1 Sp1 genotype distributions and frequencies among the control, osteopenia, and osteoporosis groups are shown in Table 2. The allelic and genotype distributions showed no significant difference among the osteoporotic patients, osteopenic patients, and controls. There was no statistical difference among the three groups for age, menopausal age, or BMI (p>0.05). The mean T scores of the groups were as follows: control group -0.4±0.6 (n=30), osteopenic group -1.8±0.4 (n=30), and osteoporotic group -3.2±0.5 (n=40).

The genotype frequencies of BB, Bb, and bb in the VDR gene were not statistically different among the control, osteopenic, and osteoporotic groups (p>0.05). Neither were the genotype frequencies of SS, Ss, and ss in the COL Iα1 gene found to be statistically different among the three groups (p>0.05).

We did not include eight individuals when evaluating VDR, Bsm I, and COL Iα1 Sp1 polymorphisms in terms of vertebral fracture risk because we did not have their lateral, lumbar, and thoracic spine radiographies. (Table 3). The distribution and frequencies of VDR, Bsm I, and COL Iα1 Sp1 genotypes among individuals with vertebral fracture and without fracture are shown in Table 3.

No significant difference was found between individuals with the vertebral fracture and "no fracture" groups in terms of BB, Bb, and bb genotypes

Table 1. Baseline demographic and clinical characteristics of the study group according to the VDR and COL Iα1 genotypes

Vitamin D receptor	BB			Bb			bb			
	n	%	Mean±SD	n	%	Mean±SD	n	%	Mean±SD	p
Patients (n)	13	13		58	58		29	29		
Age (years)			57±5.2			63.7±9.2			65.6±7.7	0.011
Age at menopause (years)			45.9±5.5			46.5±4.7			47±5.1	0.796
BMD lumbar spine (g/cm^2)			0.861±0.184			0.822±0.135			0.840±0.135	0.630
T score			-1.7±1.6			-2.0±1.3			-1.9±1.3	0.688
BMD femoral neck (g/cm^2)			0.750±0.126			0.727±0.152			0.707±0.113	0.637
T score			-0.9±1.2			-1.1±1.4			-1.3±1.0	0.644
COLIα1	SS			Ss			ss			
	n	%	Mean±SD	n	%	Mean±SD	n	%	Mean±SD	p
Patients (n)	64	64		32	32		4	4		
Age (years)			63.1±8.7			63.8±8.9			64.8±8.5	0.892
Age at menopause (years)			46.4±5.3			46.9±4.2			46.5±4.1	0.909
BMD lumbar spine (g/cm^2)			0.832±0.144			0.840±0.146			0.783±0.038	0.753
T score			-1.9±1.3			-1.9±1.3			-2.7±0.7	0.529
BMD femoral neck (g/cm^2)			0.716±0.136			0.742±0.149			0.709±0.037	0.681
T score			-1.2±1.2			-1.0±1.4			-1.5±0.3	0.563

VDR: Vitamin D receptor; SD: Standard deviation; BMD: Bone mineral density; COL Iα1: Collagen Iα1.

Table 2. Distribution of VDR Bsm I and COL Iα1 Sp1 genotypes among healthy controls and patient groups

	Control T score >-1			Osteopenia -2.5 <T score <-1			Osteoporosis T score <-2.5			
	n	%	Mean±SD	n	%	Mean±SD	n	%	Mean±SD	*p*
Patients (n)	30			30			40			
Age (years)			62.4±8.7			61.1±6.2			65.75±9.8	0.067
Age at menopause (years)			47.1±5.8			47.3±3.9			45.6±4.9	0.298
Genotype VDR Bsm I										0.774
BB	5	16.7		3	10		5	12.5		
Bb	15	50		20	66.7		23	57.5		
bb	10	33.3		7	23.3		12	30		
Allele frequency										0.968
B	25	41.7		26	43.2		33	41.3		
b	35	58.3		34	56.7		47	58.8		
Genotype COL Iα1 Sp1										0.320
SS	21	70		17	56.7		26	65		
Ss	9	30		12	40		11	27.5		
ss	0	0		1	3.3		3	7.5		
Allele frequency										0.489
S	51	85		46	76.7		63	78.8		
s	9	15		14	23.3		17	21.2		

SD: Standard deviation; VDR: Vitamin D receptor; BMD: Bone mineral density; COL Iα 1: Collagen Iα1.

in the VDR gene (p>0.05), nor was there a significant difference found between individuals in the vertebral fracture and "no fracture" groups in terms of COL Iα1 genotypes (p>0.05). In addition, the relationship between S and s allele frequencies, B and b allele frequencies, and vertebral fracture was analyzed, and no connection was found.

There was no significant difference between the VDR mutant genotype and the other bb genotypes and COL Iα1 mutant ss genotypes when compared with the other ss types (p>0.05). Also, there was no difference between the vertebral fracture group and the "no fracture" group when examining the VDR, Bsm I, and COL Iα1 Sp1 genotypes (p>0.05).

Additionally, the genotype frequency was compared in patients with and without a history of fracture, and no difference was found in the frequency of the VDR and COL Iα1 genotypes (p>0.05).

Finally, no statistically significant difference was found among the VDR and COL Iα1 genotypes and the BMD when compared with the control, osteopenic, and osteoporotic groups (p>0.05).

DISCUSSION

Most of the studies on the genetics of osteoporosis have been based largely on research into candidate genes relevant to bone metabolism. The VDR gene, the COL Iα1 gene, and the estrogen receptor-alpha (ER-α) gene are among those studied most frequently.[5]

The VDR and COL Iα1 gene polymorphisms have been associated with low BMD and an increased risk of osteoporotic fracture in several studies.[13,15] However, some studies and meta-analyses have shown no association between these polymorphisms and BMD or osteoporotic fractures.[9,16,17,22] Therefore, we need some additional information in order to discover the role of VDR and COL Iα1 genes in osteoporosis pathogenesis.

There have also been some studies suggesting that there was a relationship between the "bb" genotype of VDR gene and low BMD.[23,24]

Langdahl et al.[15] found that the VDR Bsm I "B" allele had a strong relationship with increased fracture risk in a case-controlled study. In some other studies, the relationship between VDR polymorphisms and fracture risk was shown to be independent from BMD.[13]

Garnero et al.,[25] found an association between VDR genotypes and fracture risk that was independent from BMD in a study on postmenopausal women. No relationship between VDR genotypes and whole body BMD values was shown in this study. They claimed that the Bsm I polymorphism had a correlation with the "BB" genotype and non-vertebral fracture;

Table 3. Distribution of VDR, Bsm I, and COL Iα1 Sp1 genotypes among individuals with and without vertebral fracture

	Vertebral fracture		Non-fracture		
	n	%	n	%	*p*
Patients (n)	18	74			
Genotype VDR Bsm I					0.103
BB	0	0	12	16.2	
Bb	10	55.6	43	58.1	
Bb	8	44.4	19	25.7	
Genotype COL Iα1 Sp1					0.462
SS	13	72.2	45	60.8	
Ss	4	22.2	27	36.5	
Ss	1	5.6	2	2.7	

VDR: Vitamin D receptor; COL Iα1: Collagen Iα1.

however, there was no evidence to prove that kind of a relationship existed.

A recent meta-analysis showed no relationship between VDR Bsm I polymorphisms and fracture risk.[26] Another recent meta-analysis published by Uitterlinden et al.[9] documented no significant association between fracture risk and BMD and VDR gene Bsm I, Apa I, Taq I polymorphisms. That study, which has been the most detailed report on the topic so far, was conducted by "The Genetic Markers for Osteoporosis" (GENOMOS) consortium.

Studies that claimed to identify the genetic determiner of BMD have produced contradicting data. In the GENOMOS study, they obtained some evidence that showed the effects of the VDR gene Cdx2 polymorphism on vertebral fracture risk and they concluded that there was a need for more study.[9]

The findings of our study were in concordance with the findings of the GENOMOS study.[9] Our study showed no significant association between the groups in terms of the VDR Bsm I polymorphism and BMD or this polymorphism and the risk for vertebral fracture in postmenopausal women. We examined the VDR mutant BB genotype and other Bb and bb genotypes and their relationship to the osteopenic and osteoporotic groups when compared with the control group. However, we could not find any statistically meaningful correlation.

In 1996, Grant et al.[27] defined the G-T polymorphism at the first base of the binding site for the transcription factor Sp1 in the first intron of the COL Iα1 gene and found a relationship between low bone density and osteoporotic fracture formation. They announced that the T allele (= s allele) was more prevalent in patients with osteoporosis than in the controls. Some other studies have shown that the GT (Ss) and TT (ss) genotypes have a correlation with low BMD and increased fracture risk.[27,28] However, Lidén et al.[22] and Hustmyer et al.[29] claimed that there was no difference. Bernad et al.[30] found no relationship between these genotypes and lumbar and femur BMD.

In the GENOMOS study published by Ralston et al.[10] in 2006, they could not find a relationship with BMD at the GG (SS) homozygote and GT (Ss) heterozygote, but they found a relationship with the TT (ss) genotype similar to what was discovered in previous studies. In those studies, it was emphasized that, unlike previous meta-analysis, there actually was a relationship with fracture, and they mentioned an increase independent from BMD at vertebral fracture risk, especially in women.

In our study, the relationship of the COL Iα1 mutant ss genotype and other Ss and SS genotypes (compared with the control group) between osteopenic and osteoporotic groups was investigated. We could not find a statistically meaningful correlation. Moreover, when we studied those genotypes for their relation to vertebral fracture risk, we also could find no statistically meaningful correlation. Ultimately, we could not find any relationship between Sp1 genotypes and BMD and vertebral fracture risk. The finding of our study was not in accordance with other studies that have shown an association with the COL Iα1 Sp1 polymorphism. The reason for the differences between the findings may stem from the relatively small number of cases and the study plan (properties of working population, vast samples, and data analysis), or it may be a result of the interaction of genetic and environmental factors.

Table 4. Vitamin D receptor Bsm I, and COL Iα1 Sp1 genotype frequencies in Turkish postmenopausal women

	This study (n=100)		Uysal et al.[18] (n=246)		Şimşek et al.[19] (n=111)	
	n	%	n	%	n	%
VDR Bsm I						
BB	13	13	42	17.1	–	–
Bb	58	58	126	51.2	–	–
bb	29	29	78	31.7	–	–
This study	40					
COL Iα1 Sp1						
SS	26	65	–	–	79	71.2
Ss	11	27.5	–	–	30	27.0
ss	3	7.5	–	–	2	1.8

VDR: Vitamin D receptor; COL Iα1: Collagen Iα1.

Although our study has preliminary data from a relatively small number of individuals, we compared the results of frequencies of polymorphisms with two other studies from Turkish postmenopausal women (Table 4). Uysal et al.[18] investigated the VDR, Bsm I, Taq I and Apa I polymorphisms in a group of postmenopausal Turkish women with and without osteoporosis. The Bsm I genotype frequencies estimated in our total study group were similar to the findings of that study. Şimsek et al.[19] evaluated the effects of HRT on BMD in a group of osteopenic, postmenopausal Turkish women with and without the COL Iα1 Sp1 binding site polymorphism. The SS and Ss frequencies estimated from our data were similar to the findings of Şimsek et al.[19] However, the ss frequency of our data was higher than that in the Şimsek et al.[19] study.

When we compared our findings with other frequencies from several other populations, we observed that the ss genotype frequency was similar to those from European populations. The ss genotype was 4% in French women, 3.3% in Dutch women, and 5.5% in Danish women.[31] The ss genotype frequency of the COL Iα1 Sp1 polymorphism in our total study group was 4%.

The most important limitation of our study was the small number of vertebral fracture cases for a genetic association study. This resulted in a less precise estimate. Another limitation of this study was that some samples of each genotype were not able to be confirmed with PCR, restriction fragment length polymorphism (RFLP), and/or sequence analysis.

In conclusion, our preliminary data couldn't show an association among the VDR gene Bsm I and COL Iα1 gene Sp1 polymorphisms and low BMD or vertebral fracture in postmenopausal Turkish women. Broadening the study with a wider number of cases would be a more suitable approach. Moreover, because the VDR and COL Iα1 genotypes show population specificity, it would be necessary to conduct extensive population studies in order to identify the genotypic endowment in Turkish society by confirming the genotypes with PCR, RFLP, and/or sequence analysis.

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REFERENCES

1. Rizzoli R, Bonjour JP, Ferrari SL. Osteoporosis, genetics and hormones. J Mol Endocrinol 2001;26:79-94.
2. Rizer MK. Osteoporosis. Prim Care 2006;33:943-51.
3. Lentle B, Worsley D. Osteoporosis redux. J Nucl Med 2006;47:1945-59.
4. Ralston SH, de Crombrugghe B. Genetic regulation of bone mass and susceptibility to osteoporosis. Genes Dev 2006;20:2492-506.
5. Liu YZ, Liu YJ, Recker RR, Deng HW. Molecular studies of identification of genes for osteoporosis: the 2002 update. J Endocrinol 2003;177:147-96.
6. Stewart TL, Ralston SH. Role of genetic factors in the pathogenesis of osteoporosis. J Endocrinol 2000;166:235-45.
7. Ozono K, Saito M, Miura D, Michigami T, Nakajima S, Ishizuka S. Analysis of the molecular mechanism for the antagonistic action of a novel 1alpha,25-dihydroxyvitamin D(3) analogue toward vitamin D receptor function. J Biol Chem 1999;274:32376-81.

8. Morrison NA, Qi JC, Tokita A, Kelly PJ, Crofts L, Nguyen TV, et al. Prediction of bone density from vitamin D receptor alleles. Nature 1994;367:284-7.
9. Uitterlinden AG, Ralston SH, Brandi ML, Carey AH, Grinberg D, Langdahl BL, et al. The association between common vitamin D receptor gene variations and osteoporosis: a participant-level meta-analysis. Ann Intern Med 2006;145:255-64.
10. Ralston SH, Uitterlinden AG, Brandi ML, Balcells S, Langdahl BL, Lips P, et al. Large-scale evidence for the effect of the COLIA1 Sp1 polymorphism on osteoporosis outcomes: the GENOMOS study. PLoS Med 2006;3:e90.
11. Grant SF, Reid DM, Blake G, Herd R, Fogelman I, Ralston SH. Reduced bone density and osteoporosis associated with a polymorphic Sp1 binding site in the collagen type I alpha 1 gene. Nat Genet 1996;14:203-5.
12. Garnero P, Borel O, Grant SF, Ralston SH, Delmas PD. Collagen Ialpha1 Sp1 polymorphism, bone mass, and bone turnover in healthy French premenopausal women: the OFELY study. J Bone Miner Res 1998;13:813-7.
13. Uitterlinden AG, Weel AE, Burger H, Fang Y, van Duijn CM, Hofman A, et al. Interaction between the vitamin D receptor gene and collagen type Ialpha1 gene in susceptibility for fracture. J Bone Miner Res 2001;16:379-85.
14. Nguyen TV, Esteban LM, White CP, Grant SF, Center JR, Gardiner EM, et al. Contribution of the collagen I alpha1 and vitamin D receptor genes to the risk of hip fracture in elderly women. J Clin Endocrinol Metab 2005;90:6575-9.
15. Langdahl BL, Gravholt CH, Brixen K, Eriksen EF. Polymorphisms in the vitamin D receptor gene and bone mass, bone turnover and osteoporotic fractures. Eur J Clin Invest 2000;30:608-17.
16. Hustmyer FG, Liu G, Johnston CC, Christian J, Peacock M. Polymorphism at an Sp1 binding site of COL1A1 and bone mineral density in premenopausal female twins and elderly fracture patients. Osteoporos Int 1999;9:346-50.
17. Ensrud KE, Stone K, Cauley JA, White C, Zmuda JM, Nguyen TV, et al. Vitamin D receptor gene polymorphisms and the risk of fractures in older women. For the Study of Osteoporotic Fractures Research Group. J Bone Miner Res 1999;14:1637-45.
18. Uysal AR, Sahin M, Gürsoy A, Güllü S. Vitamin D receptor gene polymorphism and osteoporosis in the Turkish population. Genet Test 2008;12:591-4.
19. Simsek M, Cetin Z, Bilgen T, Taskin O, Luleci G, Keser I. Effects of hormone replacement therapy on bone mineral density in Turkish patients with or without COL1A1 Sp1 binding site polymorphism. J Obstet Gynaecol Res 2008;34:73-7.
20. Brown JP, Josse RG; Scientific Advisory Council of the Osteoporosis Society of Canada. 2002 clinical practice guidelines for the diagnosis and management of osteoporosis in Canada. CMAJ 2002;167:S1-34.
21. Genant HK, Wu CY, van Kuijk C, Nevitt MC. Vertebral fracture assessment using a semiquantitative technique. J Bone Miner Res 1993;8:1137-48.
22. Lidén M, Wilén B, Ljunghall S, Melhus H. Polymorphism at the Sp 1 binding site in the collagen type I alpha 1 gene does not predict bone mineral density in postmenopausal women in sweden. Calcif Tissue Int 1998;63:293-5.
23. Houston LA, Grant SF, Reid DM, Ralston SH. Vitamin D receptor polymorphism, bone mineral density, and osteoporotic vertebral fracture: studies in a UK population. Bone 1996;18:249-52.
24. Douroudis K, Tarassi K, Ioannidis G, Giannakopoulos F, Moutsatsou P, Thalassinos N, et al. Association of vitamin D receptor gene polymorphisms with bone mineral density in postmenopausal women of Hellenic origin. Maturitas 2003;45:191-7.
25. Garnero P, Munoz F, Borel O, Sornay-Rendu E, Delmas PD. Vitamin D receptor gene polymorphisms are associated with the risk of fractures in postmenopausal women, independently of bone mineral density. J Clin Endocrinol Metab 2005;90:4829-35.
26. Fang Y, Rivadeneira F, van Meurs JB, Pols HA, Ioannidis JP, Uitterlinden AG. Vitamin D receptor gene BsmI and TaqI polymorphisms and fracture risk: a meta-analysis. Bone 2006;39:938-45.
27. Grant SF, Reid DM, Blake G, Herd R, Fogelman I, Ralston SH. Reduced bone density and osteoporosis associated with a polymorphic Sp1 binding site in the collagen type I alpha 1 gene. Nat Genet 1996;14:203-5.
28. Keen RW, Woodford-Richens KL, Grant SF, Ralston SH, Lanchbury JS, Spector TD. Association of polymorphism at the type I collagen (COL1A1) locus with reduced bone mineral density, increased fracture risk, and increased collagen turnover. Arthritis Rheum 1999;42:285-90.
29. Hustmyer FG, Liu G, Johnston CC, Christian J, Peacock M. Polymorphism at an Sp1 binding site of COL1A1 and bone mineral density in premenopausal female twins and elderly fracture patients. Osteoporos Int 1999;9:346-50.
30. Bernad M, Martinez ME, Escalona M, González ML, González C, Garcés MV, et al. Polymorphism in the type I collagen (COLIA1) gene and risk of fractures in postmenopausal women. Bone 2002;30:223-8.
31. Lei SF, Deng FY, Liu XH, Huang QR, Qin Y, Zhou Q, et al. Polymorphisms of four bone mineral density candidate genes in Chinese populations and comparison with other populations of different ethnicity. J Bone Miner Metab 2003;21:34-42.