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Evaluation of *ERα* and *VDR* gene polymorphisms in relation to bone mineral density in Turkish postmenopausal women

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Abstract It has been suggested that the estrogen receptor alpha (*ERα*) and vitamin D receptor (*VDR*) genes as possibly implicated in reduced bone mineral density (BMD) in osteoporosis. The present study investigated the relation of *ERα PvuII*/*Xba*I polymorphisms and *VDR Fok*I/*Taq*I polymorphisms with BMD in Turkish postmenopausal women. Eighty-one osteoporotic and 122 osteopenic postmenopausal women were recruited. For detection of the polymorphisms, polymerase chain reaction-restriction fragment lenght polymorphism techniques have been used. BMD was measured at the lumbar spine and hip by dual-energy X-ray absorptiometry. Distributions of *ERα* (*Pvu*II dbSNP: rs2234693, *Xba*I dbSNP: rs9340799) and *VDR* genotypes (*Fok*I dbSNP rs10735810, *Taq*I dbSNP: rs731236) were similar in study population. Although overall prevalence of osteoporosis had no association with these genotypes, the prevalence of decreased femoral neck BMD values were higher in the subjects with *ERα PvuII* "PP" and *ERα Xba*I "XX" genotypes than in those with "Pp/pp" genotypes and "xx" genotype, respectively ($P < 0.05$). Furthermore, subjects with *VDR Fok*I "FF" genotype had lower BMD values of femoral neck and total hip compared to those with "Ff" genotype ($P < 0.05$). In the logistic regression analysis, we confirmed the presence of relationships between the *VDR Fok*I "FF" genotypes, BMI $\leq$ 27.5, age $\geq$ 55 and the increased risk of femoral neck BMD below 0.8 value in postmenopausal women. The present data suggests that the *ERα PvuII*/*Xba*I and *VDR Fok*I polymorphisms may contribute to the determination of bone mineral density in Turkish postmenopausal women.

Keywords Osteoporosis · Estrogen receptor · Vitamin D receptor · Polymorphism · Bone mineral density

Abbreviations

ERα	Estrogen receptor alpha
VDR	Vitamin D receptor
BMD	Bone mineral density
PCR-RFLP	Polymerase chain reaction-restriction fragment lenght polymorphism
SNP	Single nucleotid polymorphism
$1,25(OH)_2D_3$	1α,25-dihydroxyvitamin D_3
BMI	Body mass index
HWE	Hardy–Weinberg equilibrium
DXA	Dual energy X-ray absorptiometry

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Introduction

Osteoporosis is the most prevalent metabolic bone disorder among developed countries. It is defined as a systemic skeletal disease characterized by low bone mass and microarchitectural deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to

nontraumatic fracture [1]. The increasing human life expectancy tends to increase the number of osteoporotic patients in the future [2]. Bone mineral density (BMD), the important risk factor for osteoporosis, is under strong genetic control with heritability estimates that range between 0.5 and 0.9 [3–5], although many environmental factors, such as dietary intakes, phsycial activities etc. play an important role in BMD [6]. In this regard, a large number of polymorphisms in multiple candidate genes have been investigated in various populations. Of them, estrogen receptor alpha (*ERα*) and vitamin D receptor (*VDR*) genes have been among of the most studied gene polymorphisms in the genetic regulation of BMD.

Estrogen plays an important role in maintaining normal bone turnover. *ERα* appears to be the major receptor mediating estrogen action in bone, and it has a prominent effect on the regulation of bone turnover and the maintenance of bone mass [1]. The human *ERα* gene is located on chromosome 6p25.1, comprimes eight exons, and spans more than 140 kb [1, 7]. *Pvu*II and *Xba*I single nucleotide polymorphisms (SNPs) have been identified in the *ERα* gene. These polymorphic sites are located in the first intron of the *ERα* gene, and so far their functional consequences are unknown. However, introns may contain regulatory elements. Although the association between *ERα* genotypes and the risk of osteoporosis in humans remains controversial, many studies have suggested a relation between *ERα* gene polymorphisms and BMD [8–13]. However, some studies yielded conflicting results [14–17].

The steroid hormon vitamin D, its receptor, *VDR*, and the metabolizing enzymes involved in the formation of the biologically active form of the hormone, together are major players in the vitamin D endocrine system. This system plays a crucial role in calcium and phosphate homeostasis and skeletal metabolism. Following renal production as the hormonal metabolite of vitamin D, 1α,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) functions as the ligand for *VDR*, with the hormone-receptor complex inducing calcemic and phosphatemic effects that result in normal bone mineralization and remodeling [18]. Therefore, *VDR* has been considered as an important candidate gene of osteoporosis. The human *VDR* gene is located on the long arm of chromosome 12 (12q13.11) [19], consists 11 exons that, together with intervening introns, spans approximately 75 kb [20]. A number of SNPs within the *VDR* gene have been studied including the *Fok*I polymorphism at the translation initiation site in the exon 2 of the gene and the *Taq*I polymorphism located in the exon 9 at the 3′ end of the gene. *VDR Fok*I and *Taq*I polymorphisms were related with low BMD values at the lumbar spine and femoral neck in some studies [21–28]. However, not all researchers found these associations [29, 30].

Relationships between *ERα* and *VDR* gene polymorphisms and BMD were investigated among various populations and different results were obtained. Therefore, the relationship between these polymorphisms and BMD will be well understood doing new researches. In the present study, we examined whether the *ERα* and *VDR* gene polymorphisms are associated with BMD values at various skeletal sites in Turkish postmenopausal women.

Methods

Subjects

The cohort of this study comprised 203 Turkish postmenopausal women (81 osteoporotic and 122 osteopenic), 40–78 years of age, attending the Uskudar State Hospital in Istanbul between June 2009 and March 2010. During ascertainment the World Health Organization (WHO) definitions and criteria for osteoporosis [31] were used. The patients received a detailed, standardized questionnaire including questions regarding the osteoporosis risk factors, such as family history of osteoporosis, menopausal status and age, cigarette smoking, alcohol consumption, medication use and other medical conditions. Only patients with a clinical diagnosis of osteoporosis and osteopenia were recruited. Exclusion criteria included conditions, diseases, and/or treatments known to interfere with bone metabolism, such as malignancies, endocrinologic disorders (hypo- and hyperparathyroidism, hyperthyroidism, Cushing's syndrome), severe liver or gastroenteral diseases, skeletal diseases (Paget's disease, osteogenesis imperfecta, osteomalacia and rheumatoid arthritis) and current pharmacological treatment with corticosteroids, anabolic androgenic steroids, estrogens or estrogen-related molecules, anticonvulsants before enrollment. None of the participants consumed alcohol. Menopause was defined as amenorrhoea of at least one year duration. The study protocol was approved by the Local Ethical Commitee of Istanbul University, Medical Faculty (Protocol No: 2006/2145) and written, informed consent was obtained from each participant prior to giving their blood sample.

BMD measurement

BMD was measured at the lumbar spine (L_1–L_4) and hip (femoral neck and total hip) by dual energy X-ray absorptiometry (DXA; Lunar DPX (GE Lunar Corporation, Madison, WI, USA).

Genotyping

Blood specimens were collected in tubes containing EDTA, and DNA samples were extracted from whole

blood with salting out procedure [32]. To detect the *Pvu*II and *Xba*I polymorphisms of the *ERα* gene, we used polymerase chain reaction-restriction fragment lenght polymorphism (PCR-RFLP) protocols performed by van Meurs et al. [33] and Onland-Moret et al. [34]. The primers (MBI Fermentas, Lithuania) used for PCR to amplify *ERα* gene fragments were forward 5′-GATATCCAGGGTTATG TGGCA-3′ and reverse 5′-AGGTGTTGCCTATTA-TATTAACCTTGA-3′ for the both *Pvu*II (rs2234693) and *Xba*I (rs9340799) restriction sites. To amplify *VDR* gene fragments, the primers were forward 5′-AGCTGGCC CTGGCACTGACTCTGCTCT-3′ and reverse 5′-ATG-GAAACACCTTGCTTCTTCTCCCTC-3′ for the *Fok*I restriction site (rs10735810), and forward 5′-CAGAGC ATGGACAGGGAGCAAG-3′ and reverse 5′-GCAA CTCCTCATGGGCTGAGGTCTCA-3′ for the *Taq*I restriction site (rs731236). PCR reactions were carried out in a final volume at 25 µl containing 10× reaction buffer (KCl), 1 mM of each nucleotide (dATP, dCTP, dGTP and dTTP) (MBI Fermentas, Lithuania), 1.5 mmol/l $MgCl_2$, 25 picomolar of each primer, 0.3 U Taq DNA polymerase (MBI Fermentas, Lithuania) and 50 ng DNA. Thermal profiles for amplification of *ERα* gene fragments consisted of an initial denaturating step of 3 min at 95°C followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 57°C for 1 min and extension at 72°C for 1 min with a final extension step for 5 min at 72°C. *VDR Fok*I genotypes was determined as described by Gross et al. [21] with slight modifications. The conditions for *VDR Fok*I gene fragments amplification consisted of an initial denaturating step of 3 min at 94°C followed by 35 cycles of denaturation at 94°C for 45 s, annealing at 60°C for 45 s, and extension at 72°C for 45 s with a final extension step for 5 min at 72°C. For detection of *VDR Taq*I RFLPs, we used the protocol performed by Curran et al. [35]. PCR conditions were 94°C initial denaturation for 4 min followed by 5 cycles of 94°C for 45 s, 64°C for 60 s and 72°C for 2 min; and a further 25 cycles of 94°C for 30 s, 64°C for 30 s and 72°C for 45 s. *ERα PvuII/XbaI and VDR FokI/TaqI* genotypes were amplified specific polymerase chain reaction product in a DNA thermal cycler (GeneAmp 9700 PCR System; Applied Biosystems, CA, USA). PCR products were then digested with restriction endonucleases (*Pvu*II and *Xba*I for *ERα* and *Fok*I and *Taq*I for *VDR*, MBI Fermentas, Lithuania). Fragments were seperated by agarose gel electrophoresis and stained with ethidium bromide to identify base pair changes. Genotypes for *Pvu*II polymorphism were classified as PP, Pp and pp; those for *Xba*I polymorphism were classified as XX, Xx and xx; genotypes for *Fok*I and *Taq*I were categorized as FF, Ff and ff and TT, Tt and tt, respectively (uppercase letters represent absence and lowercase letters represent the presence of restriction sites).

Statistical analysis

The analyses were performed using SPSS software for Windows, version 13.0. Continous variables are presented as means (±standard deviation—SD), while categorical variables are presented as frequencies. Hardy–Weinberg equilibrium (HWE) was tested using standard chi-square (χ^2) test comparing genotype frequencies. The association of genotypes with BMD were compared by analysis of variance (ANOVA). We measured the independent contribution of the *ERα* and *VDR* SNPs to the femoral neck BMD values using a stepwise logistic regression analysis with observed risk factors for osteoporosis in this study entered as covariates and odds ratios (OR) were calculated in the model. In order to determine the relative risks, odds ratio and 95% confidence intervals were used. A *P* value of less than 0.05 was considered to be statistically significant.

Results

ERα and *VDR* genotypes and allele distribution

The demographic characteristics and BMD status of the study population were presented in Table 1. The genotype frequencies of the subjects with *ERα PvuII/XbaI* and *VDR FokI/TaqI* polymorhisms were shown in Table 2. The distribution of the genotypes was consistent with the Hardy–Weinberg equilibrium in study population ($P > 0.05$).

Association of *ERα* and *VDR* polymorphisms with the BMD values

The association of *ERα* and *VDR* genotypes with the BMD values were shown in Table 3. Subjects with *ERα PvuII*

Table 1 Characteristics and BMD values of the study population

	Study population
Number	203
Age	59.45 ± 7.71
Age of menopause	46.76 ± 5.03
BMI (kg/m^2)	29.33 ± 4.74
Family history of osteoporosis (%)	42.1
Smoking (%)	8.9
Lumbar spine (L_1–L_4) BMD (g/cm^2)	0.93 ± 0.12
Lumbar spine (L_1–L_4) *T* score	−1.69 ± 0.96
Femoral neck BMD (g/cm^2)	0.81 ± 0.09
Femoral neck *T* score	−1.26 ± 0.79
Total hip BMD (g/cm^2)	0.87 ± 0.10
Total hip *T* score	−1.07 ± 0.84

Values are means ± SD except where noted

BMI body mass index, *BMD* bone mineral density

Table 2 The frequencies of *ERα* and *VDR* genotypes in study population

Genotypes	Study population *n* (%)	HWE
ERα PvuII		
PP	45 (22.4)	NS
Pp	105 (52.2)	
pp	51 (25.4)	
ERα XbaI		
XX	42 (23.1)	NS
Xx	96 (52.7)	
xx	44 (24.2)	
VDR FokI		
FF	100 (49.3)	NS
Ff	91 (44.8)	
ff	12 (5.9)	
VDR TaqI		
TT	83 (41.7)	NS
Tt	88 (44.2)	
tt	28 (14.1)	

ERα estrogen receptor α, *VDR* vitamin D receptor, *HWE* Hardy–Weinberg equilibrium, *NS* non-significant

"PP" genotype had lower BMD values of femoral neck compared to those with "Pp" ($P = 0.019$) and "pp"genotypes ($P = 0.011$) and had lower BMD values of total hip compared to the subjects with "pp" genotype ($P = 0.010$). Furthermore, "PP" genotype was associated with lower femoral neck *T* score compared to "Pp" genotype ($P = 0.039$). Subjects with *ERα XbaI* "XX" genotype had lower femoral neck BMD values than those with "xx" genotype, which closely tied to statistically significance ($P = 0.056$).

A statistically significant difference in BMD was also found among *VDR FokI* genotypes in study group. When compared with "Ff" heterozygotes, "FF" homozygotes had lower BMD values of femoral neck ($P = 0.050$) and total hip ($P = 0.027$). No significant effects of *VDR TaqI* genotypes on BMD values was found at any site in the study group ($P > 0.05$) (Table 3).

Comparison of *ERα* and *VDR* polymorphisms and osteoporosis risk factors according to the femoral neck BMD values

To assess whether these polymorphisms and osteoporosis risk factors had any effect on femoral neck BMD values, we divided the subjects into two groups according to ≤0.8 and >0.8 femoral neck BMD values among the genotype groups and osteoporotic risk factors such as low BMI, smoking, family history of osteoporosis and age (Table 4). In the ≤0.8 femoral neck BMD group, "PP" genotype frequency was found to be higher than that in the >0.8 femoral neck BMD group ($P = 0.001$, $\chi^2 = 11.403$), whereas "pp" genotype frequency was found to be higher in the >0.8 femoral neck BMD group compared to that in the ≤0.8 femoral neck BMD group ($P = 0.016$, $\chi^2 = 5.790$).

"XX" genotype frequency was higher in the ≤0.8 femoral neck BMD group than that in the >0.8 femoral neck BMD group ($P = 0.047$, $\chi^2 = 3.941$) and "xx" genotype frequency was higher in the >0.8 group compared to that in the ≤0.8 femoral neck BMD group ($P = 0.012$, $\chi^2 = 6.317$).

"FF" genotype frequency was higher in the ≤0.8 group than that in the >0.8 femoral neck BMD group ($P = 0.022$, $\chi^2 = 5.209$) and "Ff" genotype frequency was higher in the >0.8 femoral neck BMD group than that in the ≤0.8 femoral neck BMD group ($P = 0.014$, $\chi^2 = 6.031$).

The frequency of the subjects with BMI ≤ 27.5 was higher in the ≤0.8 femoral neck BMD group than that in the >0.8 femoral neck BMD group ($P = 0.001$, $\chi^2 = 10.315$). The frequency of the subjects aged 55 or over was higher in the ≤0.8 femoral neck BMD group than that in the >0.8 femoral neck BMD group ($P = 0.006$, $\chi^2 = 7.486$). The mean values of age were higher in the ≤0.8 femoral neck BMD group than those in >0.8 femoral neck BMD group ($P < 0.001$). The mean values of BMI were lower in the ≤0.8 femoral neck BMD group than those in the >0.8 femoral neck BMD group ($P = 0.015$).

There were no significant differences in *VDR TaqI* genotypes, smoking, family history of osteoporosis and age of menopause ($P > 0.05$).

Furthermore, we evaluated the risk of low femoral neck BMD using logistic regression analysis (Table 5). Femoral neck BMD values were divided into two groups as: ≤0.8 and >0.8. Femoral neck BMD value was included as the dependent variable. The *ERα PvuII* "PP", *ERα XbaI* "XX", *VDR FokI* "FF" genotypes, BMI ≤ 27.5 and age ≥ 55 were included in the model as categorical variables. There were significant differences in the risk of low femoral neck BMD values among subjects with *ERα PvuII* "PP" genotype ($P = 0.001$), *ERα XbaI* "XX" genotype ($P = 0.047$), *VDR FokI* "FF" genotype ($P = 0.022$), BMI ≤ 27.5 ($P = 0.001$), and subjects aged 55 or over (age ≥ 55) ($P = 0.006$) (Table 4). In the multivariate logistic regression analysis, *VDR FokI* "FF" genotype, BMI ≤ 27.5 and age ≥ 55 remained significant after adjustment for *ERα PvuII*/*XbaI*, *VDR FokI*, BMI and aged 55 or over (Table 5).

Discussion

Osteoporosis is characterized by a decrease both in bone mass and in bone mineral density. Osteoporosis risk may be modulated by a large number of genetic markers beyond *ERα*, including polymorphisms of *VDR* gene, and several other candidate genes [36]. Although the clinical impact of

Table 3 Association of *ERα* and *VDR* genotypes with BMD values in study population

	ERα PvuII genotypes			*ERα XbaI* genotypes		
	PP	Pp	pp	XX	Xx	xx
L_1–L_4						
BMD	0.93 ± 0.13	0.93 ± 0.11	0.93 ± 0.09	0.95 ± 0.12	0.92 ± 0.12	0.93 ± 0.10
T score	−1.66 ± 1.05	−1.69 ± 0.97	−1.72 ± 0.86	−1.51 ± 0.96	−1.73 ± 1.01	−1.73 ± 0.92
Femoral neck						
BMD	**0.77 ± 0.08**$^{\infty}$	0.81 ± 0.09	0.82 ± 0.09	0.79 ± 0.09	0.80 ± 0.08	0.83 ± 0.10
T score	**−1.49 ± 0.70**$^{\epsilon}$	−1.21 ± 0.78	−1.22 ± 0.80	−1.37 ± 0.72	−1.32 ± 0.70	−1.18 ± 0.91
Total hip						
BMD	**0.84 ± 0.09**$^{\&}$	0.87 ± 0.09	0.89 ± 0.10	0.85 ± 0.09	0.86 ± 0.09	0.89 ± 0.11
T score	−1.26 ± 0.78	−1.07 ± 0.80	−0.94 ± 0.91	−1.14 ± 0.76	−1.14 ± 0.77	−0.94 ± 1.00
	VDR FokI genotypes			*VDR TaqI* genotypes		
	FF	Ff	ff	TT	Tt	tt
L_1–L_4						
BMD	0.93 ± 0.12	0.92 ± 0.10	0.95 ± 0.12	0.93 ± 0.11	0.92 ± 0.12	0.95 ± 0.11
T score	−1.67 ± 1.04	−1.73 ± 0.85	−1.49 ± 1.02	−1.75 ± 0.93	−1.70 ± 0.98	−1.54 ± 1.00
Femoral neck						
BMD	**0.79 ± 0.09**$^{\Phi}$	0.82 ± 0.09	0.84 ± 0.08	0.81 ± 0.09	0.81 ± 0.10	0.81 ± 0.08
T score	−1.38 ± 0.80	−1.18 ± 0.77	−0.96 ± 0.70	−1.22 ± 0.77	−1.27 ± 0.80	−1.37 ± 0.79
Total hip						
BMD	**0.85 ± 0.10**$^{\Phi}$	0.88 ± 0.09	0.90 ± 0.10	0.87 ± 0.10	0.86 ± 0.10	0.88 ± 0.08
T score	−1.20 ± 0.86	−0.97 ± 0.80	−0.80 ± 0.87	−1.07 ± 0.85	−1.10 ± 0.87	−0.96 ± 0.71

The results are shown as mean ± SD

ERα estrogen receptor α, *VDR* vitamin D receptor, *BMD* bone mineral density

For *bold values*, $^{\infty}$ $P < 0.05$, PP vs. Pp and pp; $^{\epsilon}$ $P < 0.05$, PP vs. Pp; $^{\&}$ $P < 0.01$, PP vs. pp; $^{\Phi}$ $P < 0.05$, FF vs. Ff

each implicated gene polymorphism is modest, the cumulative effect may be large. Several studies has suggested that the *Pvu*II and *Xba*I polymorphisms of the *ERα* gene and *Fok*I and *Taq*I polymorphisms of the *VDR* gene may correlate with BMD, and this hypothesis has led to a great deal of interest and controversy.

In this study population-based study of postmenopausal Turkish women, we found that the *ERα PvuII*/*XbaI* and *VDR FokI* polymorphisms had effects on BMD values, while *VDR TaqI* polymorphism didn't have any effect in the study group by chi-square analysis (Tables 3, 4). Similar to our results, Ivanova et al. [11] found that lower BMD values were found in "PP" and "XX" indiviuals and higher ones in "pp" and "xx" individuals from Bulgarian population. Yamada et al. [12] found that BMD for the femoral neck was significantly lower in elderly Japanese women aged 60 years or over with the "PP" genotype and "XX" genotype than in those with the "pp" and "xx" genotype. In Finnish women, Salmen et al. [37] concluded that subjects with the "PP" and "Pp" may have a greater risk of relatively fast bone loss after menopause than those with the "pp" genotype. In contrast to our results, both the femoral neck and Ward's triangle BMD values in women with the "Pp" genotype were found to be significantly higher than those in women with the "pp" genotype in postmenopausal Korean women [13]. A significantly higher relative risk was associated with "xx" genotype rather than "XX" genotype in postmenopausal Indian women [38]. No association between *ERα PvuII* and *XbaI* genotypes and BMD were detected in Argentine postmenopausal women by Perez et al. [2]. Similar results were previously found in postmenopausal Danish women [14] and in postmenopausal Italian women [15]. A lack of association between *ERα* gene polymorphisms with baseline BMD was also found in elderly Caucasian women [39]. In recent studies with Turkish postmenopausal women, Erdogan et al. [40] have found that the average lumbar vertebrae BMD value of women with "PP" genotype was significantly higher than with "pp" genotype, and it was noted that there was no relation between *XbaI* polymorphism and BMD values and genotype frequencies. Durusu Tanriover et al. [41] could not observe a significant effect of *ERα* polymorphism on BMD or osteoporosis risk. In the present study, *ERα PvuII* "PP" and *ERα XbaI* "XX" genotypes were associated with low BMD values of femoral neck by

Table 4 Comparison of *ERα* and *VDR* genotypes and osteoporosis risk factors according to the femoral neck BMD values

Genotypes	Femoral neck BMD		*P* value (chi-square)	OR (95% CI)
	≤0.8 (*n* %)	>0.8 (*n* %)		
ERα PvuII				
PP	30 (68.2)	14 (31.8)	0.001 (11.403)	3.305 (1.61–6.74)
Pp	45 (43.3)	59 (56.7)	NS	
pp	14 (30.4)	32 (69.6)	0.016 (5.790)	0.426 (0.21–0.86)
ERα XbaI				
XX	25 (61.0)	16 (39.0)	0.047 (3.941)	2.047 (1.00–4.18)
Xx	46 (48.9)	48 (51.1)	NS	
xx	12 (30.0)	28 (70.0)	0.012 (6.317)	0.386 (0.18–0.82)
VDR FokI				
FF	52 (53.6)	45 (46.4)	0.022 (5.209)	1.936 (1.09–3.42)
Ff	31 (35.6)	56 (64.4)	0.014 (6.031)	0.487 (0.27–0.86)
ff	6 (50.0)	6 (50.0)	NS	
VDR TaqI				
TT	31 (38.3)	50 (61.7)	NS	
Tt	41 (48.2)	44 (51.8)	NS	
tt	15 (57.7)	11 (42.3)	NS	
BMI ≤ 27.5 (*n* %)	43 (60.6)	28 (39.4)	0.001 (10.315)	2.637 (1.45–4.80)
Smoking	8 (47.1)	9 (52.9)	NS	
Family history of osteoporosis	38 (46.3)	44 (53.7)	NS	
Age ≥ 55	73 (51.4)	69 (48.6)	0.006 (7.486)	2.51 (1.28–4.91)
Age (*n*, *X* ± SD)	89 (61.78 ± 7.58)	107 (57.41 ± 6.95)	0.000	
Age of menopause (*n*, *X* ± SD)	82 (47.01 ± 4.93)	97 (46.6 ± 5.14)	NS	
BMI (*n*, *X* ± SD)	89 (28.40 ± 4.41)	107 (30.05 ± 4.89)	0.015	

ERα estrogen receptor α, *VDR* vitamin D receptor, *BMD* bone mineral density, *BMI* body mass index, *OR* odds ratio, *CI* confidence interval
Femoral neck BMD values were divided into two groups as: ≤0.8 and >0.8

Table 5 Logistic regression analysis for the association between *ERα PvuII* "PP", *ERα XbaI* "XX", *VDR FokI* "FF" genotypes, BMI ≤ 27.5, age ≥ 55 and low femoral neck BMD risk in the study group (level of significance: $P < 0.05$)

	P value	Odds ratio	95% CI for OR
ERα "PP" genotype	0.188	0.437	0.128–1.497
ERα "XX" genotype	0.852	0.889	0.257–3.069
VDR "FF" genotype	0.025	0.478	0.251–0.913
BMI ≤ 27.5	0.003	0.353	0.176–0.705
Age ≥ 55	0.012	0.379	0.178–0.807

ERα estrogen receptor α, *VDR* vitamin D receptor, *OR* odds ratio, *CI* confidence interval

chi-square analysis. Hovewer, we could not find any significant association between *ERα PvuII* "PP" and *XbaI* "XX" genotypes and BMD values, in the multivariate logistic regression analysis.

In the present study, we found that *VDR* "FF" was associated with low femoral neck and total hip BMD values. In contrast to our results, in Mexican-American postmenopausal women, Gross et al. [21], Mitra et al. [23], Falchetti et al. [24] and Zajickova et al. [25] found that "ff" subjects were related with decreased BMD at the lumbar spine and increased rate of bone loss at the hip. Both lumbar and femoral BMD were observed to be highest in "FF" homozygotes by Vidal et al. [22] in postmenopausal Maltase women. On the other hand, MacDonald et al. [29] and Langdahl et al. [26] could not find any relation between BMD values and *VDR FokI* genotypes. Conflicting results may be related to differences in ethnic background, sample size and frequency and environmental factors.

The average BMD of the subjects with *VDR* "TT" and "Tt" genotypes was found significantly higher at the spine and hip than those with "tt" genotypes in postmenopausal Indian women [23]. Morita et al. [28] found in their study that in premenopausal women from Japan, bone loss at the lumbar spine in the subjects with "tt" genotype was significantly greater than that of subjects with "Tt" or "TT". Langdahl et al. [26] concluded that the BMD of the intertrochanteric region was higher in individuals with the "TT" genotype compared to "Tt" and "tt" genotypes. In contrast, "TT" genotype and "T" allele were found to have

high risk for osteoporosis by Douroudis et al. [27] in postmenopausal women of Hellenic origin. Higher prevalence of "TT" genotype was found in osteopenic and osteoporotic Polish women with lower BMD value and higher prevalence of "T" allele in these both groups was also observed by Seremak-Mrozikiewicz et al. [42]. However, some studies have not been able to show an association between BMD and *VDR TaqI* genotypes [25, 29]. Duman et al. [43] found that the osteoporotic group with the "TT" genotype had significantly higher femoral neck, trochanter, L_1–L_4 and Ward's BMD values respect to the "tt" genotype in Turkish postmenopausal women. Durusu Tanriover et al. [41] found the frequency of the subjects with "Tt" genotype was higher in the osteoporotic group compared to the control group, however, this difference did not reach statistical significance. Likewise, we found that the subjects with "Tt" genotype had lower BMD values of lumbar spine and total hip than "TT and "tt" genotypes, however these differences weren't statistical significant, either.

In the present study, the distribution of *ERα PvuII*/*XbaI* and *VDR FokI*/*TaqI* genotype frequencies were in accordance with previous Turkish population studies [40, 41, 43–45] and some other population studies [10, 11, 14, 21–30, 39] while there were differences in some [2, 12, 13, 37, 46].

The main limitation in this report is relatively small study population. We believe that a further multi-center study with a higher number of subjects with the contributions of previous studies may be necessary to conclude with greater certainty of the association between *ERα PvuII*/*XbaI* and *VDR FokI*/*TaqI* polymorphisms and predisposition to osteoporosis and low BMD status.

Our study demonstrated that *PvuII*/*XbaI* polymorphisms of the *ERα* gene and *FokI* polymorphism of the *VDR* gene may contribute to decreased bone mineral density in Turkish postmenopausal women, whereas *VDR TaqI* polymorphisms were not significantly related to bone mineral density at any skeletal sites.

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