Association of Vitamin D Receptor (FokI and BsmI) Gene Polymorphism with Bone Mineral Density and Their Effect on 25-Hydroxyvitamin D Level in North Indian Postmenopausal Women with Osteoporosis

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Abstract Osteoporosis is a systemic disease with a strong genetic component. Vitamin D receptor (VDR) has been suggested as a candidate gene for osteoporosis. Therefore the present study was aimed to investigate the pattern of allelic variants of VDR gene polymorphism (FokI and BsmI), its influence on vitamin D levels and bone mineral density (BMD) in North Indian postmenopausal women with osteoporosis for possible genetic association. 254 postmenopausal osteoporotic women and 254 postmenopausal non osteoporotic women were included in the study. VDR FokI and BsmI gene polymorphism gene were assessed by the PCR-RFLP method. Serum 25-hydroxyvitamin D was measured by the ELISA. BMD at the L₁–L₄ lumbar spine, hip, forearm and femoral neck was assessed by dual energy X-ray absorptiometry. The average BMD at spine and hip in postmenopausal women with bb and spine, hip, femoral neck and forearm with ff genotype had significantly low BMD. The frequency of ff genotype and f allele was significantly higher in postmenopausal osteoporotic women when compared with postmenopausal non osteoporotic women. However, no significant

association was found between the genotypes and vitamin D levels. Our study reveals that VDR gene FokI and BsmI polymorphism is significantly associated with low bone mineral density. Therefore the ff genotype and f allele of VDR FokI gene may be used as an important risk factor for osteoporosis.

Keywords Vitamin D receptor · Bone mineral density · Osteoporosis · Polymorphism · Postmenopausal women

Introduction

Osteoporosis is defined as a skeletal disorder characterized by compromised bone strength predisposing a person to an increased risk of fracture [1]. Osteoporosis is the most frequent metabolic bone disorder in the world, disturbing one in three women and one in eight men in elderly age [2]. In India expert groups suggested that the number of osteoporosis patients at approximately 26 million (2003 figures) with the numbers anticipated to increase to 36 million by 2013 [3]. Bone mineral density (BMD) is the most important phenotype and the key determinant for screening of osteoporosis and determining fracture risk. Twin and family studies, showed the heritability of BMD can attain 80% of bone mass [4]. Peak bone mass is attained in early adult life but decreased in postmenopausal women due to decline in production of estrogen, with effects on bone and intestinal and renal calcium absorption [5]. A number of genetic association studies have confirmed the relation between reduced bone mineral density and candidate gene polymorphisms and increased the risk of fracture [6–8]. Study reported that 25(OH) Vitamin D α hydroxylase enzyme activity decreased to convert 25-OH Vitamin D to 1, 25, (OH)₂ Vitamin D (potent Vitamin D)

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after menopause this may be due to deficiency of estrogen [9].

Vitamin D is most important factor for the bone metabolism mediated by the action of vitamin D receptor (*VDR*), is a member of nuclear receptor of transcription factors [10, 11]. *VDR* regulates the transcription process and expression of involved gene in uptake of calcium and formation of bone such as osteocalcin and calcium binding proteins [12]. The gene encoding for *VDR* is considered as a candidate gene for gene regulation of bone metabolism and it is situated at chromosome 12 cen-q12 and its contain 12 exon and spans approximately 75 kb of genomic DNA [13, 14]. The allelic variants of *VDR* gene is *Apal* (allele A/a C-A), *BsmI* (allele B/b G-A), *FokI* (allele F/f C-T) and *TaqI* (allele T/t T-C) restriction endonucleases, have been associated with BMD [15–18], and in many studies suggested bone loss in elderly ages [19, 20]. A study has been carried out in Maharastra (India) by Mitra et al. [21] shown an association between BMD at spine and hip with *VDR* gene polymorphism. To the best of our knowledge, no other study specifically genetic association of *VDR* gene with BMD in postmenopausal women have been conducted in North India (Uttar Pradesh). Therefore, the present study was carried out to investigate the pattern of allelic variants of *VDR* gene polymorphism (*FokI* and *BsmI*) with BMD in North Indian postmenopausal women with osteoporosis for possible genetic association. Further, 25-hydroxyvitamin D level were measured and correlated with *FokI* and *BsmI* gene polymorphism and BMD in postmenopausal women.

Materials and Methods

This is a case-control study. In present study, we are recruited two hundred and fifty four (254) postmenopausal osteoporotic women (age 56.12 ± 7.00) and two hundred and fifty four (254) age matched postmenopausal non osteoporotic women (55.11 ± 5.66) who attended OPD at Department of Rheumatology KG Medical University, Lucknow, from June 2011 to June 2014. All the women were without spontaneous menses for at least 1 year. Before entering this study, a questionnaire concerning health condition and medical history was taken with informed consent from every subject. In this study exclusion criteria included hormone replacement therapy, secondary osteoporosis, steroid therapy, endocrinal disorders, diabetes mellitus, Paget's disease, osteogenesis imperfecta, inflammatory arthritis, tuberculosis, cancer liver, kidney disease and use of vitamins, minerals and antioxidants. All the subjects underwent BMD measurements by dual energy X-ray absorptiometry. On the basis of the *T*-score of DEXA, the subjects were classified as osteoporotic or normal according to WHO classification of osteoporosis

after samples taken. The study protocol was approved by the institutional ethics committee of King George's Medical University (KGMU) Lucknow, India. The sample size of the study was calculated using PASS Software. The sample size was 254 samples for each group (subjects as well as control group). The sample size was calculated as per the given formula:

$$\begin{aligned} \text{Type 1 error } (\alpha) &= 0.05, \text{ Power of the test } (1 - \beta) \\ &= 0.9 \text{ (means 90\%)} \end{aligned}$$

BMD Measurements

The BMD (g/cm^2) was measured by dual energy X-ray absorptiometry (Lunar Prodigy, Madison, WI, USA) at lumbar spine L₁–L₄, femoral neck, hip forearm left skeletal site. Percent coefficient of variance ranged from 0.5 to 1.1% depending on the measurement site. According to World Health Organization criteria, the normal bone mass defined as BMD measurements at or above -1 standard deviation (SD) from the optimal peak bone density (*T*-score) of healthy young adult of the same sex. BMD measurement at or below -2.5 SD from the optimal peak bone density of healthy young adult of the same sex was osteoporotic [22].

Estimation of 25-OH Vitamin D

Serum level of total 25-hydroxyvitamin D (25-OH-VitD) were evaluated using standard commercially available ELISA Kit (MicroVUE Bone Health, Quidel, San Diego, CA, USA). The tests were conducted in duplicate and performed according to manufacturer protocol. Inter assay variation of vitamin D was between 4.3 and 4.7% and intra assay variation was 2.7–5.7%. The detection limit of the assay was 0–133 ng/ml.

VDR Genotyping

Peripheral blood for genotyping was collected in EDTA vials and DNA was extracted by using the salting-out method. DNA amplification for PCR (Applied Biosystems, Foster City, USA) analysis of *VDR* (*FokI*) gene polymorphism in exon 2 was determined by using the primers: F-5'ATGGAAACACCTTGCTTCTTCTCCCT3' and R5'AGCTGGCCCTGGCACTGACTCTGGCTCT3' producing and 265 bp fragment. The final volume of 25 μl containing 1.2 μl genomic DNA, 1.2 μl of forward and reverse primers (10 pmol conc.), 0.4 μl of 10 mM of each dNTP's and 3 unit of *Taq* (Applied Biosystems, Foster City, USA) polymerase were used to obtain PCR product. The PCR protocol was: 94 °C for 5 min followed by 30 cycles of 94 °C for 45 s 62 °C for 45 s 72 °C for 45 s and final extension at 72 °C for 7 min. After, amplification of

PCR products was digested with FokI restriction endonuclease (New England Biolabs Inc, Ipswich, MA, USA) at overnight at 37 °C. Then, 10 µl of the products were loaded onto 3% agarose gel containing ethidium bromide for electrophoresis. The presence of the restriction site, that generates two fragments of 196 and 69 bp, was indicated with f, while its absence, resulting in a single uncut 265 bp fragment, was indicated with F. Subjects were scored as ff homozygous, Ff heterozygous and FF homozygous according to the digestion pattern. Detection of the *BsmI* (*VDR* gene) site in intron 8 was performed by PCR amplification of a region carrying the *BsmI* site with primers in exon7 (Forward primer) 5'/CAACCAAGACTACAAGTACCGCGTCAGTGA-3' and intron 8 (Reverse primer)- 5'-AACCAGCGGAA-GAGGTCAAGGG-3' producing an 825-basepair (bp) fragment. To PCR products were generated in the final volume of 25 µl containing 1.2 µl genomic DNA, .4 µl of forward and reverse primers (10 pmol conc.), 0.5 µl of 10 mM of each dNTP's and 5 unit of *Taq* polymerase was used to obtain PCR product. The PCR protocol was: 94 °C for 5 min followed by 30 cycles of 94 °C for 45 s 62 °C for 45 s 72 °C for 45 s and final extension at 72 °C for 7 min. After, amplification PCR products were digested with *BsmI* (New England Biolabs Inc, Ipswich, MA, USA) restriction endonuclease at 65 °C for 1 h. Digested products were electrophoresed in a 2% agarose gel containing ethidium bromide. The presence of the *BsmI* restriction site produced 175 and 650 bp fragments, whereas the absence of this site yields one band of 825 bp fragment.

Statistical Analysis

Student's *t*-test was used to compare the anthropometric characteristics between patients and control. Shapiro–Wilks test was used to determine the parametric or non-parametric distribution of data. Student's *t*-test was used to compare the parametric variable and Mann–Whitney *U* test was used for the comparison of nonparametric variables. The association of genotype with BMD was evaluated by analysis of variance (ANOVA). BMD measurements were expressed as mean $\pm$ S.D. The analysis was carried out by using statistical package Graph Pad Prism (version 4.0). Chi square (χ^2) test or Fisher exact test (using approximation of Woolf) was used to compare the frequency distribution of genotypes and alleles between patients and control population. Association between the genotypes and osteoporosis was examined by using odds ratio (OR) with 95% confidence interval (CI) and χ^2 analysis using Graph Pad InStat version 3.06 (v3.6, Graphpad Software, Inc. San Diego, CA 92121 USA). The synergistic effect of different combinations of two SNPs (*BsmI* + *FokI*) on BMD and Vitamin D levels among study population was analyzed by one way

ANOVA using Tukey multiple comparison test. A *p*-value of < 0.05 was considered significant. Statistica version 6.0 was used for the data analysis. Hardy–Weinberg equilibrium was determined by Pearson's χ^2 goodness-of-fit tests.

Results

The baseline characteristics of 508 postmenopausal North Indian women (254 osteoporotic patients and 254 postmenopausal non osteoporotic women) are presented in Table 1. BMD of different skeletal sites were significantly ($p < 0.001$) low in osteoporotic women as compared to postmenopausal non osteoporotic women, however, BMI were significantly high in osteoporotic patients when compared with postmenopausal non osteoporotic women. There were no statistically significant difference found in age and year since menopause. Vitamin D levels were also significantly low in the osteoporotic patients in comparison of postmenopausal non osteoporotic women ($p < 0.001$).

The frequency of *VDR* (*BsmI* and *FokI*) genotypes and average BMD at lumbar spine (L₁–L₄), hip, femoral neck and forearm of 508 postmenopausal North Indian women have been presented in Table 2. The genotypes frequencies of BB, Bb and bb in postmenopausal women were 21, 57 and 22% of *BsmI* and genotypes frequency of *FokI* are FF, Ff and ff were 62.40, 33.86 and 3.74%, respectively. The average BMD of the subjects with bb genotype were found significantly lower at lumbar spine and Hip ($p < 0.006$ and $p < 0.010$) than those with BB genotype. ff genotype was also found significantly lower BMD at lumbar spine, hip and femoral neck than FF genotype ($p < 0.005$, $p < 0.035$ and 0.002, respectively). However, the differences of BMD of the subject with Bb and Ff genotypes as compared to BB and FF genotypes were not found statistically significant. The age and year since menopause were significantly higher with bb genotypes ($p < 0.044$ and $p < 0.043$) and level of vitamin D did not differ among BB, Bb, bb and FF, Ff, ff respectively ($p < 0.243$, $p < 0.637$ and $p < 0.688$, $p < 0.496$).

The distribution of genotypes and alleles of *VDR* (*BsmI* and *FokI*) gene polymorphism in postmenopausal osteoporotic women (Patients) and postmenopausal non osteoporotic women (controls) is shown in Table 3. In *BsmI* polymorphism the frequency of genotypes of BB, Bb and bb in patients were 21.26, 53.94 and 24.80%, respectively. While in controls their frequencies were 21.26, 59.84 and 18.90%, respectively. Regarding the *FokI* polymorphism the frequencies of FF, Ff and ff genotypes in patients were 58.27, 36.22 and 5.51%, respectively while their frequency in control were 66.54, 31.49, 1.97%, respectively. The ff genotype were significantly higher in patients than control ($p < 0.0403$, $\chi^2 = 4.207$). f allele of *FokI* polymorphism

Table 1 Biochemical and anthropometric characteristics of osteoporotic and non-osteoporotic postmenopausal women

S. Nos.	Variables	Osteoporotic postmenopausal women (patients) <i>N</i> = 254	Non-osteoporotic postmenopausal women (controls) <i>N</i> = 254	<i>p</i> value	95% CI
1.	Age (years)	56.12 ± 7.00	55.11 ± 5.66	0.074	−2.121–0.100
2.	Post menopause years	11.41 ± 9.70	10.18 ± 5.73	0.082	−2.621–0.159
3.	BMI (Kg/m ²)	24.93 ± 3.63	24.06 ± 2.99	0.003*	−1.449–0.290
4.	BMD Lumbar (L1-L4) (g/cm ²)	0.633 ± 0.15	0.902 ± 0.12	< 0.001*	0.2448–0.2930
5.	BMD Hip (g/cm ²)	0.548 ± 0.15	0.840 ± 0.12	< 0.001*	0.2686–0.3162
6.	BMD Femoral neck (g/cm ²)	0.641 ± 0.12	0.793 ± 0.09	< 0.001*	0.1339–0.171
7.	BMD Forearm (g/cm ²)	0.591 ± 0.14	0.819 ± 0.11	< 0.001*	0.205–0.250
8.	Vitamin D (ng/ml)	13.85 ± 5.79	26.399 ± 9.00	< 0.001*	11.235–13.875

All data are shown as mean ± SD

**p* < 0.05 is considered statistically significant

Table 2 Bone mineral density (BMD) measurements in relation to *VDR* genotypes (*N* = 508)

<i>BsmI</i>							
Variable	Genotype			BB versus bb		BB versus Bb	
	BB (<i>N</i> = 108)	Bb (<i>N</i> = 289)	bb (<i>N</i> = 111)	<i>p</i> value	95% CI	<i>p</i> value	95% CI
Genotype frequency (%)	21	57	22				
Age (years)	55.01 ± 6.30	55.44 ± 6.63	56.65 ± 5.68	0.044*	−3.239–0.040	0.551	−1.851–0.991
Post menopausal years (PMY)	9.95 ± 6.38	10.79 ± 9.19	11.60 ± 5.61	0.043*	−3.252–0.048	0.305	−2.450–0.770
BMI (Kg/m ²)	24.94 ± 3.31	24.47 ± 3.41	24.11 ± 3.17	0.059	−0.033–1.694	0.213	−0.272–1.212
BMD Lumbar (L1-L4) (g/cm ²)	0.78 ± 0.19	0.78 ± 0.18	0.71 ± 0.19	0.006*	0.019–0.120	> 0.999	−0.041–0.041
BMD Hip (g/cm ²)	0.71 ± 0.20	0.70 ± 0.19	0.64 ± 0.20	0.010*	0.016–0.123	0.653	−0.033–0.053
BMD Femoral neck (g/cm ²)	0.73 ± 0.13	0.72 ± 0.12	0.70 ± 0.13	0.089	−0.004–0.064	0.487	−0.018–0.038
BMD Forearm (g/cm ²)	0.70 ± 0.17	0.71 ± 0.16	0.67 ± 0.18	0.206	−0.016–0.076	0.596	−0.047–0.027
Vitamin D (ng/ml)	20.74 ± 9.40	20.23 ± 10.03	19.23 ± 9.72	0.243	−1.037–4.057	0.637	−1.619–2.639
<i>FokI</i>							
Variable	Genotype			FF versus ff		FF versus Ff	
	FF (<i>n</i> = 317)	Ff (<i>n</i> = 172)	ff (<i>n</i> = 19)	<i>p</i> value	95% CI	<i>p</i> value	95% CI
Genotype frequency	62.40	33.86	3.74				
Age (years)	55.72 ± 6.09	55.37 ± 7.00	56.05 ± 5.44	0.801	−3.029–2369	0.581	−0.897–1.597
Post menopausal years (PMY)	11.06 ± 8.60	10.26 ± 6.97	11.14 ± 5.62	0.954	−2.928–2.768	0.265	−0.611–2.212
BMI (Kg/m ²)	24.51 ± 3.31	24.43 ± 3.37	24.80 ± 3.95	0.757	−2.26–1.646	0.801	−0.543–0.703
BMD Lumbar (L1-L4) (g/cm ²)	0.78 ± 0.18	0.76 ± 0.18	0.58 ± 0.25	0.002*	0.078–0.321	0.241	−0.013–0.053
BMD Hip (g/cm ²)	0.71 ± 0.21	0.68 ± 0.18	0.55 ± 0.22	0.005*	0.051–0.268	0.098	−0.005–0.065
BMD Femoral neck (g/cm ²)	0.72 ± 0.13	0.73 ± 0.13	0.63 ± 0.17	0.035*	0.006–0.173	0.417	−0.034–0.014
BMD Forearm (g/cm ²)	0.71 ± 0.17	0.71 ± 0.17	0.55 ± 0.19	0.002*	0.066–0.253	0.991	−0.031–0.031
Vitamin D (ng/ml)	20.40 ± 9.41	19.74 ± 10.64	19.5 ± 9.37	0.688	3.717–5.517	0.496	−1.245–2.565

The statistical significance *p* (ANOVA) is shown between genotypes BB and bb, BB and Bb, FF and ff, FF and Ff. Parenthesis indicate the number of subjects in each group

**p* < 0.05 is considered statistically significant

was also significantly higher in patients than control (*p* < 0.024, χ^2 = 5.048). The odds ratio calculated to estimate the incidence of osteoporosis with *VDR* polymorphism genotypes: Bb (OR = 0.90, 95% CI:

0.0576–1.403), bb (1.131, 95% CI: 0.770–2.235), Ff (OR = 1.31, 95% CI: 0.905–1.905) and ff (OR = 3.19, 95% CI: 1.125–9.091). A higher risk was observed only with ff genotype.

Table 3 Distribution of genotypes and alleles of *VDR BsmI* and *FokI* gene polymorphisms in postmenopausal osteoporotic (patients) and postmenopausal non- osteoporotic women (controls)

Groups		Patients (N = 254)	Controls (N = 254)	p value	χ^2 value	Odds ratio (95% CI)
<i>BsmI B/b polymorphism</i>						
Genotypes	BB ^a	54 (21.26%)	54 (21.26%)			
	Bb	137 (53.94%)	152 (59.84%)	0.728	0.120	0.90 (0.579–1.403)
	bb	63 (24.80%)	48 (18.90%)	0.386	0.751	1.31 (0.770–2.235)
	Bb + bb	200 (78.74%)	200 (78.74%)	1.000	0.000	1.00 (0.653–1.530)
Alleles	B ^b	245 (48.23%)	260 (51.18%)	0.379	0.771	1.12 (0.879–1.439)
	b	263 (51.77%)	248 (48.82%)			
<i>FokI F/f polymorphism</i>						
Genotypes	FF ^a	148 (58.27%)	169 (66.54%)			
	Ff	92 (36.22%)	80 (31.49%)	0.179	1.800	1.31 (0.905–1.905)
	ff	14 (5.51%)	5 (1.97%)	0.0403*	4.207	3.19 (1.125–9.091)
	Ff + ff	106 (41.73%)	85 (33.46%)	0.067	3.356	1.42 (0.992–2.043)
Alleles	F ^b	388 (76.38%)	418 (82.28%)	0.024*	5.048	1.44 (1.057–1.951)
	f	120 (23.62%)	90 (17.72%)			

* $p < 0.05$ is considered statistically significant^aIn comparison with referent wild type genotypes^bIn comparison with referent wild type alleles**Table 4** Frequency distribution of different combinations of *VDR* genotypes (*BsmI* and *FokI*) in postmenopausal osteoporotic (patients) and postmenopausal non-osteoporotic women (controls) (N = 254)

S. Nos.	VDR genotypes	Patients (N = 254)	Controls (N = 254)	p value
1	BBFF	29 (11.41%)	30 (11.81%)	0.889
2	BBFf	22 (8.66%)	20 (7.87%)	0.872
3	BBff	3 (1.18%)	4 (1.57%)	0.703
4	BbFF	87 (34.25%)	114 (44.90%)	0.018*
5	BbFf	45 (17.71%)	37 (14.56%)	0.398
6	Bbff	5 (1.96%)	1 (0.40%)	0.217
7	bbFF	32 (12.59%)	25 (9.84%)	0.399
8	bbFf	25 (9.84%)	23 (9.05%)	0.879
10	bbff	6 (2.36%)	0	0.040*

 $p < 0.05$ is considered statistically significant

Frequency distribution of different combinations of *VDR* genotypes (*BsmI* and *FokI*) in patients and controls are shown in Table 4. There was significant difference between patients and controls with BbFF and bbff ($p = 0.018$ and $p = 0.040$) combined genotypes. No statistical differences were found in frequency of other combined genotype.

Synergistic effect of two SNPs (*BsmI* + *FokI*) and their genotypes are shown in Table 5. When different combinations of two SNPs (*BsmI* + *FokI*) were evaluated together among study population with BB or FF representing wild, Bb or Ff representing heterozygous and bb or ff representing mutant genotype, it was observed that the combination bearing mutant genotype of both the SNP's

(*BsmI* + *FokI*) i. e. bbff exhibited a significantly lower BMD at all the points as compared to other combinations. The lower levels of vitamin D were found in genotypes of Bbff and bbff but they were not found statistically significant.

Discussion

1,25-dihydroxyvitamin D₃ [1,25(OH)₂D₃], is the active metabolites of vitamin D and plays a vital role in calcium and bone metabolism by binding with the *VDR*. Epidemiological evidence has revealed that vitamin D deficiency can cause reduced BMD and increased risk of osteoporosis,

Table 5 Bone mineral density measurements in relation to VDR genotypes combinations (BsmI + FokI) (*N* = 508)

Variable	BBFF ^a (<i>N</i> = 59)	BBFF ^b (<i>N</i> = 42)	BBff ^c (<i>N</i> = 7)	BbFF ^d (<i>N</i> = 201)	Bbff ^e (<i>N</i> = 82)	Bbff ^f (<i>N</i> = 6)	bbFF ^g (<i>N</i> = 57)	bbff ^h (<i>N</i> = 48)	bbff ⁱ (<i>NV</i> = 6)	<i>p</i> value
BMD L1–L4	0.78 ± 0.22	0.79 ± 0.15	0.75 ± 0.25	0.80 ± 0.18	0.75 ± 0.19	0.53 ± 0.24	0.73 ± 0.17	0.74 ± 0.20	0.42 ± 0.13	<i>a</i> versus <i>f</i> < 0.05; <i>a</i> versus <i>i</i> < 0.001 <i>b</i> versus <i>f</i> < 0.05; <i>b</i> versus <i>i</i> < 0.001 <i>c</i> versus <i>i</i> < 0.05; <i>d</i> versus <i>f</i> < 0.05 <i>d</i> versus <i>i</i> < 0.001; <i>e</i> versus <i>i</i> < 0.001 <i>g</i> versus <i>i</i> < 0.05; <i>h</i> versus <i>i</i> < 0.05
BMD hip	0.71 ± 0.23	0.72 ± 0.15	0.69 ± 0.27	0.73 ± 0.20	0.67 ± 0.19	0.53 ± 0.20	0.65 ± 0.21	0.67 ± 0.19	0.43 ± 0.08	<i>a</i> versus <i>i</i> < 0.05 <i>d</i> versus <i>i</i> < 0.01
BMD forearm	0.70 ± 0.17	0.71 ± 0.18	0.65 ± 0.22	0.73 ± 0.16	0.70 ± 0.14	0.50 ± 0.20	0.66 ± 0.17	0.71 ± 0.19	0.49 ± 0.09	<i>d</i> versus <i>f</i> < 0.05 <i>d</i> versus <i>i</i> < 0.05
BMD femoral neck	0.74 ± 0.14	0.73 ± 0.11	0.69 ± 0.20	0.73 ± 0.12	0.74 ± 0.13	0.65 ± 0.16	0.66 ± 0.14	0.71 ± 0.15	0.54 ± 0.11	<i>a</i> versus <i>g</i> < 0.05; <i>a</i> versus <i>i</i> < 0.05 <i>b</i> versus <i>i</i> < 0.05; <i>d</i> versus <i>g</i> < 0.05 <i>d</i> versus <i>i</i> < 0.05; <i>e</i> versus <i>g</i> < 0.05 <i>e</i> versus <i>i</i> < 0.01
Vitamin D	20.49 ± 8.61	20.98 ± 10.65	21.5 ± 9.08	20.70 ± 9.77	19.31 ± 10.74	17.19 ± 8.62	19.23 ± 8.96	19.38 ± 10.57	18.05 ± 11.36	ns

p < 0.05 is considered statistically significant

particularly in older peoples [23, 24]. Polymorphisms of the vitamin D receptor gene (*VDR*) have been most extensively investigated with various populations. The first findings related to osteoporosis were carried out in 1994 on Australian population of Caucasian by Morrison et al. [16]. So far, to the best of our knowledge this is the first study to determine the association of *VDR* polymorphism genotypes with osteoporosis and also to correlation between *BsmI* and *FokI* genotypes with BMD and vitamin D₃ levels in North Indian postmenopausal women from Uttar Pradesh. We found a significant association of *VDR BsmI* bb genotype and *FokI* ff genotype and f allele with increased risk of osteoporosis.

This study showed bb genotype was associated with low BMD in postmenopausal women at lumbar spine and hip as compared to BB and Bb genotype. Our results is an good agreement with the finding of Pérez et al. [25] who find bb genotype with low BMD at femoral neck and lumbar spine of Argentine postmenopausal women. Many other results also supported of our finding. Tofteng et al. [26] are showed bb genotype has been associated with low BMD at lumbar spine in healthy and early postmenopausal Danish women. In a study of Canadian healthy postmenopausal women were find bb genotype had low BMD [27] and in Swedish young women [28]. Taguchi et al. [29] have confirmed that Japanese postmenopausal women carrying bb genotype less oral bone mass and lumbar spine and hip having low BMD. In postmenopausal osteoporotic Mexican women also found low BMD at lumbar spine and hip [30]. A study carried out in Hellenic origin postmenopausal women was also associated with low BMD with bb genotype at forearm [31]. These findings are supported to our results. Moreover some contrasting result are found with BB genotype had low BMD. Mitra et al. [21] was find that more than 10% higher lumbar spine and hip BMD with bb genotype in postmenopausal Indian women. Chen et al. [32] was a study carried in Chinese Women in Taiwan was also found BB genotype have lower BMD at lumbar spine and femoral neck. A study in perimenopausal black and white women, found a lower adjusted BMD at the femur neck in women with the BB genotype than the Bb and bb groups, respectively [33]. Also, there are a number of studies that do not confirm association between the *VDR* genotypes and BMD from different skeletal sites [34, 35]. The genotypes and allele frequency distribution of *BsmI* polymorphism found no statistically difference between patient and control. These observations are similar to other study [25]. On the *VDR BsmI* polymorphism with vitamin D levels, we found bb genotype individuals to have low vitamin D levels in comparison with Bb and BB genotype individuals but could not reach statistical significance. Similar findings have been reported by previous study [36].

Further we evaluated the association of the *VDR* gene *FokI* polymorphism with BMD. We found that individuals with ff genotype had lower BMD than FF or Ff individuals at lumbar spine, hip, femoral neck and fore arm. Our observations are in accordance with previous reports showing an association with FF genotype with higher BMD. Mitra et al. [21] who observed in our study, the average BMD at spine and hip with FF genotype was 10% higher than those with ff genotype and Choi et al. [37] was also reported that in their study ff genotype had 13% lower BMD at lumbar spine than the FF genotype. Another study conducted in South Indian pre and postmenopausal women by Yasovanthi et al. [38] was concluded that ff genotype had significantly low BMD in comparison with Ff and FF genotypes. A meta-analysis conducted by Zhang et al. [39] explore the association vitamin D receptor *FokI* gene polymorphism and osteoporosis in postmenopausal women and concluded that *VDR FokI* genotype is associated with increased risk of osteoporosis in Asian population but not in Caucasian population. Wang et al. [40] performed a meta-analysis focused in postmenopausal Women. They found that *FokI* polymorphism is associated with low BMD in Asian women especially Indian postmenopausal women. These findings provide evidence for a close relationship between *FokI* polymorphism and BMD, which is validates the reliability and relevance of our study. In our study we found that ff genotype had increase risk of osteoporosis (OR = 3.19, 95% CI: 1.125–9.091, $p = 0.0403$) and f allele (OR = 1.44, 95% CI: 1.057–1.951, $p = 0.024$) in postmenopausal women. These observations are similar with other study [39]. Moreover, we also found a significant association of *VDR FokI* polymorphism ff genotype and f allele are found higher in patients as compared with control. Our result is similar with other study [38]. In contrasting some results are found no association between *FokI* polymorphism with BMD [41, 42]. There are a number of possible reasons for these discrepancies including allelic heterogeneity between different ethnicity, population admixture in the studies, small or insufficient sample size and linkage disequilibrium or environmental factors which might be mask the genetic effects.

Vitamin D has been reported to be helpful in antiresorptive therapies as non-hypercalcemic analogs of 1,25-dihydroxyvitamin D could enhanced the responsiveness to estradiol and thus lower levels of estrogens may be used in treatment of osteoporosis in postmenopausal women [43]. In our study we found that vitamin D levels were significantly decreased in postmenopausal women with osteoporosis in comparison with controls. Our results are in accordance with previous report from North India [44–46]. Association of the *VDR FokI* polymorphism with vitamin D levels, we found ff genotype individuals to have low vitamin D levels in comparison with FF and Ff genotype

individuals but could not reach statistical significance. Similar findings have been reported by previous studies [38, 47]. The combined genotypes study of *BsmI* and *FokI* *VDR* polymorphism. It was found that the combined genotypes BbFF significantly higher in controls ($p = 0.018$), while bbff significantly higher in patients ($p = 0.040$).

In conclusion *VDR* gene (*BsmI* and *FokI*) polymorphism plays an important role in osteoporosis in North Indian post menopausal women. The bb genotype (*VDR* gene *BsmI* polymorphism) in osteoporotic women is associated with the lowest BMD in the L₁–L₄ lumbar spine and hip. *FokI* polymorphism of the *VDR* gene is associated with osteoporosis and the polymorphism appears to persuade peak BMD and play a key role in the progression of osteoporosis. ff genotype was associated with osteoporosis. However, the mechanisms by which *VDR* alleles regulate BMD remain poorly understood. Thus, relevance of several studies from different populations, like ours, may give support to in narrowing down the reported variation in results associated with these gene polymorphisms and disease risk.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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