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Association between vitamin D receptor BsmI (rs1544410) gene polymorphism and serum vitamin D, serum calcium, and osteoporosis risk in women over 40 years

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Abstract

Objectives: We investigated the relationships between the vitamin D receptor BsmI (rs1544410) gene polymorphism, serum vitamin D and calcium levels, and the risk of osteoporosis in women aged 40 years and older.

Methods: This case-control investigation enrolled participants (30 cases and 30 controls) classified into normal and osteoporotic groups using a dual-energy X-ray absorptiometry (DEXA) scan. Vitamin D and calcium levels were obtained from peripheral blood samples. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was performed to detect the BsmI polymorphism. Differences between groups were analyzed using an analysis of variance (ANOVA) test.

Results: 60 individuals (30 cases and 30 age-matched controls) were enrolled. The lumbar and femoral T-scores, as well as calcium levels, were significantly lower in the cases compared to the controls ($P < 0.005$). Among postmenopausal women, the lumbar T-scores significantly differed between cases with VDR polymorphisms ($P = 0.003$) and controls ($P = 0.001$). Among premenopausal women, there was only a significant difference in VDR polymorphisms in the control group ($P = 0.006$). Calcium and vitamin D levels were significantly different between the VDR polymorphisms in cases and control groups, but only among postmenopausal women ($P < 0.005$ for all comparisons). No significant differences were observed between the VDR polymorphism in terms of femoral T-scores, neither in the cases nor the controls (P for all > 0.005).

Conclusion: This study found no differences in VDR polymorphism between individuals with and without osteoporosis. Additionally, the lumbar T-scores, calcium, and vitamin D levels were significantly different between the VDR polymorphisms in the case and control groups among postmenopausal women.

Keywords: BsmI gene polymorphism, vitamin D receptor, osteoporosis, post-menopause women.

1. Background

Osteoporosis, recognized as the most prevalent chronic metabolic bone disorder, is defined by a widespread deterioration of bone mass and microarchitecture, leading to an

increased susceptibility to fragility fractures (1). In 2024, the prevalence of osteoporosis among the Iranians was reported as 38% and 25% for women and men, respectively (2). Due to the silent nature of the disease, osteoporosis remains a significant secondary health challenge until fractures occur (3). In post-menopause women, the risk of bone density reduction and osteoporosis increases due to the lack of estrogen (4)

Osteoporosis is a multifactorial condition with genetic and acquired risk factors. Aging, Caucasian race, and post-menopause are some of the crucial risk factors (5). Moreover, an insufficiency of calcium and vitamin D is strongly associated with the development of osteoporosis (6). Vitamin D levels play a crucial role in regulating mineral homeostasis and bone metabolism (7). Vitamin D is olso essential in bone metabolism and induces bone synthesis by binding to its receptor, the vitamin D receptor (VDR), which stimulates skeletal tissue and regulates bone turnover (8). VDR affects the expression and transcription of genes that are involved in the formation of bone mineral density (BMD) and calcium absorption, including osteocalcin and calcium-binding proteins (9, 10).

Based on previous investigations, the VDR gene and its polymorphisms is correlated with the development and progression of osteoporosis (11-13). Morrison and colleagues were the first to indicate that genetic variations in the VDR gene could serve as predictors for spinal and femoral BMD in Caucasians (14, 15). Thereafter, numerous studies have found that VDR genetic variants, including FokI (rs10735810), ApaI (rs7975232), and BsmI (rs1544410), are associated with BMD and osteoporosis across various ethnicities (16-19). However, the relationship between a specific VDR genotype and its potential association with reduced BMD and risk of fractures remains unclear (20, 21). Furthermore, to our knowledge, there have been no investigations into the association of VDR gene polymorphisms in the Mashhad, Iran, population.

Hence, we assessed the association between the BsmI variant and serum vitamin D and calcium levels, as well as the risk of osteoporosis among women in Mashhad City who were over 40 years old.

2. Objectives

We investigated the relationships between the vitamin D receptor BsmI (rs1544410) gene polymorphism, serum vitamin D and calcium levels, and the risk of osteoporosis in women aged 40 years and older.

3. Methods

Study participants

This case-control investigation was conducted between September 2019 and 2021, and 60 women aged over 40 years, including 30 subjects with osteoporosis (cases) and 30 age-matched subjects without osteoporosis (control group), were enrolled. Demographic data and clinical risk factors, including age, menopausal status, past medical history, and underlying diseases, were collected using a standardized questionnaire. The control group was selected from subjects referred to the hospital or private clinics in Mashhad, Iran, due to musculoskeletal complaints. The exclusion criteria for both groups were as follows: using oral or injectable corticosteroids, history of hysterectomy and/or bilateral salpingo-oophorectomy, chronic diseases, and pregnancy (Figure 1).

BMD assessment and osteoporosis definition:

BMD was assessed at the lumbar spine (L1–L4) and femoral neck using dual-energy X-ray absorptiometry (DEXA) scanning. Women exhibiting femoral neck or spinal T-scores of -2.5 or lower were classified as osteoporotic women (cases) (22).

Biochemical measurements:

A 10 mL blood sample was drawn from the antecubital vein following 12 hours of fasting. Calcium levels (mg/dL) were measured using a Prestige Chemistry Analyzer (Bionik kit, Germany) and a Roche Hitachi 704 (Germany). Vitamin D levels (IU) were measured using the

Cobas e 411 Analyzer (Roche kit, Germany) using standard protocols.

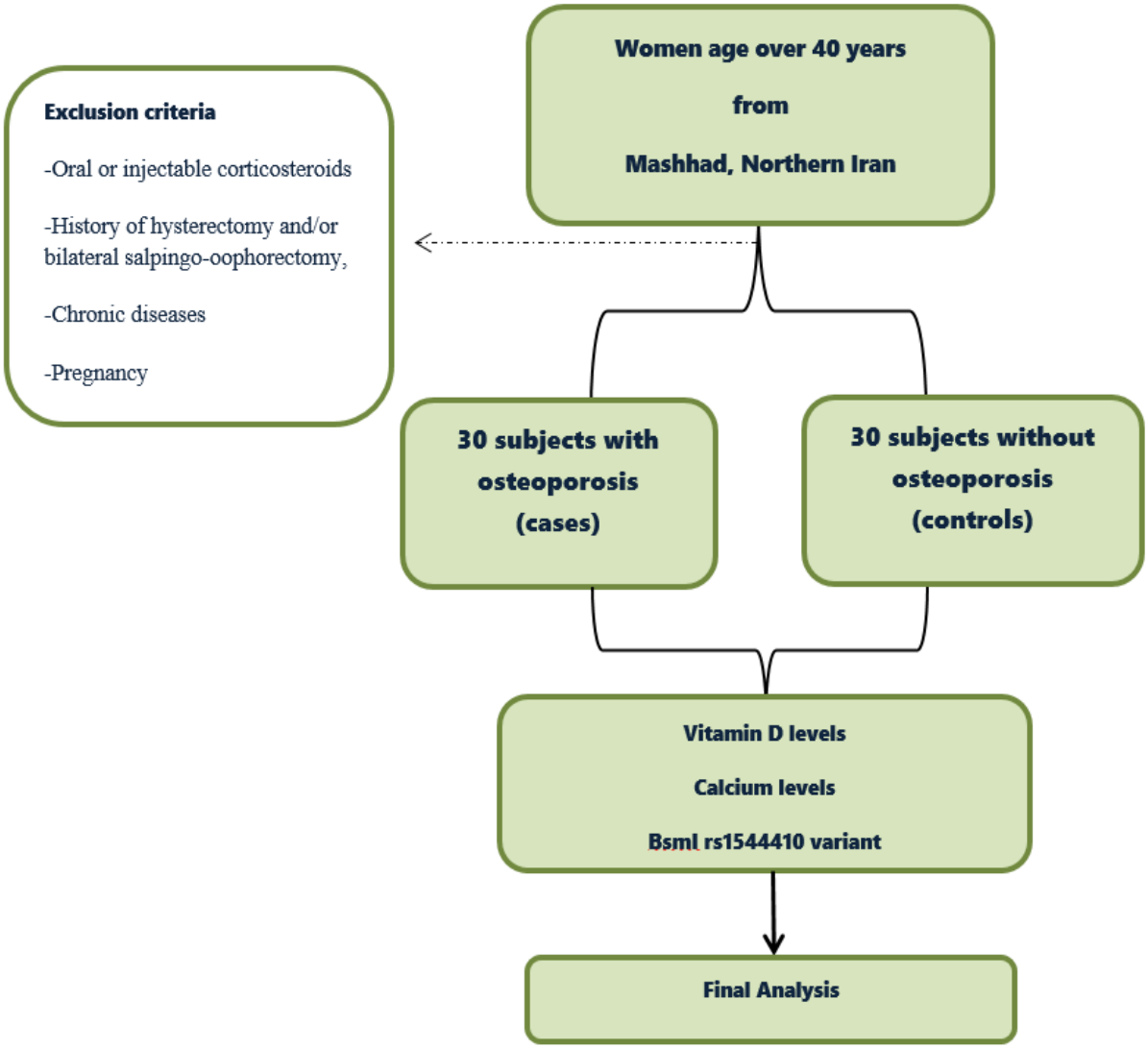

Figure 1. Flowchart of the study

DNA extraction VDR genotyping:

After using the Vacutainer system blood collection method, DNA was isolated from bloodspots dried on special NucleoSafe cards (Macherey-Nagel, Germany) utilizing standard proteinase K (GenFanAvaran, Iran) digestion, phenol-chloroform (Merck, Darmstadt, Germany) extraction, and ethanol precipitation (23). The quality and purity of DNA samples were checked using the spectrophotometric method (24). The BsmI rs1544410 polymorphism was determined using polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) analysis with specially designed primers generated using Gene Runner v5.3 software. The PCR reaction system contains 10 × buffer = 2.5 µL, 1.5 µL MgCl2 (50 mM), one µL dNTPs (40 mM), 10 forward (GGGAGACGTAGCAAAAGG) and reverse primers (AGAGGTCAAGGGTCACTG) (60 pmol), 2.3 µL DNA template (100 ng), 0.15 µL Taq polymerase (5 U/ µL), and 16.35 2.3 µL sterile water. All PCR reactions were conducted at 95° C for 15 minutes, followed by 30 cycles consisting of 99°C for 1 second,

62°C for 10 seconds, and an extension phase at 72°C for 20 seconds. The final extension step was performed at 72°C for 2 minutes using an automated thermal cycler (ASTEC, PC-808, Japan). The amplified products were analyzed by electrophoresis on an 8% polyacrylamide gel. *Bsm*I genotypes were analyzed in the vicinity of the restriction enzyme ApaI (Thermo Scientific, Ref. ER1411) for 16 hours at 37°C.

Ethical consideration:

All subjects provided written informed consent before their inclusion. Moreover, the study protocols were approved by the Ethics Committee of Islamic Azad University, Mashhad, Iran, with the ethics code IR.IAU.MSHD.REC.1399.053.

Statistical analysis:

The assessment of data normality was conducted using the Kolmogorov–Smirnov test. For comparing normally distributed data, the Student's t-test was employed; for non-normally distributed data, the Mann–Whitney U-test was used. Categorical data are expressed as counts (%) and analyzed using the chi-square test. The variable differences in VDR polymorphisms between the case and control groups were tested using the ANOVA test. All data were analyzed using SPSS version 26, with P-values < 0.005 considered statistically significant.

4. Results

A total of 60 participants (30 cases and 30 age-matched controls) were enrolled. Table 1 depicts the differences in characteristics between the cases and controls. The lumbar (-3.13±0.32 vs. -0.08±1.34) and femoral T-scores (-2.39±0.84 vs. -0.44±1.52) and calcium levels (9.30±0.50 vs 9.56±0.46) were significantly lower in the cases compared to the controls (P < 0.005). No significant differences were found between the groups in terms of age, menopausal status, vitamin D, and VDR polymorphism.

Table 1. Characteristics of the study population

Characteristics	Case (n= 30 (50%))	Control (n= 30 (50%))	P-value
Age (year)	60.86±8.45	58.96±8.00	0.375
Menopausal status Post menopause Pre menopause	25 (83.30%) 5 (16.70%)	22 (73.30%) 8 (26.70%)	0.347
Lumbar T-score	-3.13±0.32	-0.08±1.34	0.001
Femoral T-score	-2.39±0.84	-0.44±1.52	0.001
Calcium levels	9.30±0.50	9.56±0.46	0.033
Vitamin D levels	21.35±12.99	25.49±11.76	0.094
VDR polymorphism (AA) (AG) (GG)	11 (36.70%) 14 (46.70%) 5 (16.70%)	7 (23.30%) 20 (66.70%) 3 (10.00%)	0.291

Abbreviation: VDR: vitamin D receptor

As depicted in Table 2, the lumbar T-scores were significantly different between the VDR polymorphisms in the cases (P=0.003) and control groups (P=0.001) among the post-menopause women. Among premenopausal women, a significant difference was observed only in VDR polymorphisms in the control group (P = 0.006). Calcium and vitamin D levels exhibited notable differences between the VDR polymorphisms in cases and control groups, but only among postmenopausal women (P < 0.005 for all comparisons). No significant differences were observed between the VDR polymorphism and femoral T-scores in either group, whether in cases or controls.

Table 2. Comparison between the variables stratified by menopause status

Characteristics	Post menopause								Pre menopause							
	Case			P-value	Control			P-value	Case			P-value	Control			P-value
	(AA)	(AG)	(GG)		(AA)	(AG)	(GG)		(AA)	(AG)	(GG)		(AA)	(AG)	(GG)	
Lumbar T-score	-3.37±0.29	-3.12±0.27	-2.77±0.06	0.003	-1.69±0.27	-0.11±1.02	2.45±0.49	0.001	-2.92±0.12	0	-2.90±0	0.897	-1.39±0.41	0.52±0.77	1.70±0	0.060
Femoral T-score	-2.44±0.96	-2.38±0.86	-2.38±0.90	0.250	-1.08±0.71	-0.30±1.62	2.45±0.21	0.250	-2.31±0.47	0	-2.90±0	0.312	-0.89±1.11	1.25±0.79	1.20±0	0.881
Calcium levels	8.89±0.23	9.41±0.39	9.87±0.63	0.002	9.06±0.23	9.79±0.39	10.10±0.14	0.008	9.40±0.38	0	10±0	0.147	9.30±0.42	9.38±0.35	9.32±0	0.874
Vitamin D levels	12.31±5.37	24.70±15.29	30.00±13.95	0.007	12.86±3.45	25.36±8.58	45.40±4.80	0.003	32.50±5.06	0	44.00±0	0.157	18.30±3.81	27.72±9.53	54.00±0	0.122

5. Discussion

This study investigated the association between the rs1455510 gene polymorphism of VDR and serum vitamin D, calcium, and osteoporosis in women aged 40 years and older. We enrolled 60 individuals (30 with osteoporosis (cases) and 30 without osteoporosis (controls)), and the results showed that the lumbar and femoral T-scores and calcium levels were significantly lower in the cases compared to the controls. Then, we divided the participants according to menopausal status and found that among postmenopausal women, the lumbar T-scores were significantly different between the VDR polymorphisms in both the case and control groups. Among the pre-menopauses, there was only a significant difference between the VDR polymorphisms in the control group. Additionally, calcium and vitamin D levels were significantly different between the VDR polymorphisms in cases and control groups, but only among postmenopausal women. There were no significant differences in the VDR polymorphism in terms of femoral T-scores among any of the groups, either in the cases or the controls.

The VDR gene functions as a nuclear transcription factor facilitating the effects of 1, 25(2)2D3. This action influences calcium absorption, bone remodeling, and the rate of mineralization. The gene is situated on the long arm of chromosome 12, specifically at the *13q11* locus (*12q13.11*), comprising 11 exons, with exons 2 through 9 being actively transcribed (25, 26). We found that the AG-genotype of VDR gene polymorphisms was a more prevalent variant among both cases and controls. Similarly, Marozik *et al.* demonstrated that the AG genotype had the highest rate of VDR BsmI rs1544410 in postmenopausal osteoporotic women and asymptomatic controls among Lithuanian and Belarusian populations (27). Furthermore, we observed no notable differences in genotype distributions between the groups, which is consistent with the results of investigations by Tamulaitienė *et al*. (21) and Uitterlinden *et al*. (28).

The *BsmI* polymorphism is a single nucleotide polymorphism located in the VDR gene (29). Its influence on bone health has garnered significant attention, as vitamin D is essential for the absorption of calcium and the metabolism of bone. The discovery of the VDR gene and its polymorphism has led to numerous investigations that have revealed an association with BMD, resulting in diverse

findings. Banjabi reported that *ApaI* and *TaqI* were significantly related to the osteoporosis risk in the Saudi population (30). Mondockova *et al*. reported that four VDR gene polymorphisms, including *BsmI*, were associated with markers of bone turnover and an increased risk of spinal, radial, or overall fractures (31). A recent meta-analysis of 73 studies demonstrated that the ApaI rs7975232, BsmI rs1544410, and TaqI rs731236 polymorphisms influenced the risk of osteoporosis in Caucasians. In contrast, the BsmI rs1544410 and TaqI rs731236 polymorphisms were found to influence the risk of osteoporosis in the Asian population from China, Japan, Korea, and Thailand (32). Another meta-analysis suggested that *BsmI* polymorphism is a potential biomarker for lower BMD among postmenopausal subjects from North Africa and the Middle East (33). On the other hand, et al. found no relationship between the *BsmI* polymorphism and diminished axial BMD among Caucasians with systemic sclerosis (34).

These inconsistent findings are attributed to differences in populations and geographic regions, which result from variations in genetic, environmental, dietary, and lifestyle factors.

Menopause is a significant contributor to the development of osteoporosis. Reports indicate that women who have undergone menopause experience a loss of bone mass ranging from 3% to 5% annually due to the decrease in estrogen production by the ovaries. Therefore, they are at a higher risk for osteoporosis and bone fractures (35). We found that the lumbar and spinal T-scores, as well as vitamin D and calcium, were improved in the GG-genotype compared to the other genotypes among both postmenopausal osteoporotic women (cases) and healthy women (controls), which is comparable with other investigations. A weak, non-significant correlation was observed between the BMD of the lumbar spine and total hip, as well as biochemical bone markers, in females with BsmI genotypes who suffered from severe osteoporosis (21). Pedrera-Cana and colleagues found no significant associations between the rs1544410 genotype and bone loss among post-menopause Spanish women after five years (36). A study in Iran involving 146 women discovered that the GG genotype demonstrated a significant correlation with elevated BMD in the lumbar spine; however, this association was not significant in the femoral neck (37). Another study from India demonstrated that post-menopause women possessing the GG genotype exhibited the highest BMD in the femoral neck (38), which is similar to our findings.

Strengths and limitations:

This study focused on a specific VDR genetic polymorphism that could shed light on the relationship between genetic factors and the risk of osteoporosis among women over 40 years old. Additionally, the study's inclusion of serum vitamin D and calcium levels enhances its robustness, as these are critical factors in bone health. However, this study has limitations. Firstly, the sample size was limited due to the scope of this study, as the data and results were extracted from a thesis, and increasing the sample size was not feasible within the given constraints. The COVID-19 pandemic also affected the sample size and patient recruitment. Secondly, due to limitations in our dataset and sample size, we were initially unable to perform the Hardy-Weinberg equilibrium analysis. Thirdly, we did not account for potential confounding variables, including lifestyle factors (diet, physical activity, and sun exposure), within the population studied, which may affect the generalizability of the findings. Furthermore, a cross-sectional design could limit causal inferences about the relationship between the *BsmI* polymorphism and osteoporosis, emphasizing the need for longitudinal, large-scale, and multi-ethnic studies to establish temporal associations.

6. Conclusion

In our study, individuals with and without osteoporosis were not different in terms of VDR polymorphism. Additionally, the lumbar T-scores, calcium, and vitamin D levels were significantly different between the VDR polymorphisms in the case and control groups among postmenopausal women.

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Availability of data and materials: The datasets used in this study are available from the corresponding author upon reasonable request.

Conflicts of interests: All authors declared that they have no competing interests.

Consent for publication: Not applicable.

Ethics approval and consent to participate: The study protocols were approved by the Ethics Committee of Islamic Azad University, Mashhad, Iran, with the ethics code IR.IAU.MSHD.REC.1399.053.The study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki.

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Author contributions: Each author has made substantial contributions to the final manuscript, approved the submitted version, and agreed to be personally accountable for the author's contributions and to ensure that questions related to the accuracy or integrity of any part of the work.

References

1. Rachner TD, Khosla S, Hofbauer LC. Osteoporosis: now and the future. The Lancet. 2011;377(9773):1276-87. https://doi.org/10.1016/S0140-6736(10)62349-5 PMid:21450337
2. Fahimfar N, Hesari E, Mansourzadeh MJ, Khalagi K, Sanjari M, Hajivalizadeh S, et al. Prevalence of osteoporosis in the Iranian population: a systematic review and meta-analysis. Journal of Diabetes & Metabolic Disorders. 2024;23(1):229-37. https://doi.org/10.1007/s40200-023-01352-9 PMid:38932872 PMCid:PMC11196456
3. Prost S, Pesenti S, Fuentes S, Tropiano P, Blondel B. Treatment of osteoporotic vertebral fractures. Orthopaedics & Traumatology: Surgery & Research. 2021;107(1):102779. https://doi.org/10.1016/j.otsr.2020.102779 PMid:33321233
4. Unni J, Garg R, Pawar R. Bone mineral density in women above 40 years. Journal of mid-life health. 2010;1(1):19-22. https://doi.org/10.4103/0976-7800.66989 PMid:21799633 PMCid:PMC3139257
5. Tański W, Kosiorowska J, Szymańska-Chabowska A. Osteoporosis-risk factors, pharmaceutical and non-pharmaceutical treatment. European Review for Medical & Pharmacological Sciences. 2021;25(9).
6. De Martinis M, Allegra A, Sirufo MM, Tonacci A, Pioggia G, Raggiunti M, et al. Vitamin D deficiency, osteoporosis and effect on autoimmune diseases and hematopoiesis: a review. International journal of molecular sciences. 2021;22(16):8855. https://doi.org/10.3390/ijms22168855 PMid:34445560 PMCid:PMC8396272
7. Reid IR, Bolland MJ, Grey A. Effects of vitamin D supplements on bone mineral density: a systematic review and meta-analysis. The Lancet. 2014;383(9912):146-55. https://doi.org/10.1016/S0140-6736(13)61647-5 PMid:24119980
8. Houston LA, Grant SFA, Reid DM, Ralston SH. Vitamin D receptor polymorphism, bone mineral density, and osteoporotic vertebral fracture: Studies in a UK population. Bone. 1996;18(3):249-52.

https://doi.org/10.1016/8756-3282(95)00483-1 PMid:8703580
9. Fleet JC. Vitamin D-mediated regulation of intestinal calcium absorption. Nutrients. 2022;14(16):3351https://doi.org/10.3390/nu14163351 PMid:36014856 PMCid:PMC9416674
10. Beggs MR, Bhullar H, Dimke H, Alexander RT. The contribution of regulated colonic calcium absorption to the maintenance of calcium homeostasis. The Journal of Steroid Biochemistry and Molecular Biology. 2022;220:106098. https://doi.org/10.1016/j.jsbmb.2022.106098 PMid:35339651
11. Gong G, Stern H, Cheng S-C, Fong N, Mordeson J, Deng H-W, et al. The association of bone mineral density with vitamin D receptor gene polymorphisms. Osteoporosis International. 1999;9:55-64. https://doi.org/10.1007/s001980050116 https://doi.org/10.1007/s001980050152
12. Senosi MR, Fathi HM, Baki NMA, Zaki O, Magdy AM, Gheita TA. Bone mineral density, vitamin D receptor (VDR) gene polymorphisms, fracture risk assessment (FRAX), and trabecular bone score (TBS) in rheumatoid arthritis patients: connecting pieces of the puzzle. Clinical rheumatology. 2022;41(5):1333-42. https://doi.org/10.1007/s10067-022-06048-8 PMid:35048212 PMCid:PMC9056478
13. Banjabi AA, Al-Ghafari AB, Kumosani TA, Kannan K, Fallatah SM. Genetic influence of vitamin D receptor gene polymorphisms on osteoporosis risk. International journal of health sciences. 2020;14(4):22.
14. 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(6460):284-7. https://doi.org/10.1038/367284a0 PMid:8161378
15. Morrison NA, Yeoman R, Kelly PJ, Eisman JA. Contribution of trans-acting factor alleles to normal physiological variability: vitamin D receptor gene polymorphism and circulating osteocalcin. Proceedings of the National Academy of Sciences. 1992;89(15):6665-9. https://doi.org/10.1073/pnas.89.15.6665 PMid:1353882 PMCid:PMC49563
16. Li Y, Xi B, Li K, Wang C. Association between vitamin D receptor gene polymorphisms and bone mineral density in Chinese women. Molecular biology reports. 2012;39:5709-17. https://doi.org/10.1007/s11033-011-1380-3 PMid:22193625
17. Mohammadi Z, Fayyazbakhsh F, Ebrahimi M, Amoli MM, Khashayar P, Dini M, et al. Association between vitamin D receptor gene polymorphisms (Fok1 and Bsm1) and osteoporosis: a systematic review. Journal of Diabetes & Metabolic Disorders. 2014;13:1-9. https://doi.org/10.1186/s40200-014-0098-x PMid:25364703 PMCid:PMC4215021
18. Kurt O, Yilmaz-Aydogan H, Uyar M, Isbir T, Seyhan MF, Can A. Evaluation of ERα and VDR gene polymorphisms in relation to bone mineral density in Turkish postmenopausal women. Molecular biology reports. 2012;39:6723-30. https://doi.org/10.1007/s11033-012-1496-0 PMid:22311020
19. Horst-Sikorska W, Dytfeld J, Wawrzyniak A, Marcinkowska M, Michalak M, Franek E, et al. Vitamin D receptor gene polymorphisms, bone mineral density and fractures in postmenopausal women with osteoporosis. Molecular biology reports. 2013;40:383-90. https://doi.org/10.1007/s11033-012-2072-3 PMid:23070909 PMCid:PMC3518805
20. Brown MA, Haughton MA, Grant SF, Gunnell AS, Henderson NK, Eisman JA. Genetic control of bone density and turnover: role of the collagen 1α1, estrogen receptor, and vitamin D receptor genes. Journal of Bone and Mineral Research. 2001;16(4):758-64. https://doi.org/10.1359/jbmr.2001.16.4.758PMid:11316004
21. Tamulaitienė M, Marozik P, Alekna V, Mosse I, Strazdienė A, Mastavičiūtė A, et al. Association of VDR BsmI gene polymorphism, bone turnover markers and bone mineral density in severe postmenopausal osteoporosis. Gerontologija. 2012;13(4):206-13.
22. Cosman F, de Beur SJ, LeBoff MS, Lewiecki EM, Tanner B, Randall S, et al. Clinician's Guide to Prevention and Treatment of Osteoporosis. Osteoporosis International. 2014;25(10):2359-81.

https://doi.org/10.1007/s00198-014-2794-2 PMid:25182228 PMCid:PMC4176573
23. Higuchi R. Simple and rapid preparation of samples for PCR. PCR technology: principles and applications for DNA amplification: Springer; 1989. p. 31-8. https://doi.org/10.1007/978-1-349-20235-5_4
24. Lucena-Aguilar G, Sánchez-López AM, Barberán-Aceituno C, Carrillo-Ávila JA, López-Guerrero JA, Aguilar-Quesada R. DNA Source Selection for Downstream Applications Based on DNA Quality Indicators Analysis. Biopreservation and biobanking. 2016;14(4):264-70. https://doi.org/10.1089/bio.2015.0064 PMid:27158753 PMCid:PMC4991598
25. Usategui-Martín R, De Luis-Román D-A, Fernández-Gómez JM, Ruiz-Mambrilla M, Pérez-Castrillón J-L. Vitamin D receptor (VDR) gene polymorphisms modify the response to vitamin D supplementation: a systematic review and meta-analysis. Nutrients. 2022;14(2):360. https://doi.org/10.3390/nu14020360 PMid:35057541 PMCid:PMC8780067
26. Akter R, Afrose A, Sharmin S, Rezwan R, Rahman MR, Neelotpol S. A comprehensive look into the association of vitamin D levels and vitamin D receptor gene polymorphism with obesity in children. Biomedicine & Pharmacotherapy. 2022;153:113285. https://doi.org/10.1016/j.biopha.2022.113285PMid:35728355
27. Marozik PM, Tamulaitiene M, Rudenka E, Alekna V, Mosse I, Rudenka A, et al. Association of vitamin D receptor gene variation with osteoporosis risk in Belarusian and Lithuanian postmenopausal women. Frontiers in endocrinology. 2018;9:305. https://doi.org/10.3389/fendo.2018.00305 PMid:29922235 PMCid:PMC5996071
28. 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. Annals of internal medicine. 2006;145(4):255-64. https://doi.org/10.7326/0003-4819-145-4-200608150-00005 PMid:16908916
29. Ezhilarasi K, Dhamodharan U, Vijay V. BSMI single nucleotide polymorphism in vitamin D receptor gene is associated with decreased circulatory levels of serum 25-hydroxyvitamin D among micro and macrovascular complications of type 2 diabetes mellitus. International Journal of Biological Macromolecules. 2018;116:346-53. https://doi.org/10.1016/j.ijbiomac.2018.05.026 PMid:29738868
30. Banjabi AA, Al-Ghafari AB, Kumosani TA, Kannan K, Fallatah SM. Genetic influence of vitamin D receptor gene polymorphisms on osteoporosis risk. Int J Health Sci (Qassim). 2020;14(4):22-8.
31. Mondockova V, Kovacova V, Zemanova N. Vitamin D Receptor Gene Polymorphisms Affect Osteoporosis-Related Traits and Response to Antiresorptive Therapy. 2023;14(1). https://doi.org/10.3390/genes14010193PMid:36672934 PMCid:PMC9858724
32. Jiang LL, Zhang C, Zhang Y, Ma F, Guan Y. Associations between polymorphisms in VDR gene and the risk of osteoporosis: a meta-analysis. Archives of physiology and biochemistry. 2022;128(6):1637-44. https://doi.org/10.1080/13813455.2020.1787457 PMid:32757960
33. Al-Barazenji T, Allouch A, Al Husaini N, Yousef S, Ibrahim WN, Al-Haidose A, et al. Association Between Vitamin D Receptor BsmI Polymorphism and Low Bone Mineral Density in Postmenopausal Women in the MENA Region. Pathophysiology. 2025;32(1):6. https://doi.org/10.3390/pathophysiology32010006 PMid:39982362 PMCid:PMC11843856
34. Schulz N, Dischereit G, Henke L, Lange U, Klemm P. Prevalence and effects of Vitamin D receptor polymorphism on bone mineral density and metabolism in patients with systemic sclerosis: a preliminary study. Clinical and Experimental Medicine. 2024;24(1):121. https://doi.org/10.1007/s10238-024-01385-1 PMid:38847864 PMCid:PMC11161438
35. Holroyd C, Cooper C, Dennison E. Epidemiology of osteoporosis. Best practice & research Clinical endocrinology & metabolism. 2008;22(5):671-85. https://doi.org/10.1016/j.beem.2008.06.001PMid:19028351
36. Pedrera-Canal M, Moran JM, Vera V, Roncero-Martin R, Lavado-Garcia JM, Aliaga I, et al.

Lack of influence of vitamin D receptor BsmI (rs1544410) polymorphism on the rate of bone loss in a cohort of postmenopausal Spanish women affected by osteoporosis and followed for five years. PloS one. 2015;10(9):e0138606. https://doi.org/10.1371/journal.pone.0138606 PMid:26393357 PMCid:PMC4579091

37. Pouresmaeili F, Jamshidi J, Azargashb E, Samangouee S. Association between vitamin D receptor gene BsmI polymorphism and bone mineral density in a population of 146 Iranian women. Cell Journal (Yakhteh). 2013;15(1):75.
38. Górczyńska-Kosiorz S, Tabor E, Niemiec P, Pluskiewicz W, Gumprecht J. Associations between the VDR gene rs731236 (TaqI) polymorphism and bone mineral density in postmenopausal women from the RAC-OST-POL. Biomedicines. 2024;12(4):917. https://doi.org/10.3390/biomedicines12040917 PMid:38672272 PMCid:PMC11048207