The relationship between vitamin D receptor (VDR) polymorphism and the occurrence of osteoporosis in menopausal Iranian women

Raheleh Dabirnia[1]
Sanaz Mahmazi[2]
Amirhossein Taromchi[3]
Masoum Nikzad[1]
Ehsan Saburi[4]

[1] Department of Genetics, Faculty of Basic Sciences, Islamic Azad University, Zanjan Branch, Zanjan, Iran
[2] Assistant Professor, Department of Genetics, Faculty of Basic Sciences, Islamic Azad University, Zanjan Branch, Zanjan, Iran
[3] Assistant Professor, Department of Medical Biotechnology and Nanotechnology, Faculty of Medicine, Zanjan University of Medical Sciences, Zanjan, Iran
[4] Molecular Medicine & Genetics Department, Medical Faculty, Zanjan University of Medical Sciences, Zanjan, Iran

Address for correspondence:
Ehsan Saburi
Molecular Medicine & Genetics Department, Medical Faculty, Zanjan University of Medical Sciences
Zanjan, Iran
E-mail: ehsansaburi@yahoo.com

Summary

Background. **Osteoporosis, a multifactorial disease with reduced bone mineral density which increases the probability of bone fractures, is caused by calcium deficiency, and its incidence increases with age. It has been determined that mutations in functional regions of vitamin D receptor gene will affect the metabolism of minerals especially calcium and, therefore, bone density. The present study evaluates the relation between vitamin D receptor polymorphisms, TaqI (rs731236) and ApaI (rs7975232), and osteoporosis in menopausal Azari women in Zanjan province.**
Materials and methods. **This case-control study has been conducted on 50 menopausal women suffering from osteoporosis and 50 menopausal women who did not suffer from osteoporosis in Zanjan province. The diagnosis of osteoporosis was confirmed using DEXA instrument. Peripheral blood was collected from the subjects and controls to extract DNA and assess the ApaI and TaqI polymorphisms using PCR-RFLP method. The results were interpreted using independent T-test, chi-square, and Pearson correlation coefficient with a p-value less than 0.05.**
Results. **There was not a significant difference between the frequency of ApaI (AA/Aa/aa) and TaqI (TT/Tt/tt) genotypes in cases (mean age 68.72) and controls (mean age 64.7) (p=0.37 and p=0.64, respectively). In addition, ApaI/TaqI allele haplotype in osteoporotic population showed non-significant relation (p value=0.563) compared with the control group.**
Discussion and conclusion. **The relationship between the genotypes and osteoporosis, cancers, and mineral metabolism disorders has been studied for a long time. Although there has been a significant relation between the aforementioned genotypes and osteoporosis or reduced mineral density-related bone fractures in some studied, some other studies have opposing results. Therefore, it is only possible to reach an acceptable conclusion by studying the haplotype of the polymorphisms in subjects.**

KEY WORDS: osteoporosis; vitamin D receptor; polymorphism; menopause.

Background

Osteoporosis, a multifactorial disease with reduced bone mineral density (BMD) which is caused by calcium deficiency, increases the probability of bone fractures, and its incidence increases with age. According to WHO, osteoporosis is the reduction of bone density below 2.5 standard deviation from the average for healthy and mature adults with similar ethnicity and age (1). As an individual ages, the bone mass decreases. In menopausal women, the absorption of calcium in intestine and kidney is decreased because of reduced secretion of estrogen, finally leading to reduced bone density (2). According to the literature, the prevalence of osteoporosis varies among different countries, and even among different regions and ethnic groups of a single country (3). This can be due to different diets, physical activities, and life style (4). By studying genomic differences between people and the relationship of these differences with genetic and environmental factors, it will be easier to understand the role of heredity in osteoporosis. The most important genes studied in relation to osteoporosis are estrogen, collagen type I, insulin-like growth factor, interleukin-6, and vitamin D receptor (VDR) (5).
VDR gene is located on 12q13.11 and has 11 exons with a length of 5.6 kb (5). It has been determined that mutations in functional regions of the gene can highly affect the metabolism of minerals especially calcium and as a result, the occurrence of osteoporosis (6). The active form of vitamin D or calcitriol (1,25 OH VitD or D3) binds to its nuclear steroid receptor and plays an important role in calcium metabolism of osteoclasts and osteoblasts. Various single nucleotide polymorphisms (SNP) in different exons of the gene has been detected and considered as the genetic factor of osteoporosis in previous studies. Most of these SNPs are located at the 3'-end which can be detected using TaqI, ApaI, and BsmI restriction enzymes digestion (Figure 1). The present study has been conducted to assess the relationship between TaqI (rs731236) and ApaI (rs7975232) polymorphisms of VDR gene and osteoporosis in menopausal Azari women of Zanjan province.

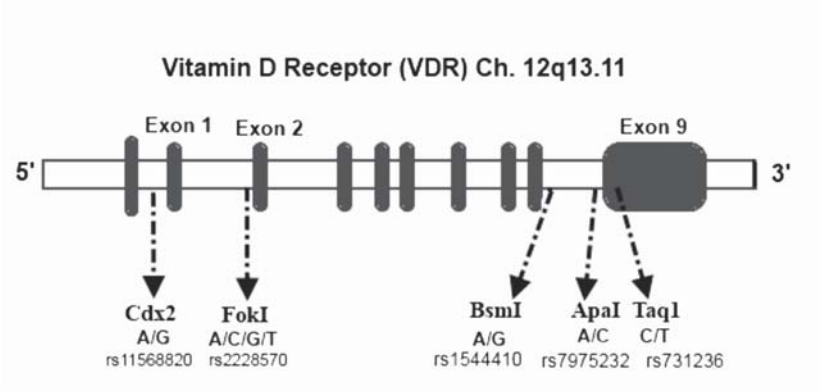

Figure 1 - The location of VDR gene polymorphisms.

Materials and methods

This case-control study was performed on Azari women attending to the hospitals and healthcare centers of Zanjan province during 2014-2015. After obtaining informed consent and medical history, 5 mL of peripheral blood was collected from 50 menopausal osteoporotic and 50 menopausal non-osteoporotic women. Osteoporosis was confirmed by performing Double-Energy X-ray Absorptiometry (DEXA) on lumbar spine (L_{1-4}) and femur neck using T-score and Z-score by dual-energy X-ray absorptiometer (DTX-200, software version 1.54((7). Demographic information was obtained by interview and questionnaire. Exclusion criteria included repeated corticosteroid use, oophorectomy, ovarian insufficiency, thyroid dysfunctions, calcium absorption defects, digestive system absorption disorders, chronic renal failure, repeated calcium supplement use, and drug abuse.

DNA was extracted using DNP™ (Cinaclone, Iran) and was stored at -20°C until use. The sequence of primers for the amplification of the target region that creates an amplicon of 490 bp length was Forward: 5′-AGCAGAGCAGAGTTCCAAGC-3′ and Reverse: 5'-GTGAGGAGGGCTGCTGAGTA-3′. PCR reaction mixture contained 2.5 μL of 10X buffer, 0.5 μL Taq DNA polymerase (5 units/μL), 1 μL of 50 mM $MgCl_2$, 0.5 μL of 10 mM dNTP mix, 5 μL DNA template, and 1 μL of 10 pmol/mL of primer mix, synthesized by Gene-All, Canada. 14.5 μL of deionized distilled water were added to reach the final volume of 25 μL. PCR was performed according to the following protocol (Corbett Life Science, Australia): 5 min at 95°C followed by 35 cycles of 30 seconds at 95°C, 30 seconds at 61°C, and 30 seconds at 72°C. A final extension step at 72°C for 10 min was considered at the end. After performing PCR, to confirm the amplification, PCR products were applied onto 1% agarose gel and electrophoresed. TaqI and ApaI restriction enzymes (Thermo Scientific) were used for PCR product digestion. TaqI cuts the 490-bp product to two fragments of 200 and 290 bp, and ApaI cuts the product to two fragments of 280 and 210 bp. TaqI cutting pattern results in determining the genotypes TT (no cut), Tt (heterozygote), and tt (cut), and ApaI cutting pattern results in determining the genotypes AA (no cut), Aa (heterozygote), and aa (cut) (8).

Independent t-test, χ^2, and Fisher exact test with odd ratio estimates as well as confidence intervals (CI) were evaluated using SPSS software (ver. 14). Logistic regression was used to evaluate the correlation between genotypes and osteoporosis occurrence. Allele frequencies and exact test using standard chi-square test for Hardy Weinberg equilibrium (HWE) were estimated by the OEGE online computer program (9) and linkage disequilibrium (10) was comparing expected and actual allele frequencies. P-value less than 0.05 were considered as statistically significant.

Results

The results of PCR-RFLP on subjects (mean age: 68.72 range: 56-88) and controls (mean age: 64.7, range: 51-81) are presented in Table 1. The TaqI and ApaI genotypes were assessed by Kurtosis-Skewness statistical test, and a normal distribution was observed. However, using Shapiro-wilk statistical test, only age distribution was shown to be normal (p=0.055), and the distribution of the two genotypes was not normal (p=0.000). TaqI and ApaI VDR allele frequencies were not similar in control and osteoporotic groups, but independent t-test (confidence interval of 95%) indicated no significant relationship between TaqI and ApaI genotypes and osteoporosis (p=0.37 and p=0.64, respectively). All SNPs were in Hardy Weinberg equilibrium, but in osteoporotic group, ApaI genotype was significantly out of Hardy-Weinberg Equilibrium. Linkage disequilibrium analysis revealed non-significant relationship between the TaqI and ApaI RFLP in osteoporosis or healthy individuals. ApaI/TaqI allele haplotypes in osteoporotic population showed no significant relation (p value=0.563) compared with control group (Table 2).

Discussion and conclusion

Although there has been scores of studies on the relationship between gene polymorphisms and osteoporosis, no research has been conducted on osteoporotic women in Zanjan province. Considering the high frequency of osteoporosis among women (around 33%) and the hypothetical role of environmental and genetic factors, this study seemed necessary. Since the past few years, study of polymorphisms of 3' region of VDR gene has captured researcher's attention (11-13). The relationship between ApaI and TaqI polymorphisms and bone disorders has been investigated in lots of studies, which has had disparate results (14-16). The relationship between TaqI, ApaI, FokI, and BsmI polymorphisms in menopausal women has been studied in different communities. In 2003, Zhang et al. reported a significant relationship between AA genotype (AA allele) of ApaI and the incidence of osteoporosis in Chinese menopausal women (17). However, their finding was not confirmed by another research group the next year

Table 1 - The frequency of genotypes in osteoporotic *vs* non-osteoporotic menopausal women based on polymorphisms.

Genotype	Non-osteoporotic (%)	Osteoporotic (%)	P value	OR (95% CI)
Apa1			0.37	
AA	30 (60%)	24 (48%)		
Aa	18 (36%)	25 (50%)		
aa	2 (4%)	1 (2%)		
HWE*	0.72	0.05		
Allele				
A	78%	73%	0.41	0.76 (0.39-1.45)
a	22%	27%	Ref	
Taq1			0.64	
TT	16 (32%)	20 (40%)		
Tt	29 (58%)	24 (48%)		
tt	5 (10%)	6 (12%)		
HWE*	0.12	0.76		
Allele				
T	61%	64%	0.66	1.13 (0.64-2.01)
t	39%	36%	Ref	

* p-value < 0.05 not consistent with HWE.

Table 2 - VDR Genotypes in osteoporotic population compared with control Group.

VDR genotype	Non-osteoporotic (%)	Osteoporotic (%)	p value
AATT	9 (18%)	9 (18%)	
AATt	17 (34%)	10 (20%)	
AAtt	4 (8%)	5 (10%)	
AaTT	7 (14%)	10 (20%)	
AaTt	10 (20%)	14 (28%)	0.563
Aatt	1 (2%)	1 (2%)	
aaTT	-	1 (2%)	
aaTt	2 (4%)	-	
aatt	-	-	

(18). In 2006, in a study on osteoporotic menopausal women in India, a significant relationship was found between aa (ApaI), bb (BsmI), FF (FokI), and TT (TaqI) genotypes and increased bone density (19). In 2013, on the other hand, in an study on osteoporotic menopausal women in India, T allele (TaqI) and AGT haplotype (ApaI, BsmI, TaqI) was determined to be related to osteoporosis (20).

In 2009, Seremak-Mrozikiewicz et al. studied osteoporotic and osteopenic menopausal women. Compared to a healthy control group, T allele and TT genotype (TaqI) in both osteoporotic and osteopenic subject has a higher frequency (21). Nonetheless, Horst-Sikorska et al. found that there was a relatiopship only between T (TaqI), b (BsmI), and a (ApaI) alleles and fractures of only non-lumbar vertebrae (22).

In 2009, Dunbar et al. studied Turkish menopausal women and showed that individuals with aa genotype had a lower bone density and higher serum calcium level in comparison with those with AA genotype (23). On the other hand, in 2004 in a similar study on Turkish menopausal women with osteoporosis, it was found that individuals with TT, Tt, and tt genotypes (TaqI) had the highest bone density and lowest serum calcitonin level (24).

In Greek menopausal women with osteoporosis, none of the polymorphisms of BsmI and TaqI had a significant relationship with bone density (BMD), osteoporosis, and bone fractures, while having a significant relationship with calcium absorption (25). On the other hand, a study on similar subjects showed the positive role of bb (BsmI), aa (ApaI), and TT (TaqI) genotypes and bAT and baT haplotypes (TaqI, ApaI, BsmI) with decreased bone mineral density (26).

Similar results was obtained from Tunisian osteoporotic menopausal women. Aa (ApaI) genotype was considered as a protective factor against osteoporosis while aa genotype was considered as an osteopenic factor related to bone fractures. In addition, Aa/TT genotype (ApaI/TaqI) was the major haplotype in individuals with normal bone mass (27). In Belarusian osteoporetic menopausal women, ApaI, TaqI, and BsmI polymorphisms were found to be an influencing factors of osteoporosis (28).

Although similar results were not obtained for the mentioned polymorphisms and osteoporosis/bone fractures in >50-year-old men in Spain, theye are not interpretable since there were too few suties on the issue (29).

Bone density and fracture in individuals with underlying disease have attracted particular attention. In a study of osteoporotic women suffering from Chronic Obstructive Pulmonary Disease (COPD) in South Korea, genotype A (ApaI) had a higher frequency in the subjects compared to healthy controls and considered as having a pivotal role in fractures in the subjects (30). In another study, the role of BsmI, TaqI, and FokI (Ff genotype) was shown to be significant in osteoporotic subjects with Rhomateuid artheritis compared with healthy controls (31). BsmI/TaqI genotypes and decreased bone density (BMD) was found to have a significant relationship in women suffering from Gaucher disease (32). However, no significant relationship was found between common VDR genotypes and bone health parameters such as osteocalcin, serum calcium, alkaline phosphatase, and bone density in Turkish subjects suffering from type 1 diabetes (6). Interestingly, although the effective role of TaqI/ApaI/BsmI genotypes and bone density in Graves hyperthyroidism was rejected, the incidence of the disease was shown to be related with the mentioned polymorphisms in Polish patients (33).

In spite of the contrasting results of the mentioned studies, in a meta-analysis review by Shen et al. on 14 studies consisting 6500 osteoporotic women, no significant relationship was found between the so far-mentioned polymorphisms and incidence of bone fracture (34). Also the results of our study are similar to Gonzá-

lez-Mercado et al. who studied on Mexican osteoporotic menopausal women (35).

It should be noted that researchers have also pursued the relationship betweenVDR gene polymorphisms and other diseases. The success of calcium (36) and calcitriol therapy has been shown in diseases such as osteoporosis (37) periodontitis (38), skin cancer (39), ovarian cancer (8), kindney and breast cancers (40), and prostate cancer (41).

Considering the different and sometimes contrasting results of the effect of polymorphisms in the incidence of bone diseases, autoimmunity, cancer, etc., it seems that the best interpretations are possible if a complex of polymorphisms and haplotypes is evaluated. In addition, even though our results showed no significant relationship between the polymorphisms of TaqI/ApaI and osteoporosis in menopausal women, by taking other studies' results into account and studying a much larger population, interpretable results can be obtained.

Conflicts of interest

The Authors declare no conflict of interest.

Acknowledgements

This study was conducted in Central laboratory of Azad University, Zanjan Branch, Zanjan, Iran.

References

1. Silver JJ, Einhorn TA. Osteoporosis and aging. Current update. Clin Orthop Relat Res. 1995;(316):10-20.
2. De Lemos ML. Seeking clarification of osteoporosis guidelines. Cmaj. 2004;171(9):1022. doi:10.1503/cmaj.1040953.
3. Wilkin TJ. Changing perceptions in osteoporosis. Bmj. 1999; 318(7187):862-4.
4. Geusens PP. Review of guidelines for testing and treatment of osteoporosis. Curr Osteoporos Rep. 2003;1(2):59-65.
5. Arai H, Miyamoto K, Taketani Y, Yamamoto H, Iemori Y, Morita K et al. A vitamin D receptor gene polymorphism in the translation initiation codon: effect on protein activity and relation to bone mineral density in Japanese women. J Bone Miner Res. 1997;12(6):915-21. doi:10.1359/jbmr. 1997;12:6:915.
6. Kocabas A, Karaguzel G, Imir N, Yavuzer U, Akcurin S. Effects of vitamin D receptor gene polymorphisms on susceptibility to disease and bone mineral density in Turkish patients with type 1 diabetes mellitus. J Pediatr Endocrinol Metab. 2010;23(12):1289-97.
7. Lorentzon M, Cummings SR. Osteoporosis: the evolution of a diagnosis. J Intern Med. 2015;277(6):650-61. doi:10.1111/joim.12369.
8. Onsory K, Bakhtiari Tajar M, Davoodi B, Heydari Ashrafi M, Abdollahi M. The role of polymorphism of TaqI in Vitamin D receptor gene and risk of ovarian cancer in women of North India. Journal of Fasa University of Medical Sciences. 2013;3(3):260-5.
9. Rodriguez T, Gaunt TR, Day LNM. Hardy-Weinberg Equilibrium Testing of Biological Ascertainment for Mendelian Randomization Studies. American Journal of EpidemiologyJ Heredity. 2009;6.
10. Gaunt T, Santiago Rodríguez S, INM D. Cubic exact solutions for the estimation of pairwise haplotype frequencies: implications for linkage disequilibrium analyses and a web tool 'CubeX'. BMC Bioinformatics. 2007;8:428.
11. Gross C, Eccleshall TR, Malloy PJ, Villa ML, Marcus R, Feldman D. The presence of a polymorphism at the translation initiation site of the vitamin D receptor gene is associated with low bone mineral density in postmenopausal Mexican-American women. J Bone Miner Res. 1996;11(12):1850-5. doi:10.1002/jbmr.5650111204.
12. 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. doi:10.1038/367284a0.
13. Nguyen TV, Kelly PJ, Morrison NA, Sambrook PN, Eisman JA. Vitamin D receptor genotypes in osteoporosis. Lancet. 1994;344(8936): 1580-1.
14. Zhang ZL, Zhao JX, Meng XW, Zhou XY, Xing XP, Xia WB. [Association of polymorphisms of vitamin D receptor gene start codon and 3'-end region with bone mineral density in postmenopausal women]. Zhonghua Yi Xue Yi Chuan Xue Za Zhi. 2003;20(1):5-8.
15. Zintzaras E, Rodopoulou P, Koukoulis GN. BsmI, TaqI, ApaI and FokI polymorphisms in the vitamin D receptor (VDR) gene and the risk of osteoporosis: a meta-analysis. Dis Markers. 2006;22(5-6):317-26.
16. Zmuda JM, Cauley JA, Danielson ME, Wolf RL, Ferrell RE. Vitamin D receptor gene polymorphisms, bone turnover, and rates of bone loss in older African-American women. J Bone Miner Res. 1997;12(9):1446-52. doi:10.1359/jbmr.1997.12.9.1446.
17. Zhang YY, Long JR, Liu PY, Liu YJ, Shen H, Zhao LJ, et al. Estrogen receptor alpha and vitamin D receptor gene polymorphisms and bone mineral density: association study of healthy pre- and postmenopausal Chinese women. Biochem Biophys Res Commun. 2003;308(4):777-83.
18. Qin YJ, Zhang ZL, Huang QR, He JM, Hu YQ, Zhao Q, et al. Association of vitamin D receptor and estrogen receptor-alpha gene polymorphism with peak bone mass and bone size in Chinese women. Acta Pharmacol Sin. 2004;25(4):462-8.
19. Mitra S, Desai M, Ikram Khatkhatay M. Vitamin D receptor gene polymorphisms and bone mineral density in postmenopausal Indian women. Maturitas. 2006;55(1):27-35. doi:10.1016/j.maturitas. 2006; 01:003.
20. Singh M, Singh P, Singh S, Kumar Juneja P, Kaur T. Vitamin D receptor (VDR) gene polymorphism influences the risk of osteoporosis in postmenopausal women of Northwest India. Arch Osteoporos. 2013;8:147. doi:10.1007/s11657-013-0147-y.
21. Seremak-Mrozikiewicz A, Drews K, Mrozikiewicz PM, Bartkowiak-Wieczorek J, Marcinkowska M, Wawrzyniak A et al. Correlation of vitamin D receptor gene (VDR) polymorphism with osteoporotic changes in Polish postmenopausal women. Neuro Endocrinol Lett. 2009;30(4):540-6.
22. 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. Mol Biol Rep. 2013;40(1):383-90. doi:10.1007/s11033-012-2072-3.
23. Dundar U, Solak M, Kavuncu V, Ozdemir M, Cakir T, Yildiz H, et al. Evidence of association of vitamin D receptor Apa I gene polymorphism with bone mineral density in postmenopausal women with osteoporosis. Clin Rheumatol. 2009;28(10):1187-91. doi:10.1007/s 10067-009-1220-1.
24. Duman BS, Tanakol R, Erensoy N, Ozturk M, Yilmazer S. Vitamin D receptor alleles, bone mineral density and turnover in postmenopausal osteoporotic and healthy women. Med Princ Pract. 2004;13(5):260-6. doi:10.1159/000079524.
25. Stathopoulou MG, Dedoussis GV, Trovas G, Theodoraki EV, Katsalira A, Dontas IA, et al. The role of vitamin D receptor gene polymorphisms in the bone mineral density of Greek postmenopausal women with low calcium intake. J Nutr Biochem. 2011;22(8):752-7. doi:10.1016/j.jnutbio.2010.06.007.
26. 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(3):191-7.
27. Sassi R, Sahli H, Souissi C, Sellami S, Ben Ammar El Gaaied A. Polymorphisms in VDR gene in Tunisian postmenopausal women are associated with osteopenia phenotype. Climacteric. 2015;18(4):624-30. doi:10.3109/13697137.2015.1007123.
28. Marozik P, Mosse I, Alekna V, Rudenko E, Tamulaitiene M, Ramanau H, et al. Association Between Polymorphisms of VDR, COL1A1, and LCT genes and bone mineral density in Belarusian women with severe postmenopausal osteoporosis. Medicina (Kaunas). 2013;49(4):177-84.
29. Alvarez-Hernandez D, Naves M, Diaz-Lopez JB, Gomez C, Santamaria I, Cannata-Andia JB. Influence of polymorphisms in VDR and COLIA1 genes on the risk of osteoporotic fractures in aged men. Kid-

ney Int Suppl. 2003;(85):S14-8. doi:10.1046/j.1523-1755.63.s85.5.x.
30. Kim SW, Lee JM, Ha JH, Kang HH, Rhee CK, Kim JW, et al. Association between vitamin D receptor polymorphisms and osteoporosis in patients with COPD. Int J Chron Obstruct Pulmon Dis. 2015;10:1809-17. doi:10.2147/COPD.S91576.
31. Mosaad YM, Hammad EM, Fawzy Z, Abdal Aal IA, Youssef HM, ElSaid TO, et al. Vitamin D receptor gene polymorphism as possible risk factor in rheumatoid arthritis and rheumatoid related osteoporosis. Hum Immunol. 2014;75(5):452-61. doi:10.1016/j.humimm.2014.02.009.
32. Greenwood A, Elstein D, Zimran A, Altarescu G. Effect of vitamin D receptor (VDR) genotypes on the risk for osteoporosis in type 1 Gaucher disease. Clin Rheumatol. 2010;29(9):1037-41. doi:10.1007/s10067-010-1464-9.
33. Horst-Sikorska W, Ignaszak-Szczepaniak M, Marcinkowska M, Kaczmarek M, Stajgis M, Slomski R. Association analysis of vitamin D receptor gene polymorphisms with bone mineral density in young women with Graves' disease. Acta Biochim Pol. 2008;55(2):371-80.
34. Shen H, Xie J, Lu H. Vitamin D receptor gene and risk of fracture in postmenopausal women: a meta-analysis. Climacteric. 2014;17(4):319-24. doi:10.3109/13697137.2013.856401.
35. Gonzalez-Mercado A, Sanchez-Lopez JY, Regla-Nava JA, Gamez-Nava JI, Gonzalez-Lopez L, Duran-Gonzalez J, et al. Association analysis of vitamin D receptor gene polymorphisms and bone mineral density in postmenopausal Mexican-Mestizo women. Genet Mol Res. 2013;12(3):2755-63. doi:10.4238/2013.July.30.13.
36. Chang B, Schlussel Y, Sukumar D, Schneider SH, Shapses SA. Influence of vitamin D and estrogen receptor gene polymorphisms on calcium absorption: BsmI predicts a greater decrease during energy restriction. Bone. 2015;81:138-44. doi:10.1016/j.bone.2015.07.011.
37. Morrison NA, George PM, Vaughan T, Tilyard MW, Frampton CM, Gilchrist NL. Vitamin D receptor genotypes influence the success of calcitriol therapy for recurrent vertebral fracture in osteoporosis. Pharmacogenet Genomics. 2005;15(2):127-35.
38. Kaarthikeyan G, Jayakumar ND, Padmalatha O, Varghese S, Anand B. Analysis of association of TaqI VDR gene polymorphism with the chronic periodontitis in Dravidian ethnicity. Indian J Hum Genet. 2013;19(4):465-8. doi:10.4103/0971-6866.124377.
39. Chen L, Smith GD, Evans DM, Cox A, Lawlor DA, Donovan J, et al. Genetic variants in the vitamin d receptor are associated with advanced prostate cancer at diagnosis: findings from the prostate testing for cancer and treatment study and a systematic review. Cancer Epidemiology Biomarkers & Prevention. 2009;18(11):2874-81.
40. Khan MI, Bielecka ZF, Najm MZ, Bartnik E, Czarnecki JS, Czarnecka AM, et al. Vitamin D receptor gene polymorphisms in breast and renal cancer: current state and future approaches (review). Int J Oncol. 2014;44(2):349-63. doi:10.3892/ijo.2013.2204.
41. Zhang Q, Shan Y. Genetic polymorphisms of vitamin D receptor and the risk of prostate cancer: a meta-analysis. J Buon. 2013;18(4):961-9.