OPEN ACCESS

Molecular Medicine Communications

DOI: 10.55627/mmc.003.02.0380

Research Article

Association of Vitamin D Gene Receptor Polymorphism with Osteoporosis in Postmenopausal Women

Mohammad Zubair[1], Saeed Khan[2*], Akhtar Ali[3], Farina Hanif[4], Uzair Abbas[1], Bilal Ahmed[2], Safia Mehmood[5], Fatima Khan[5]

[1]Department of Physiology, Dow International Medical College, Dow University of Health Sciences, Karachi, Pakistan
[2]Department of Pathology, Dow International Medical College, Dow University of Health Sciences, Karachi, Pakistan
[3]National Institute of Diabetes and Endocrinology, Dow University of Health Sciences, Karachi, Pakistan
[4]Department of Biochemistry, Dow International Medical College, Dow University of Health Sciences, Karachi, Pakistan
[5]Dow Medical College, Dow University of Health Sciences, Karachi, Pakistan
[*]Correspondence: saeed.khan@duhs.edu.pk

Abstract

Osteoporosis is a critical and complicated systemic musculoskeletal disorder that encompasses low bone density (LBD) and weakening of osseous tissue at a microarchitectural level. This predisposes to increased bone fragility and subsequent risk of fractures. It is frequently encountered in postmenopausal women and has also been seen in elderly men. Vitamin D synchronizes bone and calcium homeostasis in the body by modifying the transcription of target genes through the Vitamin D receptor (VDR) and thus aids calcium uptake and bone-forming proteins. The main objective of the present research was to study the relationship of single nucleotide polymorphisms (SNP) (rs1544410 G>A) of the *VDR* gene with postmenopausal women who have osteoporosis in a Pakistani cohort. A total of 100 postmenopausal women, 50 without osteoporosis to be in the control group, and 50 with osteoporosis as cases, were enrolled in this study. The patients had no immediate family connections with each other. All the patients underwent BMD measurements at the lumbar spine and femoral neck by dual-energy X-ray absorptiometry scan for their diagnosis of osteoporosis based on World Health Organization (WHO) criteria (T score < -2.5 SD). A total of 25 participants had at least one allele of rs1544410, of which 14 were cases and 11 controls. The number of heterozygous and homozygous were present with similar ratios in both groups (p=0.973). Age was noted to be correlated with osteoporosis (OR: 1.06; 95%CI: 1.004-1.12), (P<0.031). It was also observed that cases had a notably lower weight than the control group (P<0.0001) and the overall body mass index of cases was significantly low in comparison to control (P<0.0001). Moreover, average BMD of femoral and BMD lumbar were notably greater among the control group (P<0.0001) as compared to cases. The SNP (rs1544410) is not significantly associated with BMD but significantly correlated with age which was identified as a risk factor while body mass index was found to be a protective factor for osteoporosis.

Keywords: Osteoporosis, single nucleotide polymorphism, vitamin D receptor, bone mineral density

1. Introduction

Alzheimer's is a systemic musculoskeletal disorder comprising decreased bone mass with altered bone microarchitecture, resulting in weaker bones and an increased risk of fracture. It is the most common disease of the bone in humans; with approximately every other Caucasian woman, and one in five men above the age of fifty likely to suffer from it and encounter osteoporosis-related fractures during their lifetime. (Cosman et al., 2014). Its prevalence increases with increasing age. Consequently, it is also likely to become a major health concern in Pakistan, due to its increasing

life expectancy from 60 years in 1995 to 67 years in 2015 and a predicted 71 years in 2025 (Nagi et al., 2013). A study guided by quantitative ultrasound established that there were 9.90 million patients of osteoporosis in Pakistan, out of which 7.19 million were female while 2.71 million were male, and this number would rise to 11.3 million in 2020 and to 12.91 million in 2050 respectively (Haris, 2014). There are several known risk factors of osteoporosis including age, low bone mineral density (BMD), a lack of vitamin D intake in daily life, smoking, caffeine and alcohol intake, higher body weight, broken bones because of an accident, ethnicity, and family history. There is also an increased threat of osteoporosis among women as they age and during the menopausal period when the levels of estrogen wane. This has been linked to increased loss of bone and risk of fractures (Lane, 2006). Bone density peaks around the age of 30 among women. After that they start losing their bone mass; therefore, women are required to have an adequate intake of calcium and vitamin D in their diet to keep their bones strong for as long as possible. Hence, the focus of the present study is vitamin D insufficiency which ultimately contributes to the rising incidence of osteoporosis among women (Bouillon et al., 1995).

1, 25-Dihydroxycholecalciferol D_3 (Vitamin D_3) is a biologically essential substance that has a great impact on BMD. Calcium homeostasis, control of cell proliferation, and differentiation to many target tissues are a few of the prominent functions of Vitamin D3. Active vitamin D_3-regulated absorption of calcium has been demonstrated in the proximal and distal intestines. Vitamin D deficit is a common condition that may lead to exceptional loss of BMD and the development of osteoporosis and fracture especially in elderly female patients (Ivanova et al., 2015; Man et al., 2016).

Vitamin D receptor (VDR) is a nuclear transcription factor and the expression of target genes under its control accelerate most of the biological actions of vitamin D_3. Like other steroid hormone receptors, it can regulate various kinds of biological activities throughout many tissues. Such properties add to the impression that vitamin D deficiency may not only be harmful to bone and muscular health but may have general adverse medical outcomes (Ryan et al., 2015). The gene encoding VDR has been identified on chromosome 12, has 12 exons, and spans ~75 kb of genomic DNA (Miyamoto et al., 1997).

The role of VDR in calcium absorption in the aging intestine is debatable; some studies have reported a decline in intestinal VDR expression in humans and animals with age and others report no difference. In several reports, twin, family, and environmental analyses have concluded that genetics strongly influence BMD and bone turnover (Kelly et al., 1995; Yoshida & Stern, 2012). A single nucleotide polymorphism (SNP) of the *VDR* gene might affect the function and expression of the VDR protein. This contributes to a previously demonstrated influence on BMD and the likelihood of osteoporosis (Qin et al., 2013). Several studies have concluded that complicated connections exist between bone health and VDR (Morrison et al., 1994; Turner et al., 2012). Furthermore, numerous studies have investigated SNPs in the *VDR* gene such as BsmI (rs1544410), ApaI (rs7975232), and TaqI (rs731236) for their effect on BMD and fracture risk. According to some studies, a considerable relationship exists between *VDR* gene polymorphism (rs1544410) and BMD (Mencej-Bedrac et al., 2009), however; other studies report no change, and thus discrepancies exist (Yu et al., 2016).

Keeping in mind the impact of *VDR* and its polymorphisms on BMD, this study aimed to investigate the association of an SNP (G>A,

Table 1. Baseline Demographic Characteristics of Participants.

Variables	Control	Case	OR (95% CI)	p Value
Age (year)	53.7±7.3	57.0±7.9	1.06 (1.00-1.12)	0.031
Height (m)	150.9±5.9	149.2±9.2	0.97 (0.95-0.99)	0.325
Weight (kg)	73.6±14.8	61.4±11.8	0.93 (0.90-0.97)	<0.0001
BMI (kg/m^2)	23.9±4.7	20.5±4.8	0.84 (0.76-0.93)	<0.0001
BMD Femoral	0.9±0.2	0.7±0.1	0.000 (0.000-0.001)	<0.0001
BMD Lumber	0.8±0.3	0.7±0.1	0.002 (0.0001-0.058)	<0.0001
T Score Femoral	-0.4±0.8	-2.1±1.0	0.08 (0.03-0.23)	<0.0001
T Score Lumber	-1.2±0.9	-3.3±0.8	0.01 (0.00-0.10)	<0.0001
Age at Menarche	15.6±2.4	15.0±2.3	0.98 (0.96-0.99)	0.247
Years since	9.7±5.3	12.6±8.1	1.07 (1.00-1.15)	0.043
Number of Pregnancies	7.0±2.9	6.6±2.4	0.96 (0.95-0.99)	0.431

rs1544410) within the *VDR* gene with osteoporosis in Pakistani postmenopausal female patients.

2. Material and Methods

2.1. Patient Recruitment

A sample size of 100 was calculated with 80% power and 0.10 level of significance using OpenEpi. Females at least 5 years post-menopausal were recruited to the study. 50 cases and 50 controls were taken based on dual-energy X-ray absorptiometry (DEXA) scan reports. All patients were female, unrelated, and at least 5 years postmenopausal. Osteoporosis is diagnosed based on two criteria; WHO densitometry criteria with the DEXA scan (T score < -2.5 SD) being a gold standard or a low-energy fracture due to minor trauma or collapse from a standing position or fracture at the age of 50 or later.

Those patients who had diseases, treatments, and/or conditions which might impact bone metabolism; such as endocrine disorders like hypoparathyroidism, hyperparathyroidism, hypothyroidism, hyperthyroidism, Cushing's syndrome, diabetes mellitus as well as chronic liver disease (for more than 6 months e.g. liver cirrhosis, chronic hepatitis, hepatocellular carcinoma), chronic renal disease (as per GFR and creatinine measurements) chronic gastrointestinal disease (for more than 6 months e.g. chronic pancreatitis, chronic diarrhea) and malignancy of any type, were excluded.

Patients were recruited from the outpatient department of the orthopedic clinic in Dow University Hospital Karachi, Pakistan where they visited due to their severe musculoskeletal pain and undertook DEXA scans at the lumbar spine and femoral neck. Comprehensive personal profiles comprising medical, family, and past treatment history were acquired from all subjects. Both control groups and cases were compared for body mass index (BMI), age, height, weight, menarche, menopause, pregnancy rate, and occupation. BMD values between >-1 and <-2.5 SD (WHO criteria) were employed in this analysis. The Institutional Review Board of Dow University of Health Sciences approved this study. Written informed consent was taken from all subjects.

Table 2: Status of Smoking, Exercise, Homoeopathic Medications, and Dairy Products Intake.

Variables	Status	Controls	Cases	pValue
Smoking	No	48 (96.0%)	46 (92.0%)	0.4
	Yes	2 (4.0%)	4 (8.0%)	
Exercise/Walk	No	37 (74.0%)	35 (70.0%)	0.656
	Yes	13 (26.0%)	15 (30.0%)	
Homeopathic Medications	No	39 (78.0%)	38 (76.0%)	0.812
	Yes	11 (22.0%)	12 (24.0%)	
Dairy Products Intake	No	38 (76.0%)	33 (66.0%)	0.271
	Yes	12 (24.0%)	17 (34.0%)	

2.2. Experimental

2.2.1. Bone Marrow Density

BMD at the femoral neck (FN) and lumbar spine (L1- L4), measurements were performed by using a DEXA scan (Model: Discovery W S/N 88799). Scores were stated by way of grams per square centimeter (g/cm^2).

2.2.2. Sample Collection

Three (3) ml of blood was collected from all patients in EDTA tubes after having taken written consent.

2.2.3. DNA Extraction, PCR, & Sequencing

DNA was isolated from blood by Qiagen DNA mini kit (Qiagen, Germany) as per the manufacturer's recommendation. The region of the *VDR* gene BsmI encompassing polymorphism [G/A, rs154410] was amplified using commercially available DreamTaq green PCR master mix (Thermo Scientific, USA) with the Forward primer: 5'AGTGTGCAGGCGATTCGTAG3' and Reverse primer: 5'ATAGGCAGAACCATCTCTCAG3') from the region. The resultant PCR product was digested with BsmI to determine rs154410 polymorphism.

Amplified products of *VDR* gene fragments were sent to Macrogen for commercial sequencing. For SNP analyses, the obtained sequences of this study were aligned against the reference sequence of the *VDR* gene (NG_008731.1) by using bioinformatics tools, BioEdit, and MEGA 6.0.

2.3 Statistical analysis

A chi-square test of independence was performed to check the association of categorical variables with the status of osteoporosis. Continuous variables were initially checked for normality by Shapiro-Wilk's test. It was found that age, weight, and number of pregnancies were normally distributed among cases and controls. Therefore, an independent samples t-test was executed to compare mean scores of age, weight, and number of pregnancies between cases and controls. The average score of other continuous variables such as BMI, BMD, T score, age at menarche, and years since menopause were compared by the Mann-Whitney U test. Variables with p-value <0.05 were further processed for logistic regression analysis to measure the association of those variables with osteoporosis status. The odds ratio with a 95% confidence interval was reported from the output of logistic regression.

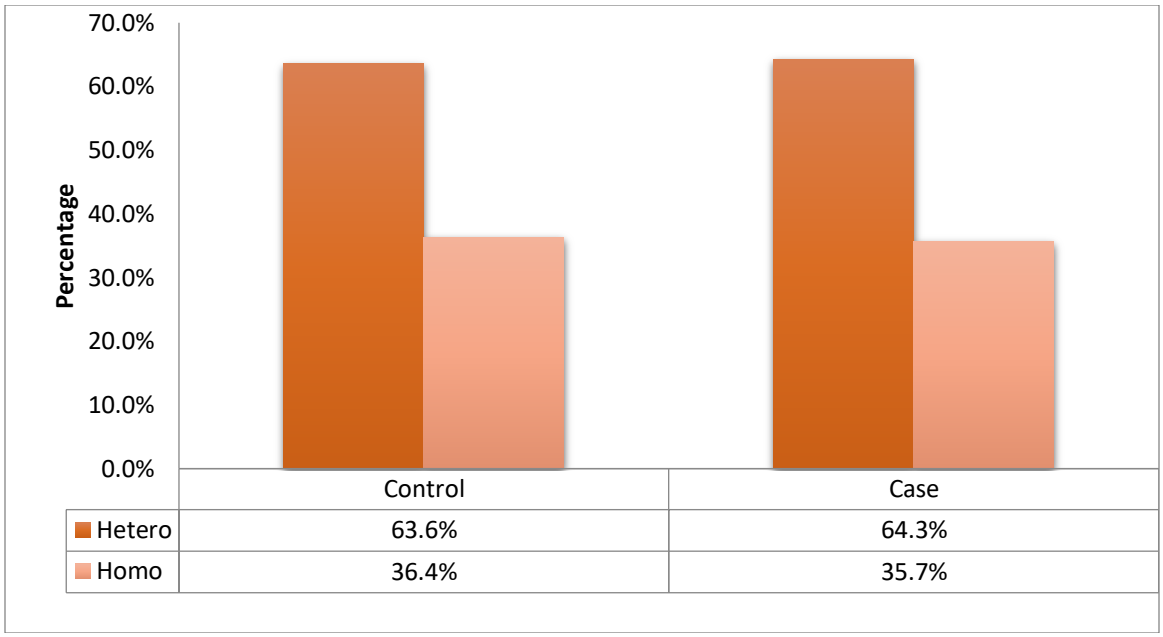

Figure 1: Comparison of zygosity between controls and cases.

3. Results

The baseline characteristics of the participants are given in Tables 1 and 2. A total of 25 participants had SNP, of which 56% (14) were cases and 44% (11) controls. The heterozygosity and homozygosity were present with similar proportions in both cases and controls (P=0.973). The output from logistic regression revealed that each addition of a single year to the age of patients increases the possibility of her being osteoporotic by 6% (OR: 1.06; 95%CI: 1.004-1.12). Logistic output also depicted that there were 16% more chances of patients being osteoporotic if BMI was reduced to 1 kg/m^2 (OR: 0.84; 95%CI: 0.76-0.93). An increase in femoral or lumbar T scores by one point elevated the chances of osteoporosis by 8% and 1% respectively.

SNPs Analysis

Partial *VDR* gene fragments from all 100 samples were sequenced and aligned against the reference sequences (NG_008731.1). The results showed that 25% (n=25) of the samples had the same SNP G>A at position g.63980 (NG_008731.1:g.63980G>A), which has been reported as an intronic variant (rs1544410). 56% (n=14) of the cases had this SNP including 64.3% (n=9) heterozygous and 35.7% (n=5) homozygous cases. Moreover, 44% (n=11) of the controls had this SNP including 63.6% (n=7) heterozygous and 36.4% (n=4) homozygous SNP. The hetero and homozygosity were present with similar proportions in both cases and controls (P=0.973) (figure 1).

4. Discussion

The results of our study are in agreement with a previous study conducted by Cosman and his colleagues that showed a positive association between osteoporosis and age (Cosman et al., 2014). The bone mass in adults is equal to the ultimate bone mass attained between 18 to 25 years of age minus the amount of bone lost thereafter. The peak bone mass is highly dependent on genetic factors, including a secondary role played by physical activity, nutrition, endocrine status, and health throughout growth. The procedure of bone remodeling that sustains a strong skeleton may

be regarded as a protective maintenance mechanism that frequently removes older bone and replaces it with new bone. Loss of bone takes place when this balance is disturbed, resulting in greater bone loss than restoration and the difference increases with age.

This investigation revealed that the average BMD femoral and BMD lumbar were notably higher among the control group as compared to cases as evidenced by a substantial difference in femoral and lumbar T scores between cases and controls. An increase in the femoral or lumbar T score increases the chances of osteoporosis. These findings are consistent with those of Ivanova and colleagues which showed that multiple fragility fractures developed in postmenopausal women who had a BMD T-score exceeding -2.5 (Ivanova et al., 2015). The use of BMD values can help comprehensive evaluation of future fracture risk, aiding one to identify women at risk and plan treatment in advance to avoid osteoporotic fractures.

The difference in femoral T scores between cases and controls is in accordance with the investigation conducted by Mittal and colleagues which, among other things, also confirmed that femoral neck fracture was more common in elderly people. However, the most common cause of femoral neck fractures is trauma. Anyone can get a fracture in their femoral neck, but trauma is more common in elderly people who have poor bone density. More than 90 percent of these fractures occur in people older than 50 years or in those who have a medical condition that weakens the bones, such as osteoporosis. This increases the risk of a fracture in the femoral neck, commonly in women presumably because of the surge in bone turnover and prompt loss of BMD (Mittal & Banerjee, 2012). Moreover, the considerable difference in the T scores of the lumbar spine between cases and controls is consistent with the study conducted by Anastasilakis and colleagues (Anastasilakis et al., 2017).

Cosman and colleagues showed that the risk of osteoporosis rises with increasing age after menopause (Cosman et al., 2014; Jasani et al., 1965). This is also corroborated by the present study as the years since menopause were significantly longer in cases as compared to the control group.

Other studies suggested that osteoporosis commonly starts in women between the ages of 45 and 50. National Osteoporosis Foundation of the USA described that roughly every other woman above the age of 50 years will suffer a bone fracture owing to osteoporosis (2019-NOF-Overview.Pdf, n.d.). The probability of breaking a hip is equivalent to the collective cancer risk of the breast, uterus, and ovary. There are several reasons for this, including genetics and levels of the hormone estrogen. Estrogen prevents resorption and increases calcium deposition in bones and its levels fall when women reach menopause, which eventually leads to bone loss and consequent osteoporosis.

The present study also demonstrated that age at menarche was not statistically significant in cases and controls. This is consistent with the other findings which report that after menopause, age is correlated with low BMD in postmenopausal females but not age at menarche (Qiu et al., 2013; Sioka et al., 2010). No significant association was found between the number of pregnancies, smoking, exercise, use of dairy products, and usage of homeopathic medicine intake with osteoporosis.

Although the results of this investigation demonstrated that one-fourth of the study participants had the SNP (rs1544410), however, there was no significant association of this variant with osteoporosis in Pakistani postmenopausal women. Some of the previous studies supported a correlation among *VDR* gene polymorphisms, BMD, and vulnerability to

postmenopausal osteoporosis (PMOP) (Dehghan & Pourahmad-Jaktaji, 2016; Mansour et al., 2010; Mitra et al., 2006; Pouresmaeili et al., 2013). However, others have been unable to find evidence of any such correlation (Fang et al., 2006; Horst-Sikorska et al., 2013; Lim et al., 1995; Pollak et al., 2001; Zhang et al., 1998). The reasons for such variability among these studies can be caused by diverse factors such as ethnicity, sample size, study design, age, and lifestyle factors (calcium intake, physical activity, obesity). Each one of these factors could affect gene regulation in distinct genotypes and subjects resulting in BMD loss or gain (Musumeci et al., 2009; Richards et al., 2008). It should also be noted that there are other genes and pathways such as those for estrogen signaling, Wnt/β-catenin as well as the transforming growth factor superfamily that may modify the effects of *VDR* polymorphisms on BMD and thus its impact on the development of osteoporosis (Fan et al., 2016; Geng et al., 2007).

There were certain limitations in the present study; firstly, the patient sample size was relatively small compared to the total population of the Karachi region and thus whether and how this SNP impacted bone microarchitecture needs further investigation in a larger cohort. Secondly, the patients were only recruited from the Karachi region. Genetic polymorphism studies should cover multiple regions and races to investigate the development of osteoporosis among various ethnicities. Lastly, future studies in a larger cohort of postmenopausal women should focus on different haplotypes, rather than SNP to explain the overall effects of common *VDR* polymorphisms.

5. Conclusions

In conclusion, we found a lack of significant association between the common *VDR* polymorphism ((rs1544410),) and BMD in a sample of Pakistani postmenopausal women. To the best of our knowledge, this is the first study that examined the association of the SNP (rs1544410) with osteoporosis in postmenopausal women in our population. Age was correlated with BMD and identified as a risk factor while BMI was positively associated with BMD and identified as a protective factor.

Author Contributions

SA conceptualized the study, MZ UA, BA, and FK performed the experiments, AA, FH, and SM analyzed the results, SA, and ZA wrote the initial manuscript, All the authors contributed to the final manuscript.

Funding

It was a self-funded study. No intramural or extramural funding was obtained from any funding agency for this research project.

Conflicts of interest

The authors of the study have no conflict of interest with this study.

Ethics Approval

This study was approved by the ethical committee of Dow University of Health Sciences, Pakistan.

Consent to Participate

Participants were included after taking written informed consent from them.

Availability of Data

The data used in this manuscript is available with the corresponding author.

References

2019-NOF-Overview.pdf. (n.d.). Retrieved March 31, 2022, from http://www.bonehealthandosteoporosis.org

/wp-content/uploads/2019-NOF-Overview.pdf

Anastasilakis, A. D., Yavropoulou, M. P., Makras, P., Sakellariou, G. T., Papadopoulou, F., Gerou, S., & Papapoulos, S. E. (2017). Increased osteoclastogenesis in patients with vertebral fractures following discontinuation of denosumab treatment. *European Journal of Endocrinology*, *176*(6), 677–683. https://doi.org/10.1530/EJE-16-1027

Bouillon, R., Okamura, W. H., & Norman, A. W. (1995). Structure-function relationships in the vitamin D endocrine system. *Endocrine Reviews*, *16*(2), 200–257. https://doi.org/10.1210/edrv-16-2-200

Cosman, F., de Beur, S. J., LeBoff, M. S., Lewiecki, E. M., Tanner, B., Randall, S., Lindsay, R., & National Osteoporosis Foundation. (2014). Clinician's Guide to Prevention and Treatment of Osteoporosis. *Osteoporosis International: A Journal Established as Result of Cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA*, *25*(10), 2359–2381. https://doi.org/10.1007/s00198-014-2794-2

Dehghan, M., & Pourahmad-Jaktaji, R. (2016). The Effect of Some Polymorphisms in Vitamin D Receptor Gene in Menopausal Women with Osteoporosis. *Journal of Clinical and Diagnostic Research : JCDR*, *10*(6), RC06-RC10. https://doi.org/10.7860/JCDR/2016/17147.8006

Fan, H., Ji, F., Lin, Y., Zhang, M., Qin, W., Zhou, Q., & Wu, Q. (2016). Electroacupuncture stimulation at CV4 prevents ovariectomy-induced osteoporosis in rats via Wnt-β-catenin signaling. *Molecular Medicine Reports*, *13*(3), 2485–2491. https://doi.org/10.3892/mmr.2016.4849

Fang, Y., Rivadeneira, F., van Meurs, J. B. J., Pols, H. A. P., Ioannidis, J. P. A., & Uitterlinden, A. G. (2006). Vitamin D receptor gene BsmI and TaqI polymorphisms and fracture risk: A meta-analysis. *Bone*, *39*(4), 938–945. https://doi.org/10.1016/j.bone.2006.04.016

Geng, L., Yao, Z., Yang, H., Luo, J., Han, L., & Lu, Q. (2007). Association of CA repeat polymorphism in estrogen receptor beta gene with postmenopausal osteoporosis in Chinese. *Journal of Genetics and Genomics = Yi Chuan Xue Bao*, *34*(10), 868–876. https://doi.org/10.1016/S1673-8527(07)60098-8

Haris, S. (2014). To Determine the Risk Factors and Prevalence of Osteoporosis among Adult Pakistani Population Residing in Karachi Using Quantitative Ultrasound Technique. *Journal of Community Medicine & Health Education*, *04*(04). https://doi.org/10.4172/2161-0711.1000299

Horst-Sikorska, W., Dytfeld, J., Wawrzyniak, A., Marcinkowska, M., Michalak, M., Franek, E., Napiórkowska, L., Drwęska, N., & Słomski, R. (2013). Vitamin D receptor gene polymorphisms, bone mineral density and fractures in postmenopausal women with osteoporosis. *Molecular Biology Reports*, *40*(1), 383–390. https://doi.org/10.1007/s11033-012-2072-3

Ivanova, S., Vasileva, L., Ivanova, S., Peikova, L., & Obreshkova, D. (2015). Osteoporosis: Therapeutic Options. *Folia Medica*, *57*(3–4), 181–190. https://doi.org/10.1515/folmed-2015-0037

Jasani, C., Nordin, B. E., Smith, D. A., & Swanson, I. (1965). SPINAL OSTEOPOROSIS AND THE MENOPAUSE. *Proceedings of the Royal Society of Medicine*, *58*, 441–444.

Kelly, P. J., Morrison, N. A., Sambrook, P. N., Nguyen, T. V., & Eisman, J. A. (1995). Genetic influences on bone turnover, bone density and fracture. *European Journal of Endocrinology*, *133*(3), 265–271. https://doi.org/10.1530/eje.0.1330265

Lane, N. E. (2006). Epidemiology, etiology, and diagnosis of osteoporosis. *American Journal of Obstetrics and Gynecology*, *194*(2 Suppl), S3-11. https://doi.org/10.1016/j.ajog.2005.08.047

Lim, S. K., Park, Y. S., Park, J. M., Song, Y. D., Lee, E. J., Kim, K. R., Lee, H. C., & Huh, K. B. (1995). Lack of association between vitamin D receptor genotypes and osteoporosis in Koreans. *The Journal of Clinical Endocrinology and Metabolism*, *80*(12), 3677–3681. https://doi.org/10.1210/jcem.80.12.8530619

Man, P. W., van der Meer, I. M., Lips, P., & Middelkoop, B. J. C. (2016). Vitamin D status and bone mineral density in the Chinese population: A review. *Archives of Osteoporosis*, *11*, 14. https://doi.org/10.1007/s11657-016-0265-4

Mansour, L., Sedky, M., AbdelKhader, M., Sabry, R., Kamal, M., & El-Sawah, H. (2010). The role of vitamin D receptor genes (FOKI and BSMI) polymorphism in osteoporosis. *Middle East Fertility Society Journal*, *15*, 79–83. https://doi.org/10.1016/j.mefs.2010.05.002

Mencej-Bedrac, S., Prezelj, J., Kocjan, T., Teskac, K., Ostanek, B., Smelcer, M., & Marc, J. (2009). The combinations of polymorphisms in vitamin D receptor, osteoprotegerin and tumour necrosis factor superfamily member 11 genes are associated with bone mineral density. *Journal of Molecular Endocrinology*, *42*(3), 239–247. https://doi.org/10.1677/JME-08-0108

Mitra, S., Desai, M., & Ikram Khatkhatay, M. (2006). Vitamin D receptor gene polymorphisms and bone mineral density in postmenopausal Indian women. *Maturitas*, *55*(1), 27–35. https://doi.org/10.1016/j.maturitas.2006.01.003

Mittal, R., & Banerjee, S. (2012). Proximal femoral fractures: Principles of management and review of literature. *Journal of Clinical Orthopaedics and Trauma*, *3*(1), 15–23. https://doi.org/10.1016/j.jcot.2012.04.001

Miyamoto, K., Kesterson, R. A., Yamamoto, H., Taketani, Y., Nishiwaki, E., Tatsumi, S., Inoue, Y., Morita, K., Takeda, E., & Pike, J. W. (1997). Structural organization of the human vitamin D receptor chromosomal gene and its promoter. *Molecular Endocrinology (Baltimore, Md.)*, *11*(8), 1165–1179. https://doi.org/10.1210/mend.11.8.9951

Morrison, N. A., Qi, J. C., Tokita, A., Kelly, P. J., Crofts, L., Nguyen, T. V., Sambrook, P. N., & Eisman, J. A. (1994). Prediction of bone density from vitamin D receptor alleles. *Nature*, *367*(6460), 284–287. https://doi.org/10.1038/367284a0

Musumeci, M., Vadalà, G., Tringali, G., Insirello, E., Roccazzello, A. M., Simpore, J., & Musumeci, S. (2009). Genetic and environmental factors in human osteoporosis from Sub-Saharan to Mediterranean areas. *Journal of Bone and Mineral Metabolism*, *27*(4), 424–434. https://doi.org/10.1007/s00774-009-0041-2

Nagi, D., Butt, Z., Farooq, F., & Aamar, A. (2013). Frequency of osteoporosis in an ambulatory setting in Lahore using quantitative calcaneal ultrasound. *Journal of the Pakistan Medical Association*, *63*, 965–968.

Pollak, R. D., Blumenfeld, A., Bejarano-Achache, I., Idelson, M., & Celinke Hochner, D. (2001). The BsmI vitamin D receptor gene polymorphism in Israeli populations and in perimenopausal and osteoporotic Ashkenazi women. *American Journal of Nephrology*, *21*(3), 185–188. https://doi.org/10.1159/000046245

Pouresmaeili, F., Jamshidi, J., Azargashb, E., & Samangouee, S. (2013). Association between Vitamin D Receptor Gene BsmI Polymorphism and Bone Mineral Density in A Population of 146 Iranian Women. *Cell Journal (Yakhteh)*, *15*(1), 75–82.

Qin, G., Dong, Z., Zeng, P., Liu, M., & Liao, X. (2013). Association of vitamin D receptor BsmI gene polymorphism with risk of osteoporosis: A meta-analysis of 41 studies. *Molecular Biology Reports*, *40*(1), 497–506. https://doi.org/10.1007/s11033-012-2086-x

Qiu, C., Chen, H., Wen, J., Zhu, P., Lin, F., Huang, B., Wu, P., Lin, Q., Lin, Y., Rao, H., Huang, H., Liang, J., Li, L., Gong, X., Peng, S., Li, M., Chen, L., Tang, K., Chen, Z., … Chen, G. (2013). Associations between age at menarche and menopause with cardiovascular disease, diabetes, and osteoporosis in Chinese women. *The Journal of Clinical Endocrinology and Metabolism*, *98*(4), 1612–1621. https://doi.org/10.1210/jc.2012-2919

Richards, J. B., Rivadeneira, F., Inouye, M., Pastinen, T. M., Soranzo, N., Wilson, S. G., Andrew, T., Falchi, M., Gwilliam, R., Ahmadi, K. R., Valdes, A. M., Arp, P., Whittaker, P., Verlaan, D. J., Jhamai, M., Kumanduri, V., Moorhouse, M., van Meurs, J. B., Hofman, A., … Spector, T. D. (2008). Bone mineral density, osteoporosis, and osteoporotic fractures: A genome-wide association study. *Lancet (London, England)*, *371*(9623), 1505–1512. https://doi.org/10.1016/S0140-6736(08)60599-1

Ryan, J. W., Anderson, P. H., & Morris, H. A. (2015). Pleiotropic Activities of Vitamin D Receptors – Adequate Activation for Multiple Health Outcomes. *The Clinical Biochemist Reviews*, *36*(2), 53–61.

Sioka, C., Fotopoulos, A., Georgiou, A., Xourgia, X., Papadopoulos, A., & Kalef-Ezra, J. A. (2010). Age at menarche, age at menopause and duration of fertility as risk factors for osteoporosis. *Climacteric: The Journal of the International Menopause Society*, *13*(1), 63–71. https://doi.org/10.3109/13697130903075337

Turner, A. G., Anderson, P. H., & Morris, H. A. (2012). Vitamin D and bone health. *Scandinavian Journal of Clinical and Laboratory Investigation. Supplementum*, *243*, 65–72. https://doi.org/10.3109/00365513.2012.681963

Yoshida, T., & Stern, P. H. (2012). How vitamin D works on bone. *Endocrinology and Metabolism Clinics of North America*, *41*(3), 557–569. https://doi.org/10.1016/j.ecl.2012.04.003

Yu, M., Chen, G.-Q., & Yu, F. (2016). Lack of association between vitamin D receptor polymorphisms ApaI (rs7975232) and BsmI (rs1544410) and osteoporosis among the Han Chinese population: A meta-analysis. *The Kaohsiung Journal of Medical Sciences*, *32*(12), 599–606. https://doi.org/10.1016/j.kjms.2016.10.002

Zhang, H., Tao, G., & Wu, Q. (1998). [Preliminary studies on the relationship between vitamin D receptor gene polymorphism and osteoporosis in Chinese women]. *Zhonghua Liu Xing Bing Xue Za Zhi = Zhonghua Liuxingbingxue Zazhi*, *19*(1), 12–14.