GENETIC TESTING AND MOLECULAR BIOMARKERS
Volume 17, Number 1, 2012

Pp. 1–5
DOI: 10.1089/gtmb.2012.0267

Vitamin D Receptor *Bsm*I Polymorphism and Osteoporosis Risk: A Meta-Analysis from 26 Studies

Fu Jia,[1,*] Rui-Fen Sun,[2,*] Qun-Hui Li,[1] Da-Xing Wang,[1] Feng Zhao,[1] Jun-Min Li,[1] Qi Pu,[1] Zhong-Zi Zhang,[1] Yan Jin,[1] Bai-Lian Liu,[1] and Ying Xiong[1]

Objective: Growing evidence has shown that *vitamin D* deficiency can cause lower bone mineral density (BMD) and an increased risk of osteoporosis. Vitamin D receptor (*VDR*) *Bsm*I polymorphism (rs1544410) can affect BMD variation and circulating osteocalcin levels. To date, a wide range of epidemiological studies have been carried out to evaluate the association between *VDR Bsm*I polymorphism and susceptibility to osteoporosis. Conflicting results, however, were obtained. The aim of this study was to evaluate the effect of *VDR Bsm*I polymorphism on osteoporosis risk using a meta-analysis. *Methods:* Twenty-six publications were identified by searching PubMed and Embase databases. The association between *VDR Bsm*I polymorphism and osteoporosis was estimated by calculating pooled odds ratios (ORs) with corresponding 95% confidence intervals (CIs). *Results:* The bb genotype was associated with a significantly decreased risk of osteoporosis in overall comparison (bb vs. BB: OR = 0.61, 95% CI, 0.40–0.92; bb vs. BB/Bb: OR = 0.70, 95% CI, 0.52–0.95, respectively). Subgroup analyses showed that the bb genotype had a decreased risk of developing osteoporosis in postmenopausal women (bb vs. BB/Bb: OR = 0.68, 95% CI, 0.46–0.98) and Africans (Bb/bb vs. BB: OR = 0.18, 95% CI, 0.09–0.37). *Conclusion:* The *VDR Bsm*I polymorphism may have a protective role against the development of osteoporosis.

Introduction

Osteoporosis is a skeletal disorder with the characterization of reduced bone mineral density (BMD), deterioration in the microarchitecture of bone tissue, and an increased risk of fracture (Kanis *et al.*, 1994). Osteoporosis is defined by the World Health Organization (WHO) as BMD values fall 2.5 standard deviations below the mean peak bone mass of young healthy adults. It is estimated that osteoporosis can affect 30% of women and 12% of men (Ralston and De Crombrugghe, 2006). Many factors so far have been identified to influence the risk of osteoporosis, including vitamin D deficiency (Nieves, 2005), excess alcohol drinking and tobacco smoking (Wong *et al.*, 2007; Berg *et al.*, 2008), medication use (Petty *et al.*, 2007), and coexisting diseases. Notably, a positive family history is another clinical risk factor, emphasizing the important roles of genetic variations playing in the pathogenesis of osteoporosis.

To date, multiple candidate genes have been investigated to determine the relationship between these genes and susceptibility to osteoporosis. Among them, vitamin D receptor (*VDR*) is the first and most extensively studied. In 1992, Morrison *et al.* reported that *VDR Bsm*I polymorphism (rs1544410) could affect BMD variation and circulating osteocalcin levels (Morrison *et al.*, 1992, 1994). Subsequently, a large number of epidemiological studies have been carried out to evaluate the association between *VDR Bsm*I polymorphism and individual susceptibility to osteoporosis. However, the results were inconclusive rather than conclusive. For example, Kanan and Mesmar (2008) reported that the *VDR Bsm*I bb genotype was significantly more frequent in patients with osteoporosis than in controls in an Asian population. On the contrary, in the same Asian population, the bb genotype was reported to be significantly higher in controls than in osteoporotic patients (Mitra *et al.*, 2006). The small sample sizes in single studies may be partly responsible for the conflicting results. Meta-analysis is a robust tool to cover the limitation of genetic association studies by pooling all the published data together, which increases the statistical power. We therefore performed a meta-analysis to determine whether the *VDR Bsm*I polymorphism was related to the risk of osteoporosis.

Materials and Methods

Selection of published studies

We performed the meta-analysis according to the guidelines of the PRISMA group (Moher *et al.*, 2009). The PubMed

[1]Department of Orthopedics, Kunming Medical University, National Clinical Key Specialty, Yanan Hospital, Kunming, People's Republic of China.
[2]Central Laboratory, Yunnan University of Chinese Traditional Medicine, Kunming, People's Republic of China.
*These two authors contributed equally to this work.

and Embase (1966–2011) databases were searched using the following algorithm: "(VDR or vitamin D receptor)" and "polymorphism" and "osteoporosis." The last search was updated on May 31, 2012. Additional records were identified through manual searching.

Inclusion and exclusion criteria

The studies were included only if they met the following criteria: (1) case–control study; (2) the study assessed the association between *VDR Bsm*I polymorphism (rs1544410) and risk of osteoporosis; (3) sufficient original data for calculating odds ratios (ORs) with corresponding 95% confidence intervals (95% CIs); (4) the full text was published in English.

If identical or overlapping data were published in different studies by the same research group, we excluded the articles with smaller sample size.

Data extraction

The following data were extracted from each study by two investigators (F.J. and R.F.) independently: the surname of the first author, year of publication, country of the corresponding author, ethnicity of study population, diagnosis of osteoporosis, matching variables, genotyping method, menopausal status, site of BMD measurement, age of cases and controls, the number of cases and controls, and genotype distribution of cases and controls. The Hardy–Weinberg equilibrium (HWE) and B allele frequency in controls were calculated based on the *Bsm*I genotyping distribution in cases and controls.

Statistical analysis

The association between *VDR Bsm*I polymorphism and osteoporosis was evaluated by calculating pooled ORs based on the individual ORs. Four comparisons were performed: Bb versus BB, bb versus BB, bb versus BB/Bb, and Bb/bb versus BB. The statistical heterogeneity between studies was tested using the Q-test and I^2 statistics (Higgins and Thompson, 2002). When there is apparent heterogeneity between studies ($p \leq 0.10$), the pooled OR was estimated using the random-effects model (the DerSimonian and Laird method; DerSimonian and Laird, 1986); otherwise, the fixed-effects model (the Mantel–Haenszel method) was used (Mantel and Haenszel, 1959). Subgroup analyses were performed according to ethnicity, menopausal status, and HWE. If the heterogeneity between studies was detected, logistic meta-regression was carried out to explore the sources of heterogeneity. Publication bias was examined with Egger's test (Egger *et al.*, 1997). Data were analyzed using STATA software, version 10.0 (STATA Corp., College Station, TX).

Results

Characteristics of studies

A total of 820 articles were identified though database and manual searching. After screening the title and abstract, 226 articles were excluded due to repetition. Then, 422 articles were excluded because they were not *Bsm*I polymorphism or osteoporosis or human study, and review articles. The remaining 172 full-text articles were assessed in detail. One hundred forty four articles were excluded with reasons for not being *Bsm*I polymorphism or osteoporosis or English studies or available data and reviews. Moreover, two studies were excluded because of overlapping data. Finally, 26 studies involving 2274 cases and 3150 controls were included in this meta-analysis (Supplementary Fig. 1; Supplementary Data are available online at www.liebertpub.com/gtmb).

The main characteristics of the studies are presented in Supplementary Table 1. In the study of Musumeci *et al.*, (2009) the genotyping distribution was separately shown based on African and Caucasian populations. It was, therefore, separately considered in subgroup analysis according to ethnicity. There were 13 studies of Caucasian population, 5 studies of Asian population, 5 studies of Turkish population, 2 studies of African population, 1 study of Jewish population, and 1 study of Mexican population. All the studies used polymerase chain reaction-restriction fragment length polymorphism (RFLP) for genotyping. Twenty studies reported that the cases were postmenopausal women. Most of the studies (81%) used the WHO criteria for the diagnosis of osteoporosis. However, only 38% (10/26) of the studies reported frequency-matched controls to cases, mainly by age. About 35% (9/26) of studies were deviated from HWE in controls.

Meta-analysis

The evaluations of the association between *VDR Bsm*I polymorphism and osteoporosis risk are summarized in Supplementary Table 2. The bb genotype was associated with a significantly decreased risk of osteoporosis when compared with BB, and BB/Bb genotypes (bb vs. BB: OR = 0.61, 95% CI, 0.40–0.92; bb vs. BB/Bb: OR = 0.70, 95% CI, 0.52–0.95, respectively) (Fig. 1). Similarly, the bb genotype had a 0.68-fold decreased risk of developing osteoporosis in postmenopausal women (bb vs. BB/Bb: OR = 0.68, 95% CI, 0.46–0.98). After subgroup analysis according to ethnicity, significantly decreased risk was observed in an African population (Bb/bb vs. BB: OR = 0.18, 95% CI, 0.09–0.37) but not in Asian, Caucasian, and Turkish populations. However, no significant association was detected both in HWE studies and in Hardy–Weinberg disequilibrium (HWD) studies.

Heterogeneity analysis and publication bias

The obvious heterogeneity between studies was present in overall comparisons and subgroup analyses. To determine the sources of heterogeneity, some potential factors were taken into consideration: ethnicity, HWE, menopausal status, and sample size (≥ 200, and < 200). However, none of the possible variables could contribute to the heterogeneity.

No evidence of publication bias for the *VDR Bsm*I polymorphism was detected in all comparisons ($p > 0.05$).

Discussion

1,25 Dihydroxyvitamin D_3 (1,25-$(OH)_2D_3$), the active form of vitamin D, plays crucial roles in calcium and bone metabolism by binding to the *VDR*. Epidemiological evidence has shown that vitamin D deficiency can cause lower BMD and an increased risk of osteoporosis, especially in older adults (Nieves, 2005; Bell *et al.*, 2010). Even though the exact mechanism is not fully known, several candidate genes have been identified to regulate genetic susceptibility to osteoporosis. A G/A variant (*Bsm*I RFLP) in intron 8 of *VDR* has been

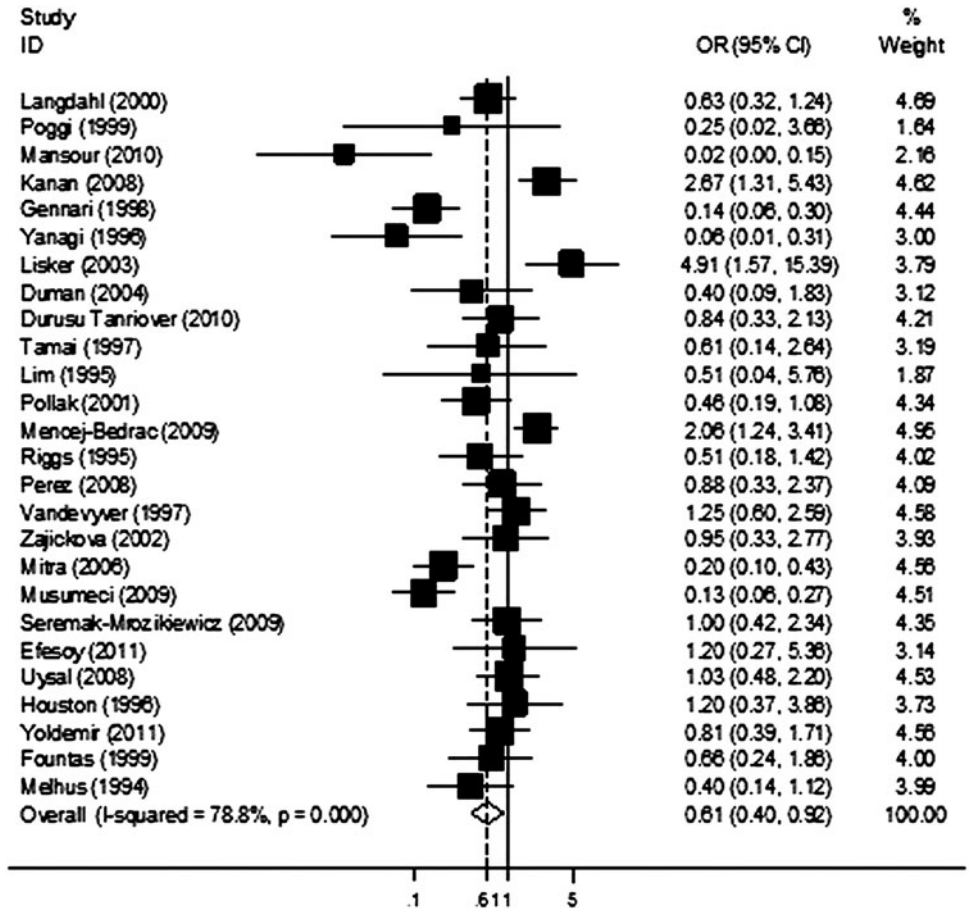

FIG. 1. Forest plot of the meta-analysis (bb vs. BB). The random-effects model was used.

detected to influence BMD variation. The presence of the polymorphism was associated with high BMD, while the absence of the polymorphism was associated with low BMD (Morrison *et al.*, 1994).

Given the potential roles of *VDR* playing in the etiology of osteoporosis, varieties of studies have been conducted to identify whether the *VDR Bsm*I polymorphism was the genetic determiner of osteoporosis. However, conflicting results have been obtained. We carried out a meta-analysis to clarify the debated issue. In this study, we found that the bb genotype was associated with a significantly decreased risk of osteoporosis in overall comparisons. Subgroup analyses showed that the bb genotype had a decreased risk of developing osteoporosis in postmenopausal women and Africans rather than in Asians and Caucasians. These findings suggest that *VDR Bsm*I polymorphism may be involved in the pathogenesis of osteoporosis. Some of our results were in agreement with the findings reported by Zintzaras *et al.* (2006) who also found no relationship between the *VDR Bsm*I polymorphism and susceptibility to osteoporosis. In contrast, some of our results were in disagreement with the findings reported by Zintzaras *et al.* (2006) who found that the bb genotype was a protective factor in East Asians. The main reason the contradictory results may be false positive was due to limited publications. Zintzaras *et al.* (2006) identified records by a search of PubMed database (last search: December 2005), and only two studies of East Asians were included. In this meta-analysis, Embase database was also searched besides searching PubMed, and the last search was performed up to May 31, 2012. Three additional studies, therefore, were included in this meta-analysis. Many more studies are needed to confirm the results by increasing the power of statistics.

After subgroup analysis according to ethnicity, we found that the bb genotype was associated with a decreased risk of developing osteoporosis in the African population but not in other populations. Although it is difficult to decipher the exact reason for the discrepancy, we could discuss some possibilities for elucidating the conflicting observations. Perhaps the different genetic backgrounds would be responsible for the inclusive results because the varied minor allele (B) distribution has been exhibited in diverse ethnicities, with the mean frequency of 16.5% in Africans (Musumeci *et al.*, 2009; Mansour *et al.*, 2010), 24.5% in Asians (Lim *et al.*, 1995; Yanagi *et al.*, 1996; Tamai *et al.*, 1997; Mitra *et al.*, 2006; Kanan and Mesmar, 2008), and 42.4% in Caucasians (Melhus *et al.*, 1994; Riggs *et al.*, 1995; Houston *et al.*, 1996; Vandevyver *et al.*, 1997; Gennari *et al.*, 1998; Fountas *et al.*, 1999; Poggi *et al.*, 1999; Langdahl *et al.*, 2000; Zajickova *et al.*, 2002; Perez *et al.*, 2008; Mencej-Bedrac *et al.*, 2009; Musumeci *et al.*, 2009; Seremak-Mrozikiewicz *et al.*, 2009). Moreover, only two studies were included in this meta-analysis to investigate the relationship between the *VDR Bsm*I polymorphism and susceptibility to osteoporosis in Africans (Musumeci *et al.*, 2009; Mansour *et al.*, 2010). There is a risk of the positive result occurring by chance due to insufficient statistical power. Further studies, therefore, are necessary to confirm this finding, especially in diverse ethnic groups.

It is well known that osteoporosis is a major public health problem in women after menopause. We therefore assessed the effect of *VDR Bsm*I polymorphism on osteoporosis risk in postmenopausal women, and we found that the bb genotype had a 0.68-fold decreased risk of developing osteoporosis, indicating that the bb genotype may protect individuals against the development of osteoporosis in postmenopausal women.

HWE is absolutely essential for a sound case–control observational study. It is probable that studies of HWD in controls have selection bias and/or genotyping error, which may cause misleading results. In this meta-analysis, nine studies were deviated from HWE (Yanagi *et al.*, 1996; Gennari *et al.*, 1998; Poggi *et al.*, 1999; Langdahl *et al.*, 2000; Lisker *et al.*, 2003; Duman *et al.*, 2004, Kanan and Mesmar, 2008; Durusu Tanriover *et al.*, 2010; Mansour *et al.*, 2010). We evaluated whether the HWE studies could disturb the results using subgroup analysis. We failed to find any association both in HWE studies and HWD studies. Full-scale prospective cohort studies are warranted to verify the results.

The between-study heterogeneity is a major concern in a meta-analysis that may influence the results. In the current study, apparent heterogeneity existed in overall comparisons and also subgroup analyses. We used logistic meta-regression to test the origin of heterogeneity when considering the possible reasons, including ethnicity, HWE, menopausal status, and sample size. Nevertheless, none of the items could be attributed to the heterogeneity, suggesting that unknown factors may account for the pooled nonhomogeneous data. Additionally, only English-language publications were included in this meta-analysis, which may result in a possibility of missing some relevant studies, especially the negative results. Even though there are limitations, our study has some advantages. For example, no evidence for publishing selected data was examined. Such data are likely to reinforce our observations about the effect of *VDR Bsm*I polymorphism on the risk of osteoporosis.

In conclusion, the meta-analysis provides evidence that bb genotype of the *VDR Bsm*I polymorphism may protect individuals against osteoporosis. The obvious heterogeneity, however, was detected in overall analysis. Therefore, the results should be interpreted with caution. Moreover, osteoporosis is a complex disease that may be involved in several other risk factors besides the *VDR Bsm*I polymorphism, such as tobacco smoking, medication use, and dietary intake. Further studies investigating gene–environment interaction are necessary to elucidate the role of genetic variations on the etiology of osteoporosis.

Author Disclosure Statement

The authors declare that they have no conflict of interest.

References

Bell TD, Demay MB, Burnett-Bowie SA (2010) The biology and pathology of vitamin D control in bone. J Cell Biochem 111: 7–13.

Berg KM, Kunins HV, Jackson JL, *et al.* (2008) Association between alcohol consumption and both osteoporotic fracture and bone density. Am J Med 121:406–418.

DerSimonian R, Laird N (1986) Meta-analysis in clinical trials. Control Clin Trials 7:177–188.

Duman BS, Tanakol R, Erensoy N, *et al.* (2004) Vitamin D receptor alleles, bone mineral density and turnover in postmenopausal osteoporotic and healthy women. Med Princ Pract 13:260–266.

Durusu Tanriover M, Bora Tatar G, Uluturk TD, *et al.* (2010) Evaluation of the effects of vitamin D receptor and estrogen receptor 1 gene polymorphisms on bone mineral density in postmenopausal women. Clin Rheumatol 29:1285–1293.

Efesoy A, Yilmaz O, Erden G, *et al.* (2011) Relationship of the vitamin D receptor and collagen I(alpha)1 gene polymorphisms with low bone mineral density and vertebral fractures in postmenopausal Turkish women. Turkish Journal of Rheumatology 26:295–302.

Egger M, Davey Smith G, Schneider M, *et al.* (1997) Bias in meta-analysis detected by a simple, graphical test. BMJ 315:629–634.

Fountas L, Moutsatsou P, Kastanias I, *et al.* (1999) The contribution of vitamin D receptor gene polymorphisms in osteoporosis and familial osteoporosis. Osteoporos Int 10:392–398.

Gennari L, Becherini L, Masi L, *et al.* (1998) Vitamin D and estrogen receptor allelic variants in Italian postmenopausal women: evidence of multiple gene contribution to bone mineral density. J Clin Endocrinol Metab 83:939–944.

Higgins JP, Thompson SG (2002) Quantifying heterogeneity in a meta-analysis. Stat Med 21:1539–1558.

Houston LA, Grant SF, Reid DM, *et al.* (1996) Vitamin D receptor polymorphism, bone mineral density, and osteoporotic vertebral fracture: studies in a UK population. Bone 18:249–252.

Kanan RM, Mesmar M (2008) The effect of vitamin D receptor and estrogen receptor gene polymorphisms on bone mineral density in healthy and osteoporotic postmenopausal Jordanian women. Int J Integr Biol 4:67–71.

Kanis JA, Melton LJ, 3rd, Christiansen C, *et al.* (1994) The diagnosis of osteoporosis. J Bone Miner Res 9:1137–1141.

Langdahl BL, Gravholt CH, Brixen K, *et al.* (2000) Polymorphisms in the vitamin D receptor gene and bone mass, bone turnover and osteoporotic fractures. Eur J Clin Invest 30:608–617.

Lim SK, Park YS, Park JM, *et al.* (1995) Lack of association between vitamin D receptor genotypes and osteoporosis in Koreans. J Clin Endocrinol Metab 80:3677–3681.

Lisker R, Lopez MA, Jasqui S, *et al.* (2003) Association of vitamin D receptor polymorphisms with osteoporosis in mexican postmenopausal women. Hum Biol 75:399–403.

Mansour L, Sedky M, AbdelKhader M, *et al.* (2010) The role of vitamin D receptor genes (FOKI and BSMI) polymorphism in osteoporosis. Middle East Fertil Soc J 15:79–83.

Mantel N, Haenszel W (1959) Statistical aspects of the analysis of data from retrospective studies of disease. J Natl Cancer Inst 22:719–748.

Melhus H, Kindmark A, Amer S, *et al.* (1994) Vitamin D receptor genotypes in osteoporosis. Lancet 344:949–950.

Mencej-Bedrac S, Prezelj J, Kocjan T, *et al.* (2009) The combinations of polymorphisms in vitamin D receptor, osteoprotegerin and tumour necrosis factor superfamily member 11 genes are associated with bone mineral density. J Mol Endocrinol 42:239–247.

Mitra S, Desai M, Ikram Khatkhatay M (2006) Vitamin D receptor gene polymorphisms and bone mineral density in postmenopausal Indian women. Maturitas 55:27–35.

Moher D, Liberati A, Tetzlaff J, *et al.* (2009) Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLoS Med 6:e1000097.

Morrison NA, Qi JC, Tokita A, *et al.* (1994) Prediction of bone density from vitamin D receptor alleles. Nature 367:284–287.

Morrison NA, Yeoman R, Kelly PJ, *et al.* (1992) Contribution of trans-acting factor alleles to normal physiological variability:

vitamin D receptor gene polymorphism and circulating osteocalcin. Proc Natl Acad Sci U S A 89:6665–6669.

Musumeci M, Vadala G, Tringali G, *et al.* (2009) Genetic and environmental factors in human osteoporosis from Sub-Saharan to Mediterranean areas. J Bone Miner Metab 27:424–434.

Nieves JW (2005) Osteoporosis: the role of micronutrients. Am J Clin Nutr 81:1232S–1239S.

Perez A, Ulla M, Garcia B, *et al.* (2008) Genotypes and clinical aspects associated with bone mineral density in Argentine postmenopausal women. J Bone Miner Metab 26:358–365.

Petty SJ, O'Brien TJ, Wark JD (2007) Anti-epileptic medication and bone health. Osteoporos Int 18:129–142.

Poggi M, Aterini S, Nicastro L, *et al.* (1999) Lack of association between body weight, bone mineral density and vitamin D receptor gene polymorphism in normal and osteoporotic women. Dis Markers 15:221–227.

Pollak RD, Blumenfeld A, Bejarano-Achache I, *et al.* (2001) The BsmI vitamin D receptor gene polymorphism in Israeli populations and in perimenopausal and osteoporotic Ashkenazi women. Am J Nephrol 21:185–188.

Ralston SH, De Crombrugghe B (2006) Genetic regulation of bone mass and susceptibility to osteoporosis. Genes Dev 20:2492–2506.

Riggs BL, Nguyen TV, Melton LJ, 3rd, *et al.* (1995) The contribution of vitamin D receptor gene alleles to the determination of bone mineral density in normal and osteoporotic women. J Bone Miner Res 10:991–996.

Seremak-Mrozikiewicz A, Drews K, Mrozikiewicz PM, *et al.* (2009) Correlation of vitamin D receptor gene (VDR) polymorphism with osteoporotic changes in Polish postmenopausal women. Neuro Endocrinol Lett 30:540–546.

Tamai M, Yokouchi M, Komiya S, *et al.* (1997) Correlation between vitamin D receptor genotypes and bone mineral density in Japanese patients with osteoporosis. Calcif Tissue Int 60:229–232.

Uysal AR, Sahin M, Gursoy A, *et al.* (2008) Vitamin D receptor gene polymorphism and osteoporosis in the Turkish population. Genet Test 12:591–594.

Vandevyver C, Wylin T, Cassiman JJ, *et al.* (1997) Influence of the vitamin D receptor gene alleles on bone mineral density in postmenopausal and osteoporotic women. J Bone Miner Res 12:241–247.

Wong PK, Christie JJ, Wark JD (2007) The effects of smoking on bone health. Clin Sci (Lond) 113:233–241.

Yanagi H, Tomura S, Kawanami K, *et al.* (1996) Vitamin D receptor gene polymorphisms are associated with osteoporosis in Japanese women. J Clin Endocrinol Metab 81:4179–4181.

Yoldemir T, Yavuz DG, Anik G, *et al.* (2011). Vitamin D receptor gene polymorphisms in a group of postmenopausal Turkish women: association with bone mineral density. Climacteric 14:384–391.

Zajickova K, Zofkova I, Bahbouh R, *et al.* (2002) Vitamin D receptor gene polymorphisms, bone mineral density and bone turnover: FokI genotype is related to postmenopausal bone mass. Physiol Res 51:501–509.

Zintzaras E, Rodopoulou P, Koukoulis GN (2006) BsmI, TaqI, ApaI and FokI polymorphisms in the vitamin D receptor (VDR) gene and the risk of osteoporosis: a meta-analysis. Dis Markers 22:317–326.

Address correspondence to:
Bai-Lian Liu, M.D.
Department of Orthopedics
Kunming Medical University
National Clinical Key Specialty, Yanan Hospital
Kunming, Yunnan 650051
People's Republic of China

E-mail: 1628322036@qq.com

Ying Xiong, M.D.
Department of Orthopedics
Kunming Medical University
National Clinical Key Specialty, Yanan Hospital
Kunming, Yunnan 650051
People's Republic of China

E-mail: xiongying789@126.com