# Association of vitamin D receptor *Bsm*I gene polymorphism with risk of low bone mineral density in post-menopausal women: a meta-analysis

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**ABSTRACT.** The vitamin D receptor *Bsm*I gene polymorphism is reportedly associated with low bone mineral density (BMD) in postmenopausal women, but results from previous studies are conflicting. In the present study, we investigated the association between this polymorphism and the risk of low BMD through a meta-analysis of published studies. A literature search of the Pubmed, Embase, and CNKI databases from inception through July 2013 was conducted. The meta-analysis was performed using the STATA 12.0 software. Crude odds ratios with 95% confidence intervals were used to assess the strength of any association. Eleven case-control studies were included for a total of 1468 low BMD cases and 2177 healthy controls. No significant variation in low BMD risk was detected in any of the genetic models. Further stratified analyses were performed to examine the effect of ethnicity. In the subgroup analysis, no significant

association was found in Caucasians and in Asians. The meta-analysis results suggest that the *Bsm*I polymorphism is not associated with low BMD risk in postmenopausal women.

**Key words:** Vitamin D receptor; *Bsm*I; Gene polymorphism; Bone mineral density; Meta-analysis

## INTRODUCTION

Osteoporosis is a common health problem, particularly in postmenopausal women, and is characterized by decreased bone mineral density (BMD) and deterioration of skeletal microarchitecture, leading to increased bone fragility and fracture risk (Kanis et al., 1994). Epidemiological studies have shown that BMD is influenced by many environmental factors, such as smoke exposure, waist to hip ratio, physical activity, and body mass index (Seo et al., 2008). In addition, many studies have shown that genetic factors play an important role in the pathogenesis of BMD. Evidence is accumulating that up to 80% of the heritability for BMD in the general population can be attributed to genetic factors (Riggs, 1997).

Vitamin D plays a crucial role in BMD metabolism via the vitamin D receptor (VDR), which is a member of the nuclear receptor family of transcription factors (Moore et al., 2006). VDR plays a role in regulating calcium homoeostasis through binding and nuclear transloca-tion of 1-$\alpha$, 25-dihydroxyvitamin $D_3$, affecting bone resorption and increasing calcium ab-sorption (Pike et al., 2002). In recent years, candidate gene association studies have explored the association between BMD and the VDR gene, which is located on chromosome 12cen-q12 and contains 14 exons. Morrison et al. (1994) first reported that VDR gene polymorphisms could predict spinal and femoral BMD in Caucasian women. Since then, a large number of studies regarding the association between VDR polymorphisms and BMD have been pub-lished, including studies examining the *Fok*I, *Bsm*I, and *Apa*I variants.

The *Bsm*I polymorphism is the most important subtype of the VDR gene polymorphisms. Several association studies have reported conflicting results of the relationship between the *Bsm*I genetic polymorphism and BMD risk. Furthermore, Qin et al. (2013) performed a meta-analysis of all studies relating the *Bsm*I polymorphism with low BMD risk and found that it was not as-sociated with low BMD in the overall population examined. However, they did not perform a subgroup analysis aimed at postmenopausal women. Osteoporosis is a major health problem in women after menopause. Remodeling imbalance plays an important role in the pathogenesis of postmenopausal osteoporosis (Turner et al., 1994). Decreased activity of the enzyme 1-$\alpha$, 25-di-hydroxyvitamin $D_3$ hydroxylase, which converts 25-OH vitamin D into 1,25-$(OH)_2$ vitamin D (potent vitamin D), has been reported after menopause due to estrogen deficiency (Gallagher et al., 2001). In this study, we performed a meta-analysis to assess the association between the *Bsm*I polymorphism of the VDR gene and low BMD in postmenopausal women.

## MATERIAL AND METHODS

### Literature search

Two reviewers searched the Pubmed, Embase, and CNKI databases to retrieve pa-

pers linking the *Bsm*I polymorphism and low BMD risk that were available up to July 2013 without language restrictions using the following key words: "vitamin D receptor", "*Bsm*I", "bone mineral density", "polymorphism", "single nucleotide polymorphism", "genetic polymorphism", and "BMD". References in the eligible articles or textbooks were also reviewed manually to identify other studies containing these key words. Any disagreement was resolved by discussion between the researchers.

## Inclusion and exclusion criteria

The following inclusion criteria were used to select studies: i) case-control studies that addressed low BMD cases and healthy controls; ii) studies that evaluated the association between the *Bsm*I polymorphism and low BMD risk; and iii) studies that included sufficient data for extraction. Studies were excluded when: i) they were not case-control studies that evaluated the association between the *Bsm*I polymorphism and low BMD risk; ii) the articles were case reports, letters, reviews, meta-analyses, or editorials; iii) the studies were based on incomplete data, no usable data were reported, or duplicate data.

## Data extraction

From each eligible report, we recorded the last name of the first author, year of publication, country of the study and ethnicity, numbers of cases and controls, gene polymorphisms, and evidence of Hardy-Weinberg equilibrium (HWE) in controls. Two investigators (Bizeng Zhao and Wei Zhang) extracted the data independently, and the results were reviewed by a third investigator (Zubin Zhou).

## Statistical analysis

The strength of the association between the *Bsm*I polymorphism and low BMD risk was measured by odds ratios (ORs) with 95% confidence intervals (CIs) under 4 genetic models, including a homozygote comparison (BB *vs* bb), a heterozygote comparison (BB *vs* Bb), a dominant model (Bb + bb *vs* BB), and a recessive model (BB + Bb *vs* bb) between groups. Heterogeneity was quantified using the $I^2$ test: $I^2 < 25\%$, no heterogeneity; $I^2 = 25\text{-}50\%$, moderate heterogeneity; $I^2 = 50\text{-}75\%$, high heterogeneity, $I^2 > 75\%$, extreme heterogeneity. When the effects were assumed to be homogeneous ($I^2 > 50\%$), the fixed-effects model was used. Otherwise, the random-effects model was more appropriate. Subgroup analysis based on ethnicity was used to explore and explain the diversity among the results of different studies. Begg's funnel plot was used to assess publication bias ($P < 0.05$ was considered to be statistically significant). Sensitivity analysis was performed by removing meta-analysis studies individually, excluding those whose genotype distributions of the control groups deviated from HWE. We assessed HWE in controls for each study using the $\chi^2$ test and $P < 0.05$ was considered to be significant disequilibrium. All analyses were conducted using the STATA Version 12.0 software (Stata Corp; College Station, TX, USA).

## RESULTS

### Studies included in the meta-analysis

   According to our inclusion criteria, a total of 11 eligible studies involving 1468 cases and 2177 controls were included in the pooled analysis (Berg et al., 1996; Vandevyver et al., 1997; Gomez et al., 1999; Valimaki et al., 2001; Zajickova et al., 2002; Douroudis et al., 2003; Zhu et al., 2004; Garnero et al., 2005; Horst-Sikorska et al., 2005; Mitra et al., 2006; Ge et al., 2009). The characteristics of selected studies are summarized in Figure 1. Genotyping in all assays was conducted using restriction fragment length polymorphism analysis. All studies were case-control studies that evaluated the association between the *Bsm*I polymorphism and low BMD risk. All the articles were written in English except for Ge et al. (2009) and Zhu et al. (2004). There were 3 studies from Asian countries and 8 studies from European countries. The publishing year of the included studies ranged from 1996 to 2009. An HWE test was conducted on genotype distribution of the controls in all included studies; all analyzed HWE except for 3 studies (Douroudis et al., 2003; Zhu et al., 2004; Ge et al., 2009). The baseline characteristics of all studies included are summarized in Table 1.
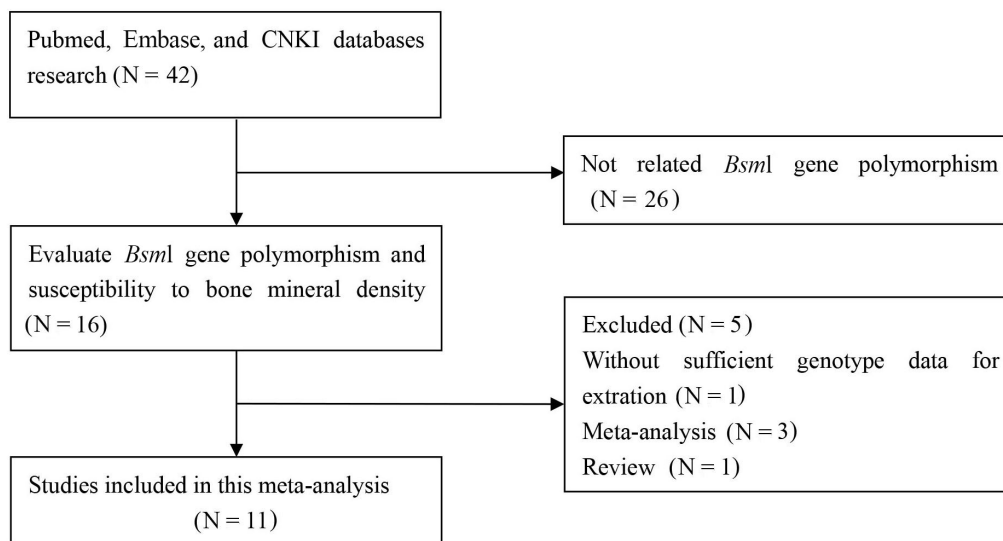


**Figure 1.** Flow diagram of study searching and selection process.

### Quantitative synthesis

   A summary of the meta-analysis findings for the association between the *Bsm*I polymorphism and low BMD risk is shown in Table 2 and Figure 2. We found no significant association between *Bsm*I polymorphisms and low BMD risk in any of the genetic models (BB *vs* bb: OR = 0.78, 95%CI = 0.42-1.45; BB *vs* Bb: OR = 0.91, 95%CI = 0.72-1.15; dominant

model: OR = 1.17, 95%CI = 0.75-1.81; recessive model: OR = 0.80, 95%CI = 0.53-1.23). In the subgroup analysis by ethnicity, the studies included were divided into Asian and Caucasian populations, and no significant association was found between *Bsm*I polymorphisms and low BMD risk in Asians (BB *vs* bb: OR = 0.92, 95%CI = 0.14-6.14; BB *vs* Bb: OR = 0.92, 95%CI = 0.25-3.38; dominant model: OR = 1.03, 95%CI = 0.22-4.73; recessive model: OR = 0.91, 95%CI= 0.31-2.68) and in Caucasians (BB *vs* bb: OR = 0.78, 95%CI = 0.41-1.47; BB *vs* Bb: OR = 0.97, 95%CI = 0.75-1.26; dominant model: OR = 1.14, 95%CI = 0.75-1.73; recessive model: OR = 0.77, 95%CI = 0.48-1.26). Furthermore, sensitivity analysis was performed with controls in HWE and the result was not altered, suggesting that the results of this meta-analysis are stable.

**Table 1.** Characteristics of literatures included in the meta-analysis.

| Study included | Year | Area | Race | Cases/Controls | Genotypes for cases | | | Genotypes for controls | | | HWE test |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | BB | Bb | bb | BB | Bb | bb | |
| Berg et al. | 1996 | Norway | Caucasian | 19/30 | 4 | 8 | 7 | 8 | 11 | 11 | 0.16 |
| Vandevyver | 1997 | Belgium | Caucasian | 86/698 | 12 | 50 | 24 | 127 | 368 | 203 | 0.08 |
| Gomez et al. | 1999 | Spain | Caucasian | 76/236 | 13 | 38 | 25 | 38 | 109 | 89 | 0.63 |
| Valimaki et al. | 2001 | Finland | Caucasian | 372/111 | 44 | 175 | 153 | 20 | 55 | 36 | 0.90 |
| Zajickova et al. | 2002 | Czech Republic | Caucasian | 65/33 | 21 | 24 | 20 | 10 | 13 | 10 | 0.22 |
| Douroudis et al. | 2003 | Greece | Caucasian | 35/44 | 3 | 12 | 20 | 10 | 29 | 5 | 0.03 |
| Zhu et al. | 2004 | China | Asian | 40/158 | 6 | 26 | 8 | 7 | 105 | 46 | 0.00 |
| Garnero et al. | 2005 | France | Caucasian | 240/349 | 50 | 124 | 66 | 40 | 162 | 147 | 0.64 |
| Mitra et al. | 2006 | India | Asian | 97/119 | 19 | 38 | 40 | 51 | 46 | 22 | 0.05 |
| Horst-Sikorska et al. | 2005 | Poland | Caucasian | 85/191 | 10 | 35 | 40 | 33 | 85 | 73 | 0.34 |
| Ge et al. | 2009 | China | Asian | 353/208 | 6 | 33 | 314 | 4 | 12 | 192 | 0.00 |

**Table 2.** Summary ORs and 95%CI of *Bsm*I gene polymorphism with bone mineral density risk.

| Subgroup | Genetic model | Sample size | | Type of model | Test of heterogeneity | | Test of association | | Test of publication bias | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Case | Control | | I² | P | OR | 95%CI | z | P |
| Overall | BB *vs* bb | 1468 | 2177 | Random | 81.8% | 0.00 | 0.78 | 0.42-1.45 | 0.00 | 1.00 |
| | BB *vs* Bb | | | Fixed | 42.8% | 0.06 | 0.91 | 0.72-1.15 | 0.00 | 1.00 |
| | Dominant model | | | Random | 71% | 0.00 | 1.17 | 0.75-1.81 | 0.00 | 1.00 |
| | Recessive model | | | Random | 81.4% | 0.00 | 0.80 | 0.53-1.23 | 0.00 | 1.00 |
| Caucasian | BB *vs* bb | 978 | 1692 | Random | 78.3% | 0.00 | 0.78 | 0.41-1.47 | 0.00 | 1.00 |
| | BB *vs* Bb | | | Fixed | 8.3% | 0.37 | 0.97 | 0.75-1.26 | 0.00 | 1.00 |
| | Dominant model | | | Random | 59.8% | 0.02 | 1.14 | 0.75-1.73 | 0.00 | 1.00 |
| | Recessive model | | | Random | 81.5% | 0.00 | 0.77 | 0.48-1.26 | 0.00 | 1.00 |
| Asians | BB *vs* bb | 490 | 485 | Random | 88.8% | 0.00 | 0.92 | 0.14-6.14 | 0.00 | 1.00 |
| | BB *vs* Bb | | | Random | 77.5% | 0.01 | 0.92 | 0.25-3.38 | 0.00 | 1.00 |
| | Dominant model | | | Random | 85.8% | 0.00 | 1.03 | 0.22-4.73 | 0.00 | 1.00 |
| | Recessive model | | | Random | 86.8% | 0.00 | 0.91 | 0.31-2.68 | 0.00 | 1.00 |
| Consistent with HWE | BB *vs* bb | 1040 | 1767 | Random | 82.3% | 0.00 | 0.78 | 0.41-1.50 | 0.24 | 0.81 |
| | BB *vs* Bb | | | Fixed | 41.1% | 0.11 | 0.89 | 0.70-1.13 | 0.24 | 0.81 |
| | Dominant model | | | Random | 73.3% | 0.00 | 1.24 | 0.77-1.99 | 0.24 | 0.81 |
| | Recessive model | | | Random | 79.8% | 0.00 | 0.83 | 0.53-1.28 | 0.24 | 0.81 |

## Publication bias

The publication bias of the meta-analysis for the association between the *Bsm*I polymorphism and low BMD risk was detected using Begg's funnel plot; all graphical funnel

plots of the included studies appeared to be symmetrical. The results of the Begg's funnel plot analyses are shown in Table 2. The results revealed no publication bias (all P < 0.05).
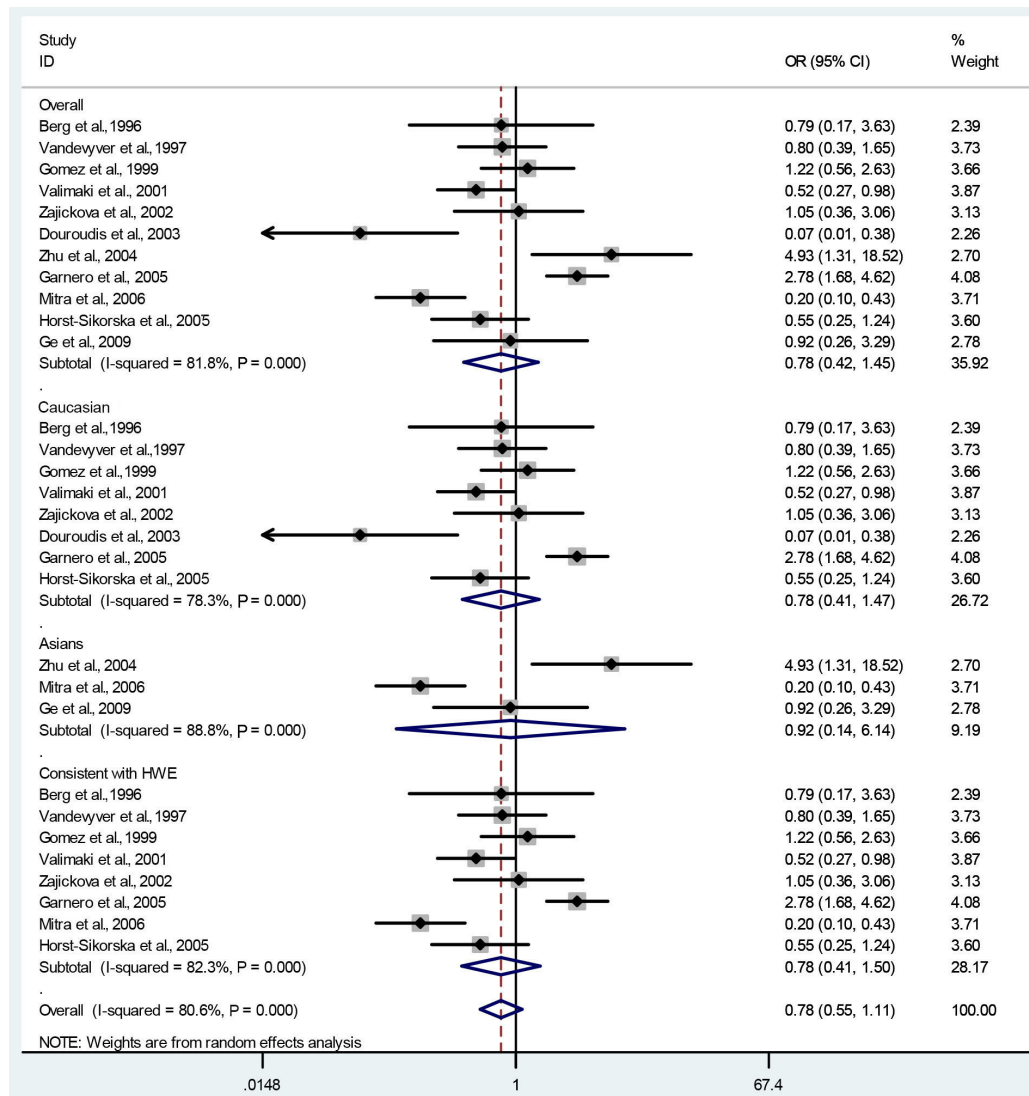


**Figure 2.** Association of vitamin D receptor *Bsm*I gene polymorphism with the risk for bone mineral density (BB *vs* bb).

## DISCUSSION

There is growing evidence that genetic variation plays an important role in the determination of individual susceptibility to complex disease traits. Low BMD is a multifactorial

disorder with a strong genetic component; the VDR gene has been suggested as a candidate gene for low BMD. The *Bsm*I polymorphism is one of most important subtypes of VDR gene polymorphisms. In recent years, the association between the *Bsm*I polymorphism and BMD has attracted increasing attention. Many studies have evaluated the association between the *Bsm*I polymorphism of the VDR gene and low BMD risk in postmenopausal women, but the results are controversial, possibly because of variations in study design, small sample sizes, and heterogeneous populations. It is possible that the observed associations between the *Bsm*I polymorphism and low BMD risk reflect chance observations rather than true associations. To help clarify these inconsistent findings, we conducted this meta-analysis to obtain a competitive result via combining more eligible studies, enlarging the sample size, and conducting a subgroup analysis. A total of 28 case-control studies were included, representing 1468 low BMD cases and 2177 healthy controls. The results of our meta-analyses show that there is no evidence supporting an association between the *Bsm*I polymorphism and the risk of low BMD. In the stratified analysis relating to ethnicity, our results indicated that the *Bsm*I polymorphism was not associated with low BMD risk in Asians and in Caucasians. Deviations of allelic distributions from HWE may contribute to between-study heterogeneity; sensitivity analysis conducted by limiting this meta-analysis to studies that are consistent with HWE revealed that these results were realistic and reliable.

The function of the XRCC1 Arg280His polymorphism may be affected by gene-gene and gene-environment interactions. A previous study demonstrated that polymorphisms in both genes evaluated (*Bsm*I polymorphism of the VDR gene and estrogen receptor genotypes) increased BMD risk, although the *Bsm*I polymorphism alone did not increase the risk of low BMD risk (Willing et al., 1998). In addition, obesity can mask the influence of the VDR genotypes on BMD, as demonstrated by Dawson-Hughes and Finneran, (1995). However, further studies of gene-gene and gene-environment interactions should be taken into consideration when assessing low BMD risk.

There were some limitations to our study. First, only published studies were included in this meta-analysis; thus, we cannot exclude the possibility of publication bias, although the results of statistical tests showed this to be unlikely. In addition, significant between-study heterogeneity was observed. Although we used the random effects model to pool ORs, this may have affected the precision of the results. Finally, although all cases and controls of each study were well defined with similar inclusion criteria, there may be potential factors that were not taken into account that influenced our results.

Despite these limitations, our meta-analysis has some advantages. This is the first meta-analysis of the relationship between the *Bsm*I polymorphism of the VDR gene and low BMD risk in postmenopausal women. Notably, we established an effective search strategy based on computer-assisted and manual searching, and the quality of studies included in the current meta-analysis was satisfactory according to our selection criteria. In addition, explicit methods for study selection, data extraction, and data analysis were well designed before initiating the meta-analysis. Finally, there was no evidence of publication bias in this meta-analysis and the sensitivity analysis indicated that the results are statistically robust.

In conclusion, our study indicates that the VDR gene *Bsm*I polymorphism is not associated with risk of low BMD in postmenopausal women. Gene-gene and gene-environment interactions should be investigated in further studies.

## Conflicts of interest

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