Association of estrogen receptor-alpha and vitamin D receptor genotypes with therapeutic response to calcium in postmenopausal Chinese women

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ABSTRACT

AIM: To investigate the correlation between calcium treatment in postmenopausal women and estrogen receptor-alpha (ER-alpha) *Xba* I and *Pvu* II genotype and vitamin D receptor (VDR) *Apa* I genotype. **METHODS:** One hundred fifteen postmenopausal Chinese women of Han population were enrolled and treated with calcichew-D_3 (1000 mg calcium and 400 U vitamin D_3) daily for 1 year. At entry and after 1 year treatment, the bone mineral density (BMD), serum and urinary bone turnover biochemical markers were evaluated. ER-alpha and VDR genotype were analyzed using PCR-restriction fragment length polymorphism. **RESULTS:** After 1 year of calcium supplementation, a significant increase of BMD and a marked reduction in serum ALP and PTH levels, and a significant increase of serum 25-(OH) vitamin D level were observed ($P<0.01$ or $P<0.05$). At entry and after 1 year of treatment, no significant association was found between *Xba* I, *Pvu* II, and *Apa* I genotypes and BMD in L1-4, Neck, and Troch, and all bone turnover marker levels. However, the percentage of change (median, Q_R) in Neck BMD was significantly different in homozygous XX [-4.14 (from -6.54 to -1.34)] in comparison with Xx [1.72 (from -1.12 to 3.20)] ($P<0.001$) or xx [1.22 (from -1.74 to 3.06)] *Xba* I ER-alpha genotype ($P=0.001$). **CONCLUSION:** Women with ER-α *Xba* I genotype XX may have a higher risk of relatively fast bone mass loss in femoral neck after menopause and that they may have a poor responsiveness to calcium supplementation. The changes in BMD are not associated with ER-alpha *Pvu* II genotype and VDR *Apa* I genotype after 1 year of calcium supplementation.

INTRODUCTION

Osteoporosis is a disease of low bone mineral mass and microarchitectural deterioration of bone, which leads to increased risk of fracture. Postmenopausal osteoporosis depends on both the peak bone mass in early adulthood and the rate of bone loss after menopause. The peak bone mass achieved and the rate of postmenopausal bone loss are under strong genetic influence[1]. The significant association between *Bsm* I polymorphism of vitamin D receptor (VDR) gene and bone mineral density (BMD) variation in Caucasian women has been reported[2]. In postmenopausal women the rate of bone loss is also associated with VDR and estrogen receptor-alpha (ER-alpha) gene polymorphism [3,4].

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Bone mass in the elderly can be maintained in some individuals with calcium and vitamin D supplementation. But, the anti-osteoporotic treatments present variability in terms of BMD. The variability may be due to genetic factors[5]. In fact, *Bsm* I polymorphism in VDR gene may modify the BMD response to calcium intake, calcium and vitamin D supplementation, and hormone replacement therapy[6,7]. However, the frequencies of distribution of *Bsm* I polymorphism in VDR gene in Chinese Han population are significantly different to those of Caucasian. *Bsm* I BB genotype is rare in Chinese population[8]. Moreover, to our knowledge, no data is available in publications regarding the *Apa* I polymorphism in VDR gene and the different BMD response to calcium supplementation. In addition, postmenopausal women can be classified as fast and slow bone losers, and postmenopausal estrogen deficiency is related closely to the risk of osteoporosis, but the association between ER-alpha genotype and responsiveness to calcium supplementation in postmenopausal women is unclear. As a result, assessing the association between VDR and ER-alpha genotypes and the effect of calcium supplementation in postmenopausal women should be helpful in choosing anti-osteoporosis treatment according to individual genotype[9,10]. In this study, our aim was to investigate the influence of VDR *Apa* I and ER-alpha *Pvu* II and *Xba* I genotypes on bone loss rates and responsiveness to calcium supplementation in postmenopausal Chinese women.

MATERIALS AND METHODS

Study population The protocol was approved by the Ethical Committee of the Sixth People's Hospital, Shanghai Jiaotong University. The study population comprised 125 unrelated postmenopausal Chinese women of Han ethnicity in Shanghai (62.6±5.5 years old) who visited outpatient clinics of the Sixth People's Hospital, Shanghai Jiaotong University. Postmenopausal women with early menopause (before 40 years of age) and those that had undergone ovariectomy were excluded. None had a history of bone disease or drug use that might affect bone turnover. Using 7 d diet record, the participants recorded daily intakes of food and beverages after oral and written instructions. We reviewed the records with the participants and calculated dietary calcium from food composition tables. The dietary calcium intake was 300-500 mg/d in study individuals. The subjects enrolled received oral calcichew-D_3 (Nycomed Pharma, AS, Oslo, Norway) daily including 1000 mg calcium and 400 U vitamin D_3. Among the 125 postmenopausal women, 115 participants could be genotyped and analyzed for BMD and bone turnover markers changes after 1 year.

Genotyping Genomic DNA was extracted and purified from EDTA blood samples using routine procedure. Genotypic analysis of VDR gene *Apa* I, ER-alpha gene *Pvu* II, and *Xba* I polymorphisms was determined by polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP). VDR gene fragment including the *Apa* I polymorphism site was amplified using primers 5′-CAGAGCATGGACAGGGAGCAA-3′ and 5′-GCAACTCCTCATGGCTGAGGTCTC-3′[3]. ER-alpha gene fragment including the *Pvu* II and *Xba* I polymorphism sites was amplified using primers 5′- CTGCCACCCTATCTGTATCTTTTCCTATTCACC-3′ and 5′- TCTTTCTCTGCCACCCTGGCGTCGATTATCTGA-3′[4]. The PCR was carried out in 30 μL of a buffer solution: Tris-HCl 10 mmol/L, KCl 50 mmol/L, $MgCl_2$ 1.5 mmol/L, 200 μmol/L each of the four deoxyribonucleotides (dNTPs), 2.5 U of *Taq* polymerase, and 0.25 μmol/L of each primer. PCR was performed with the following steps: at 94 ºC for 5 min and then at 94 ºC for 1 min, at 60 ºC for 1 min, at 72 ºC for 1 min, for 30 cycles, and at 72 ºC for 7 min. After amplification, ER-alpha gene fragment was digested with *Pvu* II and *Xba* I restriction endonuclease and electrophoresed in 2.0 % agarose gel, respectively. Absence of the *Pvu* II and *Xba* I restriction sites were indicated by "P" and "X" and presence by "p" and "x", respectively. VDR gene fragment was digested with *Apa* I restriction endonuclease and electrophoresed in 1.8 % agarose gel. Absence the restriction site was indicated by "A" and presence by "a".

BMD measurements The BMD of the lumbar spine 1-4 (L1-4) and left proximal femur including femoral neck (Neck), and trochanter (Troch) were measured using dual-energy X-ray absorptiometry (DXA) (Hologic QDR-2000, Hologic corporation, Waltham, MA, USA) before and after 1 years of treatment. The short-term reproducibilities [coefficient of variation (CV) %] of L1-4, Neck, and Troch measurements were 0.97 %, 1. 93 %, and 1.48 %, respectively. The long-term reproducibility of our DXA instrument during the trial based on weekly repeated phantom measurements was 0.45 %.

Clinical examinations and biochemical studies During the follow-up each subject visited the outpatient clinic once a year. Fasting venous blood samples

and urinary were obtained and body weight, and height were measured. At the entry and 1 year after treatment, the concentrations of serum calcium, phosphate, alkaline phosphatase (ALP), parathyroid hormone (PTH), 25-hydroxy[25-(OH)]vitamin D, osteocalcin (OC), urinary creatinine-corrected free pyridinoline (PYD), and calcium were measured. Serum intact PTH, 25-(OH) vitamin D, and OC were determined by radioimmunoassay (RIA). The intra-assay and inter-assay *CV*s were both <10 %. Urinary PYD was measured using enzyme-linked immunosorbent assay (ELISA). The intra-assay and inter-assay *CV*s for urinary PYD were 4. 2 % and 7.9 %, respectively.

Statistical analysis The x^2 test was used for Hardy-Weinberg of ER-alph *Pvu* II and *Xba* I genotypes and VDR *Apa* I genotype. One-year after calcium supple-mentation, percent change in bone turnover markers and BMD were not distributed normally and expressed as the median [interquartile range (IQR)]. One-year changes in bone turnover markers or BMD were (One-year BMD or bone turnover marker-baseline BMD or bone turnover marker)×100/baseline BMD or bone turnover marker. In the postmenopausal women completing 1-year treatment as assigned, the values of BMD and bone turnover chemical markers were compared in the baseline and 1-year after calcium treatment using an unpaired *t*-test. An association between *Apa* I, *Pvu* II , and *Xba* I genotypes and BMD was evaluated by covariance (ANCOVA). The percent changes in BMD and bone turnover markers in each genotype were analyzed using Kruskal-Wallis H test. All statistical analyses were performed with SPSS 9.0 software and $P<0.05$ was considered statistically significant.

RESULTS

Genotype frequencies of the study population The distribution of *Pvu* II genotype was as follows: PP 23.5 %, Pp 45.2 %, and pp 31.3 %, respectively. Frequencies of XX, Xx, and xx genotype were 7.8 %, 40.9 %, and 51.3 %, respectively. The distribution of *Apa* I genotype was as follows: AA 7.8 %, Aa 38.9 %, and aa 53.3 %, respectively. The genotypes distribution of *Pvu* II, *Xba* I, and *Apa* I were compatible with the population in the Hardy-Weinberg equilibrium.

Characteristics of the study population One-year after supplementation for 1000 mg calcium and 400 U vitamin D_3 daily, compared with bone turnover markers at the baseline, the concentrations of serum ALP and PTH were significantly decreased ($P<0.05$ or $P<0.01$), and serum 25-(OH) vitamin D was markedly increased ($P<0.01$), but the values of serum OC and urinary PYD and calcium had no significant difference. After 1-year treatment of calcium, the BMD at L1-4, neck, and troch sites was significantly increased compared with that of baseline ($P<0.01$ or $P<0.05$) (Tab 1).

Association between the genotype and BMD and bone turnover markers At baseline and after 1-year treatment, no significant association was found between *Pvu* II, *Xba* I, and *Apa* I genotypes and BMD at L1-4 and any sites of proximal femur adjusted for age, weight, and years since menopause. However, *Xba* I genotype was significantly associated with percent change in BMD of neck after 1 year of calcium supplementation (Tab 2). The percent change (median, Q_R) in neck BMD was significantly different in homozygous XX [-4.14 (from -6.54 to -1.37)] in comparison with Xx [1.72 (from -1.12 to 3.20)] ($P<0.01$) or xx [1.22 (from -1.74 to 3.20)] *Xba* I ER-alpha genotype ($P=0.001$). However, the percent change BMD in L1-4 and troch was not statistically different between *Xba* I genotype. Moreover, no significant association was found between the percent changes in BMD and *Pvu* II and *Apa* I genotypes after calcium supplementation.

At baseline and after 1-year treatment, serum and urinary levels of bone turnover markers was not significantly associated with *Xba* I, *Pvu* II, and *Apa* I genotypes (Tab 2-4). In addition, the percent changes in bone turnover markers were not significantly different neither in ER-alpha, nor VDR gene polymorphisms.

DISCUSSION

Osteoporosis is a worldwide health issue, with a high prevalence of disease not only in Western countries but also in Asia. Osteoporosis is also a multifactorial diseases with a genetic background as essential hypertension[11,12]. Genetic factors play important roles in the pathogenesis of postmenopausal osteoporosis[1,13]. Several candidate genes that may contribute to bone turnover and BMD have been identified. VDR and ER-alpha genes are two important candidate genes that can potentially regulate BMD. Several studies reported that the *Bsm* I polymorphism of VDR gene and *Pvu* II and *Xba* I polymorphism of ER-alpha gene had significant effect on BMD in Caucasian women[2,3,14]. Recently, we have reported that *Pvu* II and *Xba* I polymorphism

Tab 1. Characteristics of postmenopausal women at baseline and 1 year after calcium supplementation. Median changes and 25th to 75th percentile (interquartile) range in bone turnover markers and BMD in comparison with baseline in each postmenopausal woman supplemented with calcium 1000 mg daily for 1 year. *n*=115. Mean±SD.

	Baseline	After calcium supplementation	Bone turnover markers and BMD change (%)	*P* value
Age/year	62±5	63±5	-	0.801
Years since menopause/year	13±6	14±6	-	0.709
Height/cm	154±5	152±6	-	0.638
Weight/kg	57±8	58±8	-	0.395
ALP/IU·L^{-1}	71±20	67±15	-7.3 (-20.0~0.9)	0.019
BGP/mg·L^{-1}	13±8	12±5	-3.8 (-36.5~46.0)	0.556
PTH/ng·L^{-1}	31±9	23±9	-27.8 (-44.0~-7.0)	0.000
25-(OH) vitamin D/mg·L^{-1}	10±9	17±9	126.7 (34.0~320.0)	0.000
Urinary PYD/ nM·mM^{-1} Cr	41±35	41±17	29.2 (-24.9~93.2)	0.975
Urinary Ca/nM·mM^{-1} Cr	0.44±0.21	0.43±0.24	-0.4 (-33.3~46.8)	0.708
L1-4 BMD/g·cm^{-2}	0.77±0.10	0.78±0.10	0.73 (-0.86~2.72)	0.001
Neck BMD/g·cm^{-2}	0.61±0.07	0.62±0.07	1.15 (-1.74~3.05)	0.000
Troch BMD/g·cm^{-2}	0.49±0.06	0.50±0.06	1.03 (-0.85~3.17)	0.018

of ER-alpha gene was associated with peak BMD and bone size in Chinese women[15,16]. Up to now, the biological candidate genes of osteoporosis can be ordered into 5 categories: (i) calcium homeostasis; (ii) hormonal dysfunction; (iii) osteoblast and osteoclast development and regulation; (vi) cartilage matrix metabolism; and (v) lipoprotein metabolism. However, little data are available in the literature regarding the genetic factors that can influence the clinical response to anti-osteoporotic therapies. Therefore, it is very useful to choose efficacious drugs targeting osteoporosis according to different genotypes[10,17].

At present, our study evaluated the different bone mass gains after calcium supplementation according to ER-alpha and VDR genotype in postmenopausal Chinese women. The frequencies of *Xba* I, *Pvu* II, and *Apa* I genotypes in our group of unrelated postmenopausal Chinese women was very similar to the previous report in Chinese population[15,16,18], and significantly different to those in Caucasians[2,14]. Although no significant association was found between VDR *Apa* I genotype, ER-alpha *Pvu* II, and *Xba* I genotypes and BMD at baseline and 1-year after calcium supplementation, *Xba* I genotype was significantly associated with bone loss in femoral neck. The percent change in femoral neck BMD was significantly different in homozygous XX (-4.14 %) in comparison with Xx (1.72 %) or xx (1.22 %) genotype. Moreover, at entry, and after 1 year of calcium supplementation, there was no significant difference between *Xba* I genotype group with respect to serum and urinary levels of bone turnover markers. The results suggested that postmenopausal women with XX genotype had a fast bone loss after menopause, and poor responsiveness to calcium supplementation. However, the action mechanism of *Xba* I polymorphism on bone loss is not completely understood. Salmen *et al*[19] reported that 177 postmenopausal women were administered calcium lactate, 500 mg alone or in combination with vitamin D_3, 100-300 U/d for 5 years. The results showed that lumbar spine BMD decreased more in subjects with the ER-alpha genotypes PP (6.4 %) and Pp (5.2 %) than in subjects with the pp genotype (2.9 %) after 5 years. But, *Pvu* II genotype was not associated with the rate of bone loss in femoral neck. Unfortunately, in their report data regarding the genetic evaluation of ER-alpha *Xba* I genotype was not available. Therefore, several studies have investigated the effect of VDR gene *Bsm* I polymorphism on response to calcium or vitamin D_3 supplementation. Graafmans *et al*[7] analyzed 81 women, age 70 years or older, who participated in a placebo-controlled clinical trial on the effect of vitamin D_3 supplementation (400 U daily for at least 2 years) on BMD. The results showed that VDR *Bsm* I genotype was not associated with baseline BMD at the femoral neck, but the mean increase of femoral neck BMD in the vitamin D_3 group relative to the placebo group, expressed as percentage of baseline BMD, was significantly higher in

Tab 2. Characteristics of the subjects according to ER-alpha *Xba* I genotype. *n*=115. Mean±SD. $^{c}P<0.01$ vs Xx or xx genotype.

	XX	Xx	xx	*P* value
n (%)	9 (0.078)	47 (0.409)	59 (0.513)	
Age/year	61±5	63±5	62±5	0.432
Years since menopause /year	12±7	14±6	13±6	0.080
Height/cm	155±5	154±6	153±5	0.810
Weight/kg	57±10	57±6	57±9	0.581
Bone markers at baseline				
ALP	67±15	75±23	67±16	0.161
BGP	9±6	12±6	14±9	0.195
PTH	36±14	31±8	31±9	0.386
Pyd	31±4	31±19	46±21	0.239
25-(OH) vitamin D	16±7	12±8	11±8	0.785
Urinary Ca	0.61±0.21	0.39±0.20	0.44±0.21	0.061
Bone markers after 1 year				
ALP	70±17	67±16	66±15	0.861
BGP	11±4	12±5	12±5	0.919
PTH	27±8	22±9	24±10	0.246
Pyd	42±25	44±19	37±18	0.934
25-(OH) vitamin D	17±9	16±8	18±10	0.767
Urinary Ca	0.46±0.29	0.46±0.22	0.40±0.25	0.525
Bone markers change/%				
ALP	6.5 (-21.8~21.3)	-11.5 (-20.7~6.6)	-3.7 (-19.2~15.8)	0.202
BGP	-4.7 (-43.1~26.8)	5.1 (-20.3~69.1)	-27.6 (-38.7~13.1)	0.181
PTH	-35.1 (-52.1~0.8)	-26.0 (-46.0~-13.3)	-27.5 (-44.0~3.3)	0.701
Pyd	-3.5 (12.5~27.9)	12.0 (8.9~109.9)	-2.4 (-43.1~70.9)	0.068
25-(OH) vitamin D	98.6 (13.3~326.4)	312.2 (256.4~384.2)	133.6 (39.6~277.5)	0.924
Urinary Ca	-3.5 (-12.5~27.9)	52.0 (8.9~109.9)	-2.4 (46.9~71.8)	0.062
BMD at baseline /g·cm^{-2}				
L1-4	0.79±0.12	0.77±0.10	0.77±0.10	0.773
Neck	0.62±0.10	0.62±0.06	0.60±0.08	0.340
Troch	0.50±0.07	0.50±0.06	0.48±0.06	0.210
BMD after 1 year/g·cm^{-2}				
L1-4	0.80±0.13	0.78±0.11	0.78±0.10	0.839
Neck	0.59±0.10	0.63±0.07	0.61±0.08	0.347
Troch	0.49±0.07	0.50±0.06	0.49±0.06	0.382
BMD change/%				
L1-4	0.73 (-0.86~2.72)	0.73 (-1.29~2.76)	0.66 (-0.64~2.84)	0.965
Neck	-4.14 (-6.54~-1.34)^{c}	1.72 (-1.12~3.20)	1.22 (-1.74~3.06)	0.003
Troch	0.00 (-2.65~1.06)	1.03 (-0.66~3.51)	1.40 (-0.85~3.18)	0.361

the BB (4.4 %) and Bb genotype (4.2 %) compared with the bb genotype (-0.3 %). Ferrari *et al*[20] investigated the influence of VDR *Bsm* I genotype on bone loss rates and responsiveness to calcium supplementation in Switzerland elders. Calcium supplementation (800 mg daily as calcium carbonate or osseino-mineral complex) was given to 72 elderly subjects for 18 months. The results showed that 30 subjects had net loss of lumbar spine BMD of more than 0.48 % per year, and 7 subjects of BB genotype had losses greater than 0.48 % per year, compared with 15 of Bb and 8 of bb subjects. Moreover, the rate of change in lumbar spine BMD was significantly greater in BB genotype (-2.3 % per year) than in homozygote bb (0.7 % per year) or in heterozygote Bb subjects (1.0 % per year). The data suggested that VDR gene *Bsm* I polymorphism was related to the response of bone mass to calcium supplementation in elderly Caucasians. Because VDR BB genotype is rare in

Tab 3. Characteristics of the subjects according to ER-alpha *Pvu* II genotype. *n*=115. Mean±SD.

	PP	Pp	pp	*P* value
n (%)	27 (0.235)	52 (0.452)	36 (0.313)	
Age/year	61±7	62±5	62±5	0.074
Years since menopause /year	12±7	14±6	13±6	0.069
Height/cm	155±5	154±6	153±5	0.052
Weight/kg	57±10	57±6	57±9	0.078
Bone markers at baseline				
ALP	70±24	72±19	71±19	0.917
BGP	9±6	12±6	14±9	0.871
PTH	36±14	31±8	31±9	0.992
Pyd	45±16	33±15	45±18	0.331
25-(OH) vitamin D	16±7	12±6	11±6	0.770
Urinary Ca	0.61±0.21	0.39±0.20	0.44±0.21	0.601
Bone markers after 1 year				
ALP	66±16	67±14	68±17	0.542
BGP	11±4	13±5	11±5	0.406
PTH	25±11	22±8	24±10	0.616
Pyd	42±23	41±23	41±19	0.941
25-(OH) vitamin D	16±7	16±8	18±10	0.616
Urinary Ca	0.44±0.27	0.46±0.23	0.38±0.23	0.492
Bone markers change/%				
ALP	-3.7 (-22.2~10.9)	-3.2 (-17.5~21.0)	-11.1 (-20.2~9.8)	0.832
BGP	-12.1 (-39.9~40.1)	8.4 (-27.6~77.1)	-23.3 (-37.9~5.7)	0.263
PTH	-13.0 (-52.9~8.5)	-31.0 (-41.3~-14.9)	-28.8 (-44.0~-8.1)	0.587
Pyd	6.6 (-23.0~60.8)	28.7 (-14.0~106.4)	38.5 (-38.1~116.5)	0.648
25-(OH) vitamin D	109.9 (20.3~196.1)	116.7 (14.3~325.6)	214.0 (43.5~492.4)	0.329
Urinary Ca	10.4 (-46.1~51.9)	20.9 (-20.8~88.1)	-16.3 (-41.7~8.6)	0.107
BMD at baseline /g•cm^{-2}				
L1-4	0.76±0.02	0.78±0.02	0.78±0.02	0.801
Neck	0.61±0.02	0.63±0.01	0.60±0.01	0.116
Troch	0.50±0.01	0.51±0.01	0.50±0.01	0.625
BMD after 1year /g•cm^{-2}				
L1-4	0.76±0.03	0.78±0.02	0.78±0.02	0.829
Neck	0.62±0.02	0.63±0.01	0.61±0.01	0.427
Troch	0.50±0.01	0.51±0.01	0.50±0.01	0.473
BMD change/%				
L1-4	0.421 (-0.86~2.71)	0.31 (-0.80~3.0)	0.31 (-1.11~2.02)	0.625
Neck	0.37 (-2.18~3.58)	0.81 (-2.71~2.64)	1.21 (-1.70~3.06)	0.615
Troch	1.06 (-2.65~4.01)	1.27 (-0.66~3.90)	0.26 (-0.87~2.06)	0.305

Chinese population, in present study, we investigated the association between VDR *Apa* I genotype and change of BMD to calcium supplementation in postmenopausal women. No significant genotypic differences was found in the change in lumbar spine and proximal femur BMD before and after 1 year of calcium supplementation.

A high calcium intake suppressed the loss of bone in weight-stable pre- and postmenopausal women. Women who increased calcium intake to greater than 800 mg/d for 1-2 years showed a 0.5 % -1 % decrease in bone loss compared with women consuming less than 800 mg/d[21,22]. In the present study, the data confirmed that daily administration of 1000 mg of calcium and 400 U of vitamin D_3 for 1 year slightly increased in lumbar spine and proximal femur BMD and significantly decreased the age-related increases in serum ALP and PTH levels.

Tab 4. Characteristics of the subjects according to VDR *Apa* I genotype. *n*=115. Mean±SD.

	AA	Aa	aa	*P* value
n (%)	9 (0.078)	45 (0.389)	61 (0.533)	
Age/year	63±5	63±4	62±5	0.299
Years since menopause/year	15±9	14±5	13±6	0.609
Height/cm	151±4	154±6	154±5	0.312
Weight/kg	58±6	57±8	57±8	0.846
Bone markers at baseline				
ALP	75±15	75±21	67±18	0.154
BGP	11±7	12±6	13±9	0.638
PTH	16±10	22±8	24±10	0.213
Pyd	28±15	42±39	40±33	0.331
25-(OH) vitamin D	10±6	12±13	12±23	0.942
Urinary Ca	0.55±0.15	0.43±0.22	0.42±0.20	0.412
Bone markers after 1 year				
ALP	61±15	73±15	63±15	0.058
BGP	12±3	12±4	12±5	0.837
PTH	16±10	22±8	24±10	0.183
Pyd	35±10	44±20	39±19	0.941
25-(OH) vitamin D	18±9	16±9	17±9	0.637
Urinary Ca	0.66±0.28	0.42±0.23	0.41±0.23	0.492
Bone markers change/%				
ALP	-11.5 (-28.8~-2.5)	-9.0 (-20.3~10.3)	-3.5 (-19.4~18.7)	0.832
BGP	62.3 (-35.0~88.4)	-5.3 (-32.4~21.9)	-5.4 (-39.1~49.6)	0.263
PTH	-55.7 (-73.1~23.3)	-27.5 (-42.0~-14.3)	-26.4 (-44.1~-6.3)	0.587
Pyd	60.8 (-23.4~138.9)	48.9 (-11.4~132.3)	13.1 (-28.3~76.0)	0.648
25-(OH) vitamin D	103.3 (7.3~475.3)	81.8 (12.2~298.7)	134.0 (45.1~325.5)	0.329
Urinary Ca	25.5 (-4.1~45.2)	-0.4 (-32.7~48.0)	-6.7 (-38.2~36.9)	0.107
BMD at baseline /g•cm^{-2}				
L1-4	0.79±0.04	0.78±0.02	0.77±0.02	0.899
Neck	0.65±0.03	0.61±0.01	0.62±0.01	0.379
Troch	0.52±0.02	0.51±0.01	0.50±0.01	0.660
BMD at 1 year /g•cm^{-2}				
L1-4	0.74±0.05	0.78±0.02	0.78±0.02	0.783
Neck	0.69±0.03	0.61±0.01	0.62±0.01	0.098
Troch	0.53±0.03	0.50±0.01	0.50±0.01	0.603
BMD change/%				
L1-4	1.42 (-1.21~3.19)	0.73 (-1.00~2.52)	0.92 (-0.83~2.84)	0.876
Neck	1.49 (-0.74~5.68)	1.22 (-1.74~3.12)	1.10 (-2.23~2.83)	0.710
Troch	1.55 (-5.97~5.85)	1.029 (-2.51~3.17)	1.14 (-0.50~2.61)	0.781

In conclusion, our results suggested that Chinese women of Han population with ER-alpha *Xba* I genotype XX had a greater risk of relatively fast bone mass loss in femoral neck after menopause than those with the Xx and xx genotype and that they might have a poor responsiveness to calcium supplementation, and they needed to apply more efficacious drugs of anti-osteoporosis. ER-alpha *Pvu* II and VDR *Apa* I genotypes were not associated with therapeutic response to calcium for postmenopausal Chinese women. Considering the limited number of participants in the present study, we aim to carry out an investigation with a sufficiently large number of participants and of placebo-controlled in the future to determine a certain association between candidate gene polymorphisms and the effect of anti-osteoporosis drugs.

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