

Raloxifene administration in post-menopausal women with osteoporosis: effect of different *BsmI* vitamin D receptor genotypes

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BACKGROUND: The vitamin D receptor (VDR) gene polymorphism has been considered a factor influencing the effectiveness of the anti-osteoporotic treatments. The aim of this study was to correlate the effectiveness of raloxifene treatment in post-menopausal women with osteoporosis to *BsmI* VDR genotypes. **METHODS:** Between January and August 2000, 75 Italian osteoporotic women were enrolled and treated with raloxifene at a dose of 60 mg/day. At entry and after 1 year of treatment, lumbar bone mineral density (BMD), serum osteocalcin (OC) and urinary creatinine-corrected free deoxyypyridinoline (DPD) levels were evaluated. DNA was extracted from blood and analysed with restriction endonuclease *BsmI* for VDR gene. **RESULTS:** After treatment, a significant increase in lumbar BMD and a significant reduction in serum OC and urinary DPD levels were observed. The percentage of change (mean \pm SD) in lumbar BMD, and in serum OC and urinary DPD levels was significantly different in homozygous bb (1.58 ± 0.80 , -5.15 ± 2.36 and -7.71 ± 2.89 for BMD, OC and DPD respectively) in comparison with BB (4.13 ± 2.26 , -13.59 ± 4.68 and -15.16 ± 4.65 for BMD, OC and DPD respectively) *BsmI* VDR genotypes. Heterozygous Bb VDR patients showed an intermediate percentage (mean \pm SD) of BMD, serum OC and urinary DPD change (2.49 ± 1.54 , -8.69 ± 2.60 and -10.52 ± 2.56 for BMD, OC and DPD respectively) not significantly different in comparison with homozygous BB and bb. **CONCLUSIONS:** In post-menopausal women with osteoporosis the effectiveness of raloxifene treatment on bone metabolism seems to be controlled by different *BsmI* VDR genotypes.

Key words: bone mineral density/osteoporosis/post-menopause/raloxifene/vitamin D receptor gene polymorphisms

Introduction

Raloxifene is a synthetic non-steroidal drug derived from benzothiophene and afferent to selective estrogen receptor modulators (SERM), a group of compounds that interact with estrogen receptors (ER) eliciting tissue-specific responses (Seeman, 2001).

Raloxifene at a standard dose of 60 mg daily has been successfully used in preventing post-menopausal bone loss in women without osteoporosis, but also as treatment for established post-menopausal osteoporosis (Clemett and Spencer, 2000). Raloxifene administration also reduces the risk of vertebral fractures by ~40% in post-menopausal osteoporotic women with or without pre-existing fractures in comparison with placebo treatment (Lufkin *et al.*, 1998; Ettinger *et al.*, 1999; Meunier *et al.*, 1999).

The anti-osteoporotic treatments present variability in terms of bone mineral density (BMD) gain response. The variability may be due to the phenomenon of the regression to the mean (Cummings *et al.*, 2000), but also to genetic

factors (Keen and Kelly, 1997; Eisman, 1999). According to this view, the vitamin D receptor (VDR) gene polymorphism could be considered a pivotal factor influencing the effectiveness of the anti-osteoporotic treatments (Eisman, 1999, 2001). It has been suggested, in fact, that VDR gene polymorphisms may modify the BMD response to calcium (Ca) intake (Dawson-Hughes *et al.*, 1995; Ferrari *et al.*, 1995; Kiel *et al.*, 1997), Ca and vitamin D supplementation (Howard *et al.*, 1995; Graafmans *et al.*, 1997; Wishart *et al.*, 1997), cyclic etidronate treatment (Marc *et al.*, 1999) and hormone replacement therapy (HRT) (Deng *et al.*, 1998; Kurabayashi *et al.*, 1999).

To our knowledge, no data are available in the English literature regarding the VDR gene polymorphisms and the different BMD response to raloxifene treatment. Based on these considerations, the aim of the present study was to examine the correlation between a VDR gene polymorphism and the effectiveness of raloxifene treatment in post-menopausal women with osteoporosis.

Materials and methods

The procedures used during the study were in accordance with the guidelines of the Helsinki Declaration on human experimentation. The protocol was approved by the Local Ethics Committee of the University of Naples. Before entering the study, the purpose of the protocol was explained to all women attending the Departments of Gynaecology of the Universities of Catanzaro and Naples. A written informed consent was obtained by all subjects.

Patients

Between January and August 2000, 75 consecutive ambulatory post-menopausal women with osteoporosis were enrolled in the study protocol.

Inclusion criteria were spontaneous post-menopause and BMD values ≥ 2.5 SD below the mean bone density of the peak value for sex-matched healthy young adults (-2.5 T-score) at posterior–anterior lumbar spine. All women enrolled were Caucasian and genetically unrelated.

Exclusion criteria were: active rheumatoid arthritis, gastrointestinal or liver disease, metabolic, neoplastic or endocrine diseases, history of acute or recurrent vascular thrombosis; secondary causes of osteoporosis, such as hyperparathyroidism, Paget's disease of bone, or renal osteodystrophy; previous treatment with bisphosphonates, sodium fluoride, calcitonin, estroprogestins, anabolic steroids, corticosteroids, Ca, vitamin D, or phosphate (P); treatment with corticosteroids, thiazidic diuretics or other drugs interfering with bone metabolism; serum creatinine (Cr) >133 $\mu\text{mol/l}$; history of gastrointestinal side-effects; body mass index (BMI) <18 or >30 kg/m^2 . Women with plasma 25OH-vitamin D levels <25 nmol/l were excluded from the study. Also excluded were women who regularly used any medication that had the potential of causing gastrointestinal irritation or drugs to inhibit gastric acid secretion, women smoking >20 cigarettes per day, and women drinking more than three alcoholic beverages per day.

Treatment and study protocol

The subjects enrolled received oral raloxifene hydrochloride (Evista; Eli Lilly, Sesto Fiorentino, Florence, Italy) at a dose of 60 mg/day. The duration of the treatments was 12 months.

At baseline and after treatment, BMD and bone metabolism were measured in all groups. Both patients and clinicians were blind with respect to these results throughout the study period.

The study of biochemical parameters and the measurements of BMD are detailed below.

At baseline and every 3 months, Ca intake, alcohol consumption and physical activity were evaluated as previously described (Palomba *et al.*, 1999, 2002). Ca intake and alcohol consumption were assessed by a dietary history of patients using a semi-quantitative diet questionnaire developed by dieticians of the University of Naples. Ca intake was expressed as a score ranging from 1 to 3, according to the following scale: score 1 for a Ca intake <500 mg/day, score 2 for a Ca intake 500–1000 mg/day, and score 3 for a Ca intake >1000 mg/day. Alcohol consumption was also expressed as a score ranging from 1 to 3, according to the following scale: score 1 for an alcohol consumption <1000 mg/day, score 2 for an alcohol consumption 1000–2000 mg/day, and score 3 for an alcohol consumption >2000 mg/day. A semi-quantitative questionnaire was also used to evaluate patients' daily physical activity. Physical activity was expressed as a score ranging from 1 to 3, according to the following scale: score 3 (high physical activity) was assigned to women who exercised regularly; score 2 (moderate physical activity) was assigned to women who did not exercise regularly but participated daily in activities, such as house-cleaning, climbing stairs, or walking to work, to the bus stop, or

to a restaurant; score 1 (low physical activity) was assigned to women who did not participate in any of above-mentioned activities.

No dietary restriction or changes were implemented during the study. To ensure adequate Ca intake, all patients with a Ca intake <1000 mg received daily supplements of elemental Ca in the form of effervescent tablets composed of Ca carbonate (Cacit; Procter & Gamble, Rome, Italy) to achieve a total daily Ca intake of ≥ 1000 mg. This supplement was taken at lunch.

BMD measurement

The BMD was determined by dual energy X-ray absorptiometry (Hologic QDR 1000; Waltham, MA, USA) at posterior–anterior lumbar spine (vertebrae L2–L4). The precision of the measurements expressed as coefficient of variation (CV) *in vitro* for repeated BMD determinations in two standard phantoms in our laboratory was 0.41 and 0.43% for the University of Naples and Catanzaro respectively. The CV *in vivo* had been evaluated comparing two measurements performed at 7 day intervals in 40 volunteers and was 1.0 and 1.2% for the University of Naples and Catanzaro respectively.

The absorptiometries were examined by the same observer blinded with respect to different treatment regimens and VDR genotypes. We used the mean of three scans to calculate bone mineral content. The BMD values were calculated by the software of the bone densitometer dividing the bone mineral content (g/cm) by the bone width (cm) and expressed directly as an index (g/cm²).

Vertebral fractures were assessed from thoracic and lumbar (T4–L4) lateral and anterior–posterior spinal radiographs taken at baseline and from lateral spinal radiographs taken at the end of the study. Potential fractures were identified by quantitative morphometry according to the guideline of the US National Osteoporotic Foundation Working Group on Vertebral Fractures (Kiel, 1995), and subsequent visual verification of incident fractures was performed by a qualified radiologist.

Biochemical assays

At entry, serum FSH and estradiol (E₂) levels were assayed in all women to confirm the post-menopausal status. At entry and after 1 year of treatment, Ca, phosphate, parathyroid hormone (PTH), osteocalcin (OC) and urinary Cr and deoxypyridinoline (DPD) levels were determined using commercial kits (Palomba *et al.*, 2002).

In particular, serum OC levels and urinary Cr-corrected free DPD were used as markers of bone formation and resorption respectively. Serum OC levels (reference range: 3.1–13.7 ng/ml) were assayed by an immunoradiometric assay (IRMA; Diagnostic Products Corporation, Los Angeles, CA, USA) with a sensitivity of 0.1 ng/ml, and an intra-assay and inter-assay CV of 4.5 and 3.5% respectively. Serum PTH levels (reference range: 10–65 ng/l) were determined using an intact PTH IRMA (Diagnostic System Laboratories Inc., Webster, TX, USA) with a sensitivity of 1.0 ng/l, and an intra- and inter-assay CV of 7.1 and 3.5% respectively.

Urinary DPD concentrations (reference range normalized for Cr levels: 3.0–7.4 nmol/mmol) were assayed by an enzyme immunoassay (Metra Biosystems, Milan, Italy) with a sensitivity of 1.1 nmol/l, and an intra-assay and inter-assay CV of 7.6 and 5.5% respectively. Urinary concentrations of Cr (reference range: 8.8–14.1 mmol/24 h) were measured using an auto-analyser (Monarch 1000; Instrumentation Laboratory, Milan, Italy). Cr-corrected values were calculated by dividing DPD by urinary Cr measured using a standard colorimetric assay.

Blood and 24 h urine samples were collected between 08:30 and 09:30 after an overnight fast to avoid the interference of circadian changes. Patients were asked to refrain from eating foods containing

Table I. Characteristics of the subjects according to VDR gene *BsmI* polymorphism

	VDR genotypes			P
	BB	Bb	bb	
No. (%)	12 (18.2)	30 (45.4)	24 (36.4)	
Age (years)	65.5 ± 3.2	65.6 ± 5.6	64.9 ± 3.5	NS
Body mass index (kg/m ²)	25.6 ± 1.9	25.5 ± 1.6	25.2 ± 1.4	NS
Time since menopause (years)	14.4 ± 4.2	14.6 ± 3.5	14.7 ± 2.9	NS
Parity (number)	2.0 ± 0.4	2.1 ± 0.4	1.8 ± 0.3	NS
Smoking history				NS
Never smoked (%)	4 (33.3)	10 (33.3)	10 (41.7)	
Past smoker (%)	4 (33.3)	12 (40.0)	9 (37.5)	
Current smoker (%)	4 (33.3)	8 (26.7)	5 (20.8)	
Cigarettes smoked (number/day)	7.2 ± 3.6	7.4 ± 2.6	7.6 ± 2.8	NS
25-OH vitamin D (nmol/l)	70.1 ± 14.9	71.2 ± 15.6	64.3 ± 14.8	NS
Ca (mmol/l)	2.31 ± 0.25	2.30 ± 0.24	2.32 ± 0.22	NS
Phosphorus (nmol/l)	1.22 ± 0.13	1.21 ± 0.12	1.21 ± 0.12	NS
Ca intake score ^a	1.8 ± 0.8	1.9 ± 0.8	2.0 ± 0.8	NS
Women assuming Ca supplementation (%)	8 (66.6)	22 (73.3)	18 (75.0)	NS
Alcohol intake score ^b	1.3 ± 0.5	1.3 ± 0.4	1.4 ± 0.5	NS
Physical activity score ^c	1.6 ± 0.8	1.4 ± 0.7	1.4 ± 0.7	NS
Lumbar spine T-score	-3.64 ± 0.60	-3.65 ± 0.81	-3.56 ± 0.58	NS

Values are expressed as mean ± SD; statistical significance based on one-way analysis of variance (NS = not significant).

^a1 = <500 mg/day; 2 = 500–1000 mg/day; 3 = >1000 mg/day.

^b1 = <1000 mg/day; 2 = 1000–2000 mg/day; 3 = >2000 mg/day.

^c1 = low; 2 = moderate; 3 = high.

fat or gelatine within 12 h of their clinic visit. Serum samples were separated within 1 h of collection and kept frozen at -80°C, and urine was stored at -20°C until biochemical analysis. All samples from the same woman were analysed blind by a central laboratory in the same assay (University of Catanzaro).

DNA analysis

The DNA analysis was performed in the Department of Clinical and Experimental Medicine of the University of Naples. At entry, DNA was extracted from whole blood samples with a 'salting out' procedure (Miller *et al.*, 1988). Genotypic analysis of VDR gene polymorphisms was determined by PCR amplification and enzymatic digestion of the products with *BsmI* restriction enzyme. The forward primer for amplification of the *BsmI* VDR polymorphism was modified from a published method (Sainz *et al.*, 1997) (5'-AAGACTACAAGTACCGCGTCAGTG-3'). The reverse primer for amplification of the *BsmI* VDR polymorphism was the same as that published elsewhere (Morrison *et al.*, 1994) (5'-AACCAGCGGGAAGAGGTCAAGGG-3').

PCR was performed with a Techne Progene Thermal Cycler (Cambridge, UK) under standard conditions for 30 cycles and at 65°C annealing temperature. DNA was digested with *BsmI* under standard conditions and the products were analysed by electrophoresis on a 1.5% agarose gel containing ethidium bromide. The alleles were defined as 'B' or 'b' in the absence or presence of the restriction site respectively.

Safety evaluation

Standard clinical evaluations and laboratory analyses, including haematological, renal function and liver function tests, and microscopic examinations of sediment from midstream urine specimens were performed before treatment and after every 6 months. A mammography was performed before treatment and then yearly. The subjects were instructed to record the appearance of adverse experiences (AE) in a daily diary.

Statistical analysis

Analysis of variance followed by the Newman-Keuls multiple range test was used to compare multiple measures of age, time since menopause, BMI, BMD, and biochemical data. Wilcoxon's signed-rank test was used to compare parity, cigarettes smoked, alcohol consumption, Ca intake and physical activity. The proportion of women receiving Ca supplements in the three groups of polymorphism was compared using the χ^2 -test. Fisher's exact test was used to compare the incidence of AE between groups. Statistical analysis was performed using SPSS 9.0 (SPSS Inc., Chicago, IL, USA).

The statistical significance was set at $P < 0.01$. Data were normally distributed and were expressed as mean ± SD.

Results

Sixty-six of 75 (88%) enrolled osteoporotic post-menopausal women completed the study. Six subjects dropped out from the study due to lack of compliance with treatment and three missed the control visit after 1 year of treatment for personal reasons. No drop-outs were due to drug-related side-effects.

Eight women reported the appearance of 'mild' hot flushes. Three women reported a worsening of their mild vasomotor symptoms. In particular, two and one women reported the presence of 'moderate' and 'severe' hot flushes respectively. Two women reported the presence of leg cramps. No other drug-related AE were reported. During the study, no serious AE were observed. One woman had an asymptomatic vertebral fracture.

After 12 months of raloxifene treatment, we observed a significant ($P = 0.0009$) increase in lumbar spine BMD (0.545 ± 0.056 versus 0.558 ± 0.061 mg/cm², before versus after treatment respectively) in our study population. The percentage of BMD change (%) from baseline was of 2.46 ± 1.71 . At the same time, the serum OC (6.17 ± 0.66 ng/ml) and urinary DPD

Table II. Bone mineral density (BMD) and biochemical markers before and after 12 months of raloxifene treatment (60 mg/day) according to VDR gene *BsmI* polymorphism

	VDR genotypes			P
	BB	Bb	bb	
BMD lumbar spine (g/cm ²)				
Baseline	0.546 ± 0.060	0.547 ± 0.058	0.541 ± 0.056	NS
12 months	0.569 ± 0.067 ^a	0.561 ± 0.063 ^a	0.549 ± 0.058 ^a	NS
Parathyroid hormone (ng/l)				
Baseline	46.8 ± 8.2	48.1 ± 7.0	47.7 ± 6.9	NS
12 months	47.1 ± 5.1	48.6 ± 5.9	48.7 ± 5.0	NS
Osteocalcin (ng/ml)				
Baseline	6.78 ± 0.68	6.69 ± 0.75	6.77 ± 0.61	NS
12 months	5.85 ± 0.29 ^a	6.11 ± 0.74 ^a	6.43 ± 0.62 ^a	NS
DPD (nmol/mmol)				
Baseline	6.25 ± 0.80	6.29 ± 0.59	6.43 ± 0.51	NS
12 months	5.29 ± 0.60 ^a	5.64 ± 0.58 ^a	5.93 ± 0.44 ^a	0.004

Values are expressed as mean ± SD.

^aP < 0.01 versus baseline.

DPD = deoxyypyridoline/creatinine ratio; NS = non-significant.

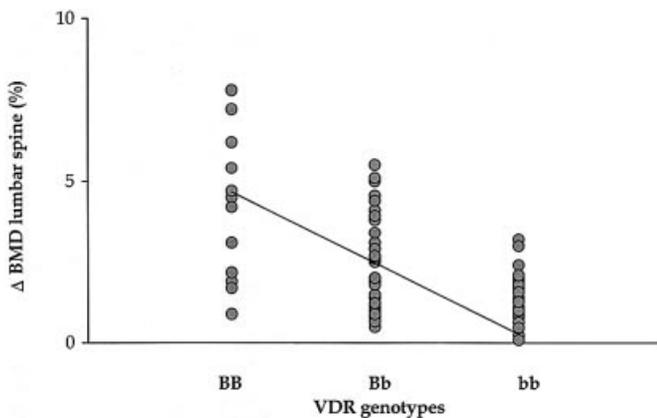


Figure 1. Percentage change in lumbar spine bone mineral density (BMD) in comparison with baseline in each osteoporotic postmenopausal woman treated with raloxifene 60 mg daily for 1 year according to *BsmI* VDR genotypes. A significant ($P = 0.0001$) difference was detected between BB and bb genotypes.

levels (5.68 ± 0.57 nmol/mmol) were significantly ($P = 0.0009$) lower in comparison with basal values (6.73 ± 0.64 and 6.33 ± 0.59 nmol/mmol for OC and DPD respectively).

The VDR genotype prevalence in study population is shown in Table I. In our population study, the *BsmI* VDR genotypes frequencies were in Hardy–Weinberg equilibrium (Ott, 1999) and the allelic frequencies were comparable with published data in Caucasian unrelated subjects (Hustmyer *et al.*, 1993).

Considering the different *BsmI* VDR genotypes, no significant differences in baseline data were detected between the three groups of women (Table I). In particular, no significant differences in age, BMI, time since menopause, parity, number of cigarettes smoked daily, Ca and alcohol intake scores, physical activity score, and T-score were detected between the three groups of women. The three groups were also similar in serum 25OH-vitamin D, Ca and phosphate levels.

At entry, there was also no significant difference between VDR genotype group with respect to lumbar BMD, serum OC and urinary DPD levels (Table II). After 1 year of raloxifene treatment, a significant ($P = 0.0009$) difference in lumbar BMD and in serum OC and urinary DPD levels was observed in each group of VDR genotype in comparison with baseline values (Table II). At the same time, no statistically significant difference was observed between the three groups for lumbar BMD, serum PTH levels, or serum OC ($P = NS$). However, urinary DPD levels were significantly ($P = 0.004$) different (Table II).

In Figures 1 and 2 are shown the individual percentages of change in lumbar BMD, and in serum OC and urinary DPD-Cr levels in each woman according to *BsmI* VDR genotypes after 1 year of raloxifene administration.

The percentage of change (mean ± SD) of lumbar BMD, and of serum OC and urinary DPD levels was significantly ($P = 0.0009$) different in women homozygous for bb (1.58 ± 0.80 , -5.15 ± 2.36 and -7.71 ± 2.89 for BMD, OC and DPD respectively) in comparison with those homozygous for BB (4.13 ± 2.26 , -13.59 ± 4.68 and -15.16 ± 4.65 for BMD, OC and DPD respectively) genotypes.

An intermediate percentage of change (mean ± SD) in BMD, serum OC and urinary DPD (2.49 ± 1.54 , -8.69 ± 2.60 and -10.52 ± 2.56 for BMD, OC and DPD respectively) was observed in patients heterozygous for Bb in comparison with those homozygous for BB and for bb genotypes.

Discussion

Alleles of the VDR gene are related to bone density and to a particular bone density pattern which varies with chronological age, sex, and anatomical site (Eisman, 1999; Gomez *et al.*, 1999; Gong *et al.*, 1999). In addition, some VDR gene polymorphisms are related to a higher prevalence in vertebral fractures (Gomez *et al.*, 1999; Uitterlinden *et al.*, 2001), probably as independent factors from BMD (Uitterlinden *et al.*, 2001).

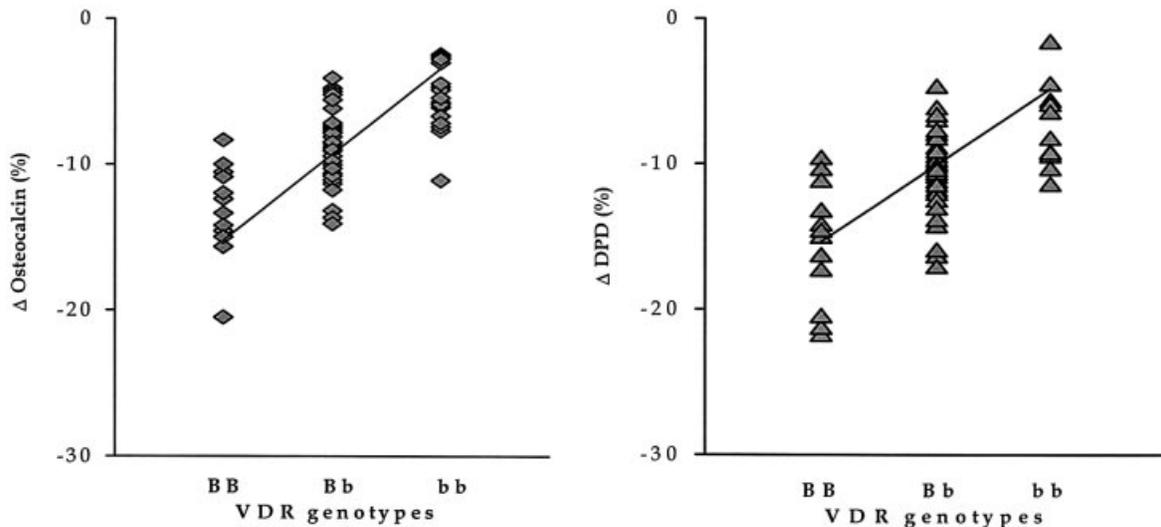


Figure 2. Percentage change (Δ) in serum osteocalcin (OC) and urinary deoxypyridoline (DPD) levels in comparison with baseline in each osteoporotic post-menopausal woman treated with raloxifene 60 mg daily for 1 year according to *BsmI* VDR genotypes. A significant ($P = 0.0001$) difference was detected between BB and bb genotypes.

Several therapeutic options are available for the prevention and treatment of post-menopausal osteoporosis (Altkorn and Vokes, 2001), but few data are available in the literature regarding the genetic factors that could influence the clinical response to anti-osteoporotic therapies (Keen and Kelly, 1997; Eisman, 2001).

Raloxifene is a non-steroidal drug that inhibits bone resorption and reduces the risk of vertebral fractures in post-menopausal women (Clemett and Spencer, 2000; Seeman, 2001). Our results confirm that 1 year of continuous oral raloxifene administration at doses of 60 mg daily induces a significant increase in lumbar spine BMD in post-menopausal women with osteoporosis (Ettinger *et al.*, 1999). As previously reported (Palomba *et al.*, 2001; Davies *et al.*, 1999), the tolerability of the drug was very good. Hot flushes and leg cramps were the only drug-related events. Moreover, the incidence of these AE was very low and no woman discontinued the treatment as a result of her appearance.

In the present study, the lumbar spine BMD increased significantly more in women homozygous for BB than in those homozygous for the bb genotype. Serum and urinary levels of bone turnover markers also decreased significantly more in women homozygous for BB than in those homozygous for bb. An intermediate percentage change of lumbar BMD, serum OC and urinary DPD was observed in women heterozygous for Bb compared with those homozygous genotypes.

At the present, our study is the first evaluating the different bone gains after raloxifene treatment according to VDR genotypes.

The mechanism of action of raloxifene on bone is currently not completely understood. In several studies (Sano *et al.*, 1995; Kobayashi *et al.*, 1996; Mizunuma *et al.*, 1997; Gennari *et al.*, 1998) polymorphisms of the ER gene have been associated with differences in BMD, suggesting that the mechanism of action of raloxifene may also be modulated by different ER gene polymorphisms.

Experimental studies indicate that estrogen status influences VDR abundance in human osteoblastic cells (Liel *et al.*, 1992; Ishibe *et al.*, 1995; Byrne *et al.*, 2000). In fact, it has been demonstrated that E_2 up-regulates VDR mRNA levels in human osteoblast-like cells (Ishibe *et al.*, 1995). Byrne *et al.* have suggested that the mechanism by which this agent up-regulates VDR cellular expression is mediated by the ER and may involve, at least partially, direct regulation of VDR transcription (Byrne *et al.*, 2000). In particular, a hormone-responsive promoter immediately upstream of exon 1c in the human VDR gene has been identified. The activity of this promoter was up-regulated by E_2 and its biological effect is blocked by SERM. Despite considerable effort to relate VDR and ER genotypes, the results are actually contrasting (Han *et al.*, 1997; Gennari *et al.*, 1998; Willing *et al.*, 1998; Deng *et al.*, 1999; Brown *et al.*, 2001; Kim *et al.*, 2001). Willing *et al.* have demonstrated an interaction between ER and VDR loci (Willing *et al.*, 1998). In fact, in women with *PvuII* ER genotype, the presence of a bb VDR genotype is related more significantly to BMD than is a BB VDR genotype (Willing *et al.*, 1998). The combining between VDR *FokI* and ER genotypes is significantly related to BMD, showing that ER polymorphisms, singly and in relation to VDR *FokI* polymorphism, influence bone mass in Korean women (Kim *et al.*, 2001). In addition, the association between VDR and ER allelic variants plays a key role in genetic determination of BMD also in Italian post-menopausal women (Gennari *et al.*, 1998). On the contrary, in other studies (Han *et al.*, 1997; Deng *et al.*, 1999; Brown *et al.*, 2001) no significant association was observed between the ER polymorphism alone or in combination with VDR genotypes, with BMD and/or its rate of change.

These discrepancies in the results are probably due to the inappropriate control of the factors influencing bone (Deng *et al.*, 1999). Unfortunately, at present the data regarding the genetic evaluation of ER polymorphisms were not available.

Our data, obtained without a placebo-controlled group, are confirmation that the bone metabolism is largely controlled by genetic mechanisms, and that the bone loss and gain are genetically determined also with respect to anti-osteoporotic treatments. In particular, the significant difference in lumbar spine BMD change observed after 1 year of raloxifene administration between homozygous bb and BB VDR subjects supports the initial hypothesis that different *BsmI* VDR genotypes modify the pharmacological response to raloxifene treatment in osteoporotic women.

With regard to the effect of VDR gene polymorphism on pharmacological response to anti-osteoporotic treatment, Marc *et al.* have already shown a higher response to etidronate treatment in BB VDR patients in comparison with bb women (Marc *et al.*, 1999). Indeed, VDR and ER genotypes singly or in association have a genotypic effect on the change in BMD observed in post-menopausal women with or without HRT varying from 1 to 18.7% (Deng *et al.*, 1998). In a recent paper (Kurabayashi *et al.*, 1999), the VDR and ER gene polymorphism has been related to the effectiveness of HRT on bone density in Japanese women. In this retrospective study (Kurabayashi *et al.*, 1999), it was shown that subjects with genotype bb (TT in Japanese women) had a percentage change in BMD that was significantly higher than those with Bb genotype after 2 years of HRT. Our results seem to contradict this and to show a different impact of genetic factor on raloxifene and HRT treatment on bone. Furthermore, in the Kurabayashi *et al.* (1999) study only one woman was homozygous for BB and she was not included in the results. Indeed, the population studied was very heterogeneous and unselected, overall with regard to dietary, BMD and progestin balance. On the contrary, in the present study in order to obtain results with minimal confounding factors we enrolled women with no significant differences in demographic characteristics. Furthermore, because of the dietary Ca intake and the fact that vitamin D metabolites are factors affecting the responsiveness of VDR genes (Dawson-Hughes *et al.*, 1995; Howard *et al.*, 1995; Graafmans *et al.*, 1997; Kiel *et al.*, 1997; Wishart *et al.*, 1997), a similar Ca dietary intake of ≥ 1000 mg daily was provided for each woman, and subjects with serum 25OH-vitamin D levels lower than 25 nmol/L were excluded from the study.

Finally, we feel that in the future it will be possible to select the optimal anti-osteoporotic regimen of treatment, as raloxifene administration, for post-menopausal women with osteoporosis on the basis of a complete genetic evaluation.

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