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Genetic Influences on Bone Density: Physiological Correlates of Vitamin D Receptor Gene Alleles in Premenopausal Women

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ABSTRACT

Common vitamin D receptor (VDR) gene alleles have recently been shown to contribute to the genetic variability in bone mass and bone turnover; however, the physiological mechanisms involved are unknown. To examine this, the response to 7 days of 2 μg oral 1,25-dihydroxyvitamin D[1,25-$(OH)_2$D] (calcitriol) stimulation was assessed in 21 premenopausal women, homozygous for one or other of the common VDR alleles (bb, n = 11; BB, n = 10). Indices of bone turnover and calcium homeostasis were measured during 2 weeks. Baseline osteocalcin, 1,25-$(OH)_2$D, type I collagen carboxyterminal telopeptide, and inorganic phosphate levels were significantly higher and spinal bone mineral density was significantly lower in the BB allelic group. After calcitriol administration, similar levels of 1,25-$(OH)_2$D were attained throughout the study in both genotypic groups. The increase in serum osteocalcin levels in the BB group was significantly less than that in the bb group (11% *vs.* 32%, $P = 0.01$). The genotype-related baseline difference in osteocalcin levels was not apparent at the similar serum 1,25-$(OH)_2$D levels. By contrast, the baseline differences in phosphate and type I collagen carboxyterminal telopeptide persisted throughout the study. Serum ionized calcium levels did not differ between genotypes, nor did it move out of normal range values. However, parathyroid hormone was less suppressed in the low bone density group (38% *vs.* 11%, $P = 0.01$). These data indicate that the VDR alleles are associated with differences in the vitamin D endocrine system and may have important implications in relation to the pathophysiology of osteoporosis. (*J Clin Endocrinol Metab* **80:** 2800–2805, 1995)

STUDIES IN adult twins indicate a strong genetic influence on bone density that relates to the genetic effect on bone turnover (1). Recent evidence indicates that common vitamin D receptor (VDR) gene alleles predict osteocalcin, a marker of osteoblastic activity, and collagen turnover marker levels and bone mineral density in a normal Caucasian population in both twin and population-based studies (1–4). Other (but not all) studies have observed similar relationships between bone density and VDR gene alleles in different populations (5–7).

1,25-Dihydroxyvitamin D_3 stimulates the synthesis of osteocalcin through interaction with a vitamin D response element in the osteocalcin gene (8). *In vivo* this translates into a significant increase in serum osteocalcin within 24 h of the administration of calcitriol (9). Previous studies of responses in osteocalcin to calcitriol stimulation *in vivo* have shown highly variable responses (10, 11). There are also conflicting data regarding the effects of both dietary calcium and calcitriol on the pathogenesis and treatment of osteoporosis (12–17). This variability may be explained at least in part by genetic factors. The VDR gene allele associated with the lower bone density is present in approximately 17% of the Caucasian population and may result in physiological differences in the vitamin D endocrine system and possibly in response to 1,25-dihydroxyvitamin D [1,25-$(OH)_2$D]. We therefore studied the response to calcitriol stimulation in two groups of normal premenopausal women selected on the basis of homozygosity for VDR gene alleles.

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Subjects and Methods

Subjects

Twenty-one Caucasian premenopausal women were studied. Although 4 were amenorrheic (3 had had hysterectomies and 1 was 8 months postpartum), serum estradiol and FSH levels were all within premenopausal ranges. Fifteen subjects were from a cohort of twins recruited from the Australian National Health and Medical Research Council Twin Registry for studies of the genetics of osteoporosis. Of these, 6 were from monozygotic pairs, and 9 were from dizygotic (DZ) twin pairs. Both individuals from 2 pairs of DZ twins were included. Six of the 21 subjects were recruited through the nursing staff at St. Vincent's Hospital, Sydney. None of these patients had a history of bone disease, illness, or drug use that might affect bone turnover. All subjects had normal renal function as assessed by serum creatinine. All of these premenopausal women were chosen randomly on the basis of their VDR genotype.

The subjects were selected on the basis of their VDR gene allele, as previously determined by polymerase chain reaction on isolated blood leukocytes to detect the region of DNA susceptible to the *Bsm*-I endonuclease. The presence of the restriction site was coded as "b", and the absence of the site was coded as "B". Ten of these healthy women were BB, and 11 were bb. If the VDR gene alleles have a significant affect on bone physiology, one would expect to see this difference in the homozygotes. Therefore, to minimize the sample size required, heterozygotes were not included in this study. Each woman received 2 μg oral calcitriol for 7 days. Blood and urine samples were collected in the

morning after an overnight fast, at baseline, and also second daily for 12 days. Capsules were counted to assess compliance at each visit.

Serum osteocalcin was measured by using a polyethylene glycol separation step by an in-house RIA, using rabbit antiovine osteocalcin antiserum and [^{125}I]ovine osteocalcin as the tracer (18). The normal reference interval is 3–18 μg/L with a sensitivity of 1 μg/L and an interassay coefficient of variation of 7%. 1,25-$(OH)_2$D was measured after extraction with acetonitrile and purification on C_{18}OH columns using the Nichols Institute kit (San Juan Capistrano, CA). The assay sensitivity is 10 pmol/L with a normal reference interval of 38–150 pmol/L (16–62 pg/mL) and a coefficient of variation of 11%. Parathyroid hormone (PTH) was measured by using an immunoradiometric assay of the intact parathyroid hormone (iPTH) molecule (Nichols Institute Diagnostics kit, San Juan Capristrano, CA). The reference interval for iPTH is 1–7 pmol/L with a sensitivity of 1 pmol/L and an interassay coefficient of variation of 7%. Urinary calcium (Ca), creatinine (Crt), phosphate, and total hydroxyproline (OHP) were measured on samples collected after an overnight fast, with calculation of the Ca/Crt and OHP/Crt ratios. The overnight fast is particularly important to avoid dietary influences on Ca excretion and particularly OHP excretion (19). Urinary Crt and Ca were both measured by colorimetry on a Hitachi 717 (Boehringer-Mannheim, Germany). OHP was quantified by using a manual spectrophotometric method (20), and urinary excretion was corrected for Crt excretion and expressed as urinary OHP/Crt. The tubular maximum of phosphate ($TmPO_4$) was calculated by using the formula for tubular reabsorption of phosphate and was converted to $TmPO_4$ (21, 22). Serum concentrations of LH and FSH were measured by using the Abbott IM analyzer, on the basis of a microparticle enzyme immunoassay, and estradiol levels were measured by the Clinical Assays RIA kit. The interassay coefficient of variation was 6% for the LH assay, 7% for the FSH assay, and 11% for the estradiol assay. The assays were performed within the Department of Chemical Pathology, St. Vincent's Hospital.

Serum for collagen turnover markers was stored at −70 C until all samples for a subject were collected and then analyzed in a single assay. Procollagen I propeptide (PICP) was measured by an immunoradiometric assay (Farmos Diagnostica, Turku, Finland) using an antibody directed at the trimeric carboxyterminal extension of procollagen. The assay has a sensitivity of 15 ng/mL with inter- and intraassay coefficients of 4.1% and 2.1%, respectively. The telopeptide of type I collagen (ICTP) was also measured with an immunoradiometric assay (Farmos Diagnostica), using an antibody directed at the carboxyterminal ICTP. The assay has a sensitivity of 0.5 μg/L with inter- and intraassay coefficients of variation of 7.9% and 6.2%, respectively.

Bone densitometry was performed by using a Lunar DEXA densitometer (DPX-L, Lunar, Madison, WI). The reproducibility of bone mineral density measurements by DEXA is 1.5% at the lumbar spine and 1.3% at the femoral neck (23). Dietary calcium intake was assessed by questionnaire, as previously described (24).

Statistical methods

Data for each parameter were first examined for deviation from the normal distribution by using the Kolmogorov-Smirnov test. When the deviation was statistically significant, a suitable transformation based on the Box-Cox method was made (25). Baseline comparability between genotypes with respect to demographic variables was assessed by the unpaired *t* test. Responses, simple change, and percentage change (mean of treatment visits 2, 3, and 4) from baseline of each parameter before, during, and after calcitriol stimulation were examined by a repeated analysis of variance model. In this model, the effects of genotype and day of study were considered fixed, whereas the effects of subjects were considered random. The model parameters were estimated by the least squares method, using the SAS statistical analysis system (26).

Results

At baseline there were no significant differences between the two genotypes for age, height, weight, and dietary calcium intake as shown in Table 1. Subjects with the BB genotype had significantly higher osteocalcin, inorganic phosphate, ICTP, and 1,25-$(OH)_2$D levels than those with the bb genotype, and bone density at the lumbar spine was 6% lower (Table 1).

TABLE 1. Baseline anthropometric and biochemical indices according to vitamin D receptor gene alleles

	BB	bb	*P* value
Age (yr)	32.7 ± 5.9	37.9 ± 10.1	0.30
Height (cm)	163.9 ± 8.6	162.3 ± 9.1	0.47
Weight (kg)	60.1 ± 8.2	62.0 ± 8.9	0.84
Dietary calcium (g/day)	740 ± 335	811 ± 264	0.14
Lumbar spine bone density (g/cm^2)	1.16 ± 0.09	1.23 ± 0.09	0.04[a]
Femoral neck bone density (g/cm^2)	0.94 ± 0.14	0.92 ± 0.09	0.6[a]
Osteocalcin (μg/L)	10.1 ± 3.6	7.9 ± 2.0	0.05
Ionized calcium (mmol/L)	1.27 ± 0.04	1.28 ± 0.05	0.64
PO_4 (mmol/L)	1.20 ± 0.18	1.01 ± 0.11	0.008
PTH (pmol/L)	2.4 ± 1.0	2.7 ± 0.7	0.31
1,25-$(OH)_2$D (pmol/L)	125 ± 19	86 ± 30	0.003
Alkaline phosphatase (μkat/L)	1.09 ± 0.34	0.93 ± 0.23	0.23
PICP (mmol/L)	117 ± 39	108 ± 33	0.57
ICTP (mg/L)	3.9 ± 1.1	3.1 ± 0.6	0.05
OHP/Crt (μmol/mmol)	14 ± 1	13 ± 1	0.12
Urinary Ca/Crt (mmol/mmol)	0.26 ± 0.07	0.29 ± 0.08	0.8

Values are mean ± SD. *P* = level of significance between two genotypes.

[a] *P* values derived on the basis of analysis of covariance (adjusted for body weight). All other *P* values derived on the basis of unpaired *t* test and logarithmic transformed data.

With calcitriol administration, there were significant increases in serum 1,25-$(OH)_2$D by day 2 in both groups. These levels dropped to below baseline by 24 h after oral calcitriol ceased. The bb genotype had a significantly greater increase compared with that of the BB genotype (135 ± 32 *vs.* 39 ± 15%, mean ± SE, $P = 0.03$). However, the values attained both during and after calcitriol stimulation were not significantly different between the two allelic groups (Fig. 1). Thus the administration of calcitriol removed at least temporarily the genotype-related differences in 1,25-$(OH)_2$D levels.

Despite the increase in 1,25-$(OH)_2$D levels, fasting serum-ionized calcium remained within reference limits throughout the study, without any significant changes within or differences between genotypes (Fig. 1). In both groups, serum inorganic phosphate levels increased above baseline without any significant difference in the magnitude of that response between genotypes (Fig. 1). Although the BB group had significantly higher serum phosphate throughout the study, there were no significant differences in $TmPO_4$ between genotypes at baseline or throughout the study. In both genotypes there was a significant increase in $TmPO_4$ from baseline ($P < 0.05$), which persisted throughout the observation period. The iPTH response was significantly different between the two genotypes [$P = 0.01$, analysis of variance (ANOVA)]. There was a 38 ± 7% decrease in iPTH levels from baseline in the higher bone density group (bb), which was apparent by day 2 and persisted throughout calcitriol administration ($P = 0.006$) (Fig. 1). By contrast, in the low bone density group (BB), there was no significant decrease in iPTH levels (11 ± 15%, $P = 0.4$).

After administration of calcitriol, serum osteocalcin levels

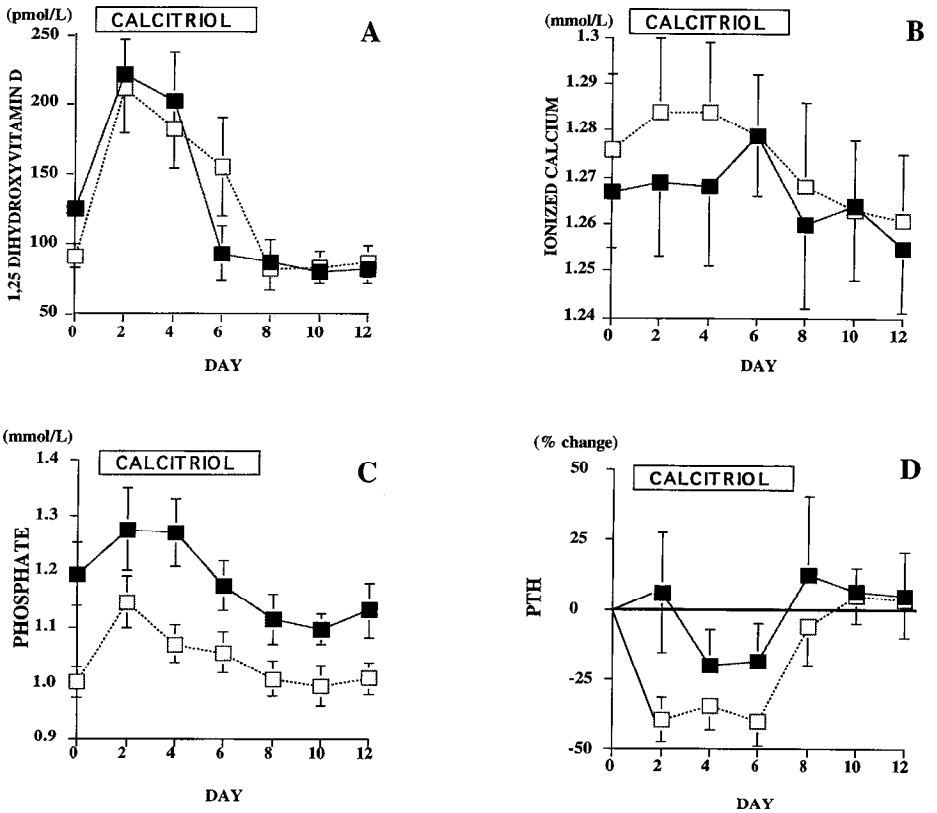

FIG. 1. Response of serum biochemical parameters to calcitriol stimulation (mean ± SE): A, 1,25-$(OH)_2D$ (pmol/L); B, ionized calcium (mmol/L); C, inorganic phosphate (mmol/L); and D, intact PTH (% change from baseline). □ represents the high bone mass genotype (bb); ■ represents the low bone mass genotype.

increased in both VDR allelic groups, with a significant increase from baseline in BB ($P = 0.03$) and in bb ($P = 0.0001$) by day 2 (Fig. 2). When serum 1,25-$(OH)_2D$ levels were similar between the VDR gene allelic groups, *i.e.* from day 2 onward, there was no significant difference in serum osteocalcin levels. The overall osteocalcin response, expressed as percentage change from baseline or mean change, was predicted by VDR allele genotype ($P = 0.006$, ANOVA). During the period of administration of calcitriol, there was a significantly greater increase in osteocalcin levels in the bb genotype, after adjusting for baseline differences (32% *vs.* 11%, $P = 0.01$) (Fig. 2). Osteocalcin levels then gradually declined for the remainder of the observation period, yet baseline genetic differences had not been restored by the end of the study.

The difference in serum ICTP present at baseline, with the higher level seen in the low bone density genotype, BB, persisted throughout the study (Fig. 2). A significant increase in serum PICP in response to calcitriol was observed in both groups, although there was no significant difference between the groups at any time during the study. However, in contrast to osteocalcin response, a greater mean increase in PICP during the treatment period was observed in the BB genotype rather than the bb genotype (13.5 ± 2.1% *vs.* 4.6 ± 2.0%, $P = 0.02$). There was a significantly greater response in urinary Ca excretion to calcitriol stimulation in the BB genotype. It increased rapidly in the BB group by day 2 ($P = 0.02$) and remained significantly greater than baseline throughout the study, whereas the bb group did not increase significantly above baseline at any stage throughout the study. The mean response during the treatment period was significantly greater in the BB genotype (ratio increased by 0.20 ± 0.03 *vs.* 0.05 ± 0.03, $P = 0.01$, ANOVA). The BB genotype had a rapid increase in OHP/Crt that peaked at 48 h ($P = 0.05$), remained significantly greater than baseline at day 4, and then returned to baseline by day 6. The bb genotype, on the other hand, did not have a statistically significant increase from baseline. This difference in response was significantly related to the VDR genotype ($P = 0.001$, ANOVA). The overall response of OHP during the treatment period was significantly greater in the BB group (22% *vs.* 8%, $P = 0.03$, ANOVA). This difference was also seen in the mean change during the treatment period (6.9 *vs.* 2.5 μmol/mmol, $P = 0.03$).

Discussion

The present study extends observations relating VDR alleles to variation in bone mineral density and indicates that in normal premenopausal women there are differences in bone and calcium homeostasis in relation to VDR gene alleles, including variability in response to calcitriol stimulation. In this group of subjects, significant VDR gene-bone mineral density associations were observed at the lumbar spine; however, this was not observed at the proximal femur, consistent with previous studies showing a weaker overall genetic and specific VDR gene affect at this site (4). In larger population-based studies, the VDR genotype with low bone density (BB) is associated with higher basal osteocalcin and 1,25-$(OH)_2D$ (2, 4), as seen in this smaller study. However, this is the first report of higher fasting serum inorganic phosphate and ICTP levels in those with the low bone density

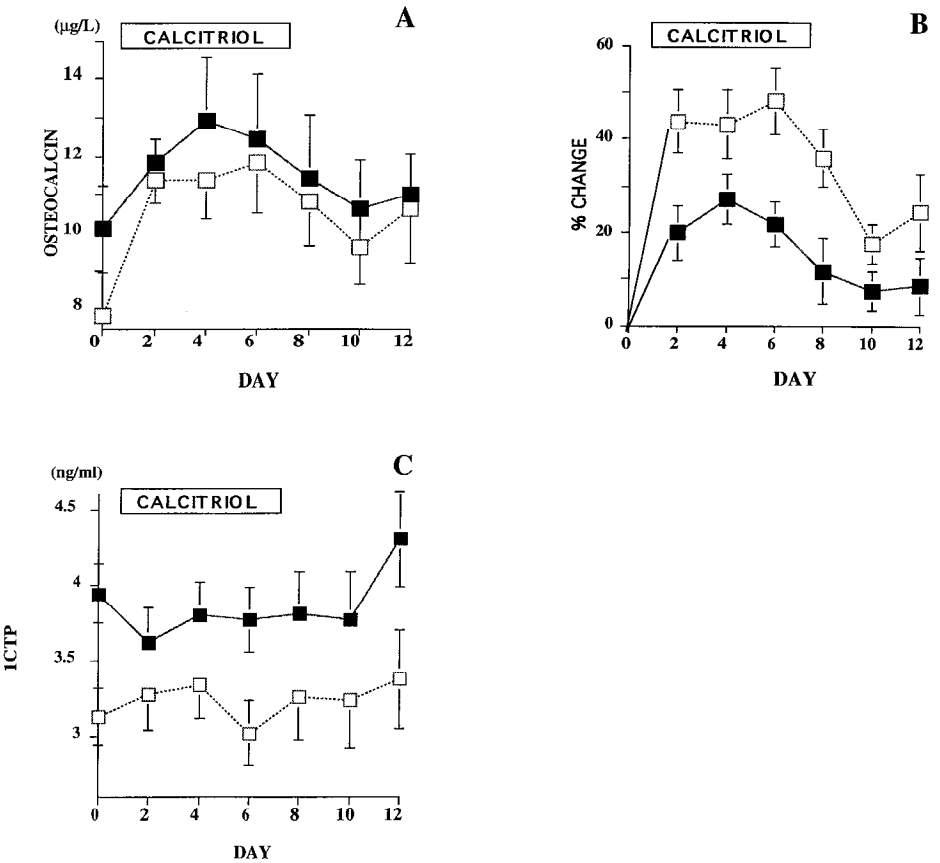

FIG. 2. Changes in serum markers of calcium homeostasis (mean ± SE): A, osteocalcin (μg/L); B, osteocalcin (% change over baseline); C, ICTP (mg/L); D, OHP (% change over baseline); E, Ca/Crt (% change over baseline). □ represents the high bone mass genotype (bb); ■ represents the low bone mass genotype.

genotype, despite comparable baseline serum calcium and intact PTH levels.

In these premenopausal women there was a modest increase in serum 1,25-$(OH)_2D$ levels with calcitriol administration, followed by a sustained decrease after cessation of exogenous 1,25-$(OH)_2D$, which presumably reflects homeostatic feedback to renal 1,25-$(OH)_2D$ synthesis by the raised serum 1,25-$(OH)_2D$ and phosphate and the decreased iPTH (27). Calcium levels remained within the reference range, and there was an increase in serum phosphate levels with an elevation in maximal tubular phosphate reabsorption, as PTH tended to be suppressed with increasing 1,25-$(OH)_2D$ levels. Osteocalcin, PICP, and fasting urinary calcium excretion levels also increased with treatment, with a trend toward increases in urinary OHP excretion without any changes in ICTP. These findings are consistent overall with increased bone turnover, as in similar previous studies involving calcitriol stimulation (11, 12).

When analyzed with regard to VDR genotype, variability in response was evident. There was a difference in percentage increase in serum 1,25-$(OH)_2D$ (BB = 39 ± 15%, bb = 135 ± 32%, $P = 0.01$), such that by 48 h both genotypes attained similar levels despite different baselines. After calcitriol stimulation, fasting serum phosphate levels increased equally in the VDR genotypes and thus maintained the difference seen at baseline with a higher level in the BB genotype. Serum iPTH was inhibited by 38 ± 7%, $P = 0.006$ in the bb genotype, yet there was a nonsignificant decline from baseline in the BB genotype (11 ± 15%, $P = 0.04$), despite similar levels of 1,25-$(OH)_2D$ and fasting Ca. This may reflect a higher set point of the parathyroids to 1,25-$(OH)_2D$ feedback inhibition of PTH synthesis in the BB genotype (28).

Although serum osteocalcin levels were initially higher in the BB genotype, exogenous 1,25-$(OH)_2D$ treatment resulted in a significantly greater increase in the bb genotype compared with that in the BB genotype, resulting in similar serum levels by 48 h. After equalizing 1,25-$(OH)_2D$ levels, both genotypes had similar short-term overall osteoblast responses to calcitriol treatment. Therefore, the osteoblasts in the BB genotype may have normal sensitivity to calcitriol, resulting in higher osteocalcin levels with higher 1,25-$(OH)_2D$ levels. The relatively short duration of this study did not allow any observations regarding long-term changes reflecting osteoblast or osteoclast recruitment (10, 11).

On the other hand, the baseline differences in ICTP persisted throughout the study, with little overall influence of this short-term calcitriol stimulation. As for serum phosphate, this may reflect long-term stimulation of mature osteoclasts by higher 1,25-$(OH)_2D$ levels in the low bone mineral density genotype. However, significant differences in response of urinary OHP and fasting Ca/Crt were also observed with the greater response in the genotype associated with the low bone density, BB. The implications drawn from these data regarding bone resorption are limited somewhat by the lack of unequivocal markers of bone resorption, particularly inasmuch as the effects of 1,25-dihydroxyvitamin D_3 on bone resorption are complex (11), involving both recruitment of osteoclasts (29) and stimulation of existing osteoclasts via osteoblasts (30). However, on the basis of ICTP, serum phosphate, Ca/Crt excretion, and urinary OHP data,

the study suggests a higher level of bone resorption and type I collagen breakdown in the low bone density genotype. The use of other markers of bone turnover, such as pyridinoline cross-links and bone-specific alkaline phosphatase, may have been useful to explore the effect on bone turnover.

The physiology of these differences in the vitamin D endocrine system is of considerable interest. Although the mechanism for the higher baseline 1,25-$(OH)_2$D level is unclear, it could result in accelerated bone remodeling. Increased osteoblastic and osteoclastic activity or numbers are suggested by the higher basal osteocalcin, ICTP, and phosphate and in the long-term could result in a lower bone mineral density. Long-term high bone turnover states such as postmenopause and hyperparathyroidism do result in an overall loss of skeletal mineral (31, 32). If the higher 1,25-$(OH)_2$D level is indeed forcing this higher rate of bone turnover, it remains to be determined what causes this difference in basal 1,25-$(OH)_2$D in healthy women.

The different steady-state baseline 1,25-$(OH)_2$D levels, together with equivalent levels of PTH and serum Ca, suggest that different 1,25-$(OH)_2$D levels are required for the maintenance of normocalcemia, *i.e.* to produce similar calcitropic responses in the different homozygotes of the VDR gene. The simplest mechanism would relate to differences in the concentration, as suggested by minigene analysis (3), or quality of the vitamin D receptors in target tissues, resulting in differing sensitivities to 1,25-$(OH)_2$D in different target tissues. VDR levels vary in different target tissues and are modified by the level of cell differentiation, hormones such as estrogen, FSH, and corticosteroids, as well as by different physiological states (33, 34). It is therefore possible that the already complex nature of VDR receptor expression in different tissues could play a role in relation to VDR gene alleles.

If there are qualitative or quantitative differences in the functional capacity of the VDR gene in the gastrointestinal tract, then the BB genotype may require higher levels of 1,25-$(OH)_2$D to attain similar Ca absorption. Similarly, the parathyroid gland in the low bone density group would seem to be less sensitive to the negative feedback of 1,25-$(OH)_2$D, in view of similar PTH levels, despite higher 1,25-$(OH)_2$D levels in the BB genotype at baseline, and less suppression of PTH despite equivalent 1,25-$(OH)_2$D levels after calcitriol administration. Yet in bone, when 1,25-$(OH)_2$D levels were similar, markers of bone formation such as osteocalcin were also similar, indicating that differences in bone turnover indices seen at baseline may relate to different 1,25-$(OH)_2$D levels. However, markers of bone resorption and type I collagen breakdown, urinary Ca/Crt, OHP, and ICTP, were greater in the low bone density genotype with equivalent 1,25-$(OH)_2$D levels, suggesting differing sensitivities between VDR genotypes of bone resorption to 1,25-$(OH)_2$D.

The precise molecular mechanism driving the physiological effect of VDR gene alleles on the vitamin D endocrine system, and the different responses seen in this study, remain to be elucidated. However, it may be that the net result of an altered VDR functional capacity in the BB genotype in those tissues involved with calcium regulation drive 1,25-$(OH)_2$D to higher levels. This in turn may also force an elevated bone turnover state and in the long-term may result in a lowered bone mineral density. Physiological studies of renal and gastrointestinal calcium handling may help to determine why baseline 1,25-$(OH)_2$D levels differ. For example, a recent study suggests different responses in bone mineral density over time to Ca intake in relation to VDR alleles (35), which requires additional investigation into the significance of differing responses between genotypes to treatment regimes, including those that may modify 1,25-$(OH)_2$D levels. Regardless of the primary mechanism, the VDR alleles account for a major part of the genetic effect on bone mass in twins, and in these normal premenopausal women significant differences in the vitamin D endocrine system relating to these frequent VDR gene alleles are present. Understanding these pathways and the underlying molecular physiological mechanisms has potentially important implications for targeting and tailoring osteoporosis prevention and treatment based on pathophysiological mechanisms.

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References

1. **Kelly PJ, Hopper JL, Macaskill GT, Pocock NA, Sambrook PN, Eisman JA.** 1991 Genetic factors in bone turnover. J Clin Endocrinol Metab. 72:808–813.
2. **Morrison NA, Yeoman R, Kelly PJ, Eisman JA.** 1991 Contribution of trans-acting factor alleles to normal physiological variability: vitamin D receptor gene polymorphisms and circulating osteocalcin. Proc Natl Acad Sci USA. 89:6665–6669.
3. **Tokita A, Kelly PJ, Nguyen TU, et al.** 1994 Genetic influence on type L collagen synthesis and degradation: further evidence for genetic regulation of bone turnover. J Clin Endocrinol Metab. 78:1461–1466.
4. **Morrison NA, Qi JC, Tokita A, et al.** 1994 Prediction of bone density from vitamin D receptor alleles. Nature. 367:284–287.
5. **Yamagata Z, Miyamura T, Iijima S, et al.** 1994 Vitamin D receptor gene polymorphism and bone mineral density in healthy Japanese women. Lancet. 344:1027.
6. **Spector TD, Keen RW, Arden NK, et al.** 1994 Vitamin D gene alleles and bone density in postmenopausal women: a UK twin study. J Bone Miner Res. 9(Suppl. 1):s143.
7. **Hustmyer F, Peacock M, Hui S, Johnston C, Christian J.** 1994 Bone mineral density in relation to polymorphism at the vitamin D receptor locus. J Clin Invest. 94:2130–2134.
8. **Kerner S, Scott R, Pike J.** 1989 Sequence elements in the human osteocalcin gene confer basal activation and inducible response to hormonal vitamin D_3. Proc Nat Acad Sci USA. 86:4455–4459.
9. **Zerwekh J, Sakhae K, Pak C.** 1985 Short term 1,25-dihydroxyvitamin D_3 administration raises serum osteocalcin in patients with postmenopausal osteoporosis. J Clin Endocrinol Metab. 60:615–617.
10. **Duda RJ, Kumar R, Nelson KI, Zinsmeister AR.** 1987 1,25-Dihydroxyvitamin D stimulation test for osteoblast function in normal and osteoporotic women. J Clin Invest. 79:1249–1253.
11. **Bollerslev J, Gram J, Neilsen K, et al.** 1991 Effect of a short course of 1,25-dihydroxyvitamin D_3 on biochemical markers of bone remodelling in adult male volunteers. Bone. 12:339–343.
12. **Ott SM, Chestnut III CH.** 1989 Calcitriol treatment is not effective in postmenopausal osteoporosis. Ann Intern Med. 110:267–274.
13. **Ott SM, Chestnut III CH.** 1990 Tolerance to dose of calcitriol in association with improved bone density in women with postmenopausal osteoporosis. J Bone Miner Res. 5:449 (Abstract).
14. **Gallagher JC, Riggs BL.** 1990 Action of 1,25-dihydroxyvitamin D_3 on calcium balance and bone turnover and its effect on vertebral fracture rate. Metabolism. 39:30–34.
15. **Aloia JF, Vaswami A, Yeh JK, Ellis K, Yasumura S, Cohn SH.** 1988

Calcitriol in the treatment of postmenopausal osteoporosis. Am J Med. 84:401–408.
16. **Need AG, Nordin BEC, Horowitz M, Morris HA.** 1990 Calcium and calcitriol therapy in osteoporotic postmenopausal women with impaired calcium absorption. Metabolism. 39(Suppl. 1):53–54.
17. **Tilyard M, Spears G, Thompson J, Dovey S.** 1992 Treatment of postmenopausal osteoporosis with calcitriol or calcium. N Engl J Med. 326:357–362.
18. **Kelly PJ, Pocock NA, Sambrook PN, Eisman JA.** 1989 Age and menopause-related changes in indices of bone turnover. J Clin Endocrinol Metab. 69:1160–1165.
19. **Nordin BEC.** 1978 Diagnostic procedures in disorders of calcium metabolism. Clin Endocrinol. 8:55–67.
20. **Hodgkinson A, Thompson T.** 1982 Measure of the fasting urinary hydroxyproline:creatine ratio in normal adults and its variation with age and sex. J Clin Pathol. 35:807–811.
21. **Favus MJ, Gagel RF, Christakos S, et al.** 1993 Primer on the metabolic bone diseases and disorders of mineral metabolism. 2nd ed. New York: Raven Press; 418–421.
22. **Walton R, Bijvoet O.** 1975 Nomogram for renal threshold phosphate concentration. Lancet. 2:309.
23. **Nguyen T, Sambrook P, Kelly P, et al.** 1993 Prediction of osteoporotic fractures by postural instability and bone density. Br Med J. 307:1111–1115.
24. **Angus R, Sambrook PN, Pocock N, Eisman JA.** 1989 A simple method for assessing calcium intake in Caucasian women. J Am Diet Assoc. 89:209–214.
25. **Box GEP, Cox DR.** 1964 An analysis of transformations. J R Stat Soc. Ser B 26:211–243.
26. **SAS Institute Inc.** 1991 SAS/STAT User's guide. Cary, NC: SAS Institute Inc.
27. **Walters MR.** 1992 Newly identified actions of the vitamin D endocrine system. Endocr Rev. 13:719–764.
28. **Silver J, Naveh-Many T.** 1994 Regulation of parathyroid hormone synthesis and degradation. Semin Nephrol. 14:175–94.
29. **Bar-Shavit Z, Teitel Baum S, Reitsma P, et al.** 1983 Induction of monocytic differentiation and bone resorption by 1,25-dihydroxyvitamin D. Proc Natl Acad Sci USA. 80:5907–5911.
30. **Reichel H, Koefler H, Norman A.** 1989 The role of the vitamin D endocrine system in health and disease. N Engl J Med. 320:980–991.
31. **Kelly PJ, Pocock NA, Sambrook PN, Eisman JA.** 1989 Age and menopause-related changes in indices of bone turnover. J Clin Endocrinol Metab. 69:1160–1165.
32. **Nikkila MT, Saoristo JJ, Koivula TA.** 1989 Clinical and biochemical features in primary hyperparathyroidism. 105:148–153.
33. **Chen TL, Feldman D.** 1981 Regulation of 1,25-dihydroxyvitamin D_3 receptors in cultured mouse bone cells: correlation of receptor concentration with the rate of cell division. J Biol Chem. 256:5561–5566.
34. **Huang Y, Lee S, Stolz R, et al.** 1989 Effects of hormones and development on the expression of the rat 1,25-dihydroxyvitamin D_3 receptor gene. J Biol Chem. 264:17454–17461.
35. **Ferrari S, Rizzoli R, Chevalley T, et al.** 1995 Vitamin-D-receptor-gene polymorphisms and change in lumbar-spine bone mineral density. Lancet. 345:423–424.