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Vitamin D Receptor Gene Polymorphisms Are Associated with the Risk of Fractures in Postmenopausal Women, Independently of Bone Mineral Density

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Context: Osteoporosis is a systemic disease with a strong genetic component. Vitamin D receptor (VDR) gene polymorphisms explain only a small part of the genetic influence on the level of bone mineral density (BMD), whereas their effect on fracture remains uncertain.

Objective: The objective of this study was to investigate the relationships between VDR genotypes and fracture risk

Design: A prospective population-based cohort was studied.

Subjects: A total of 589 postmenopausal women (mean age, 62 yr) were followed prospectively during a median (interquartile) of 11 (1.1) yr.

Main Outcome Measure: The study measured incidents of vertebral and nonvertebral fractures.

Results: VDR allele B was significantly and dose dependently overrepresented in women who fractured, including 34 and 86 women with first incident vertebral and nonvertebral fragility fractures, respectively. This corresponded to an odds ratio of 1.5 (95% confidence interval, 0.95–2.40) for heterozygous carriers (bB, n = 286) and 2.10 (95% confidence interval, 1.16–3.79) for homozygous carriers (BB, n = 90) of the B allele, compared with women with the bb genotype (n = 213). VDR genotype groups did not differ for demographics, physical activity, grip strength, personal and maternal history of fracture, and calcium intake. The association was independent of BMD of the spine, hip, and radius, and of the BMD loss at the radius. The relationship between VDR polymorphisms and fracture risk was not altered after adjustment for baseline circulating levels of bone turnover markers, estradiol, dehydroepiandrosterone sulfate, SHBG, IGF-I, intact PTH, and 25 hydroxyvitamin D.

Conclusion: VDR genotypes are associated with the risk of fracture in postmenopausal women independently of BMD, rate of postmenopausal forearm BMD loss, bone turnover, and endogenous hormones. The mechanisms by which VDR genotypes influence bone strength remain to be determined. **(*J Clin Endocrinol Metab* 90: 4829–4835, 2005)**

OSTEOPOROSIS IS A systemic disease characterized by decreased bone mass and microarchitectural deterioration of bone tissue, leading to increased bone fragility and fracture. Although this disease is clearly multifactorial involving several environmental influences, genetic factors play a major role in its pathogenesis. Twin and pedigree studies have shown that genetic influences account for 50–80% of the interindividual variability of bone mineral density (BMD) in young adults (1–3). Conversely, the magnitude of the influence of genetic factors on the rate of postmenopausal bone loss in postmenopausal women remains controversial (4, 5), with one study finding a significant genetic effect for spine, but not hip, BMD loss (5), and another reporting no significant influence (4).

Because a low BMD in postmenopausal women, which results from a low peak BMD and or a faster rate of bone loss, is a strong determinant of fracture risk, most genetic studies in osteoporosis have been dedicated to identifying gene polymorphisms that could explain the interindividual variance in these two components. During the past 10 yr, since the initial report from Morrison *et al.* (6), several genetic polymorphisms in candidate genes for osteoporosis, such as vitamin D receptor (VDR), estrogen receptor, PTH receptor, calcitonin receptor, IL-1 receptor antagonist, IL-6, TGF-β, IGF-I, type I collagen, osteocalcin, apolipoprotein E, methylenetetrahydrofolate reductase, peroxisome proliferators-activated receptor, osteoprotegerin, and GnRH have been investigated (for reviews see Refs. 7 and 8). More recently, associations were reported between BMD and gene polymorphisms for lipoprotein receptor-related protein 5 (9–11), cathepsin K (12), and pituitary glutaminyl cyclase (13). However, their relation with BMD and/or postmenopausal bone loss was inconsistent between studies, and when present, the association was of low magnitude.

Among these candidate genes, the first and by far most extensively investigated is that coding for the VDR and, more specifically, the *Bsm*I restriction fragment length polymorphism (RFLP). The VDR plays a role in regulating calcium homoeostasis through binding and nuclear translocation of $1\alpha 25(OH)_2D_3$, affecting bone resorption, and increasing calcium absorption (14). The most recent metaanalysis of the influence of VDR on BMD and BMD change in women indicated a significant but very modest effect on spine BMD in postmenopausal women; individuals carrying the BB genotype have a 2.4% lower BMD than the Bb/bb genotype

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Abbreviations: BMD, Bone mineral density; CTX, C-terminal cross-linking telopeptide of type I collagen; DHEA, dehydroepiandrosterone; DXA, dual-energy x-ray absorptiometry; OFELY, Os des Femmes de Lyon; 25 (OH) D, 25 hydroxyvitamin D; OR, odds ratio; RFLP, restriction fragment length polymorphism; VDR, vitamin D receptor.

groups (15). No significant association could be detected with spine BMD in premenopausal women, with pre- and postmenopausal femoral neck BMD and with BMD change at the femoral neck (15). However, analyzing the relationship between BMD and VDR polymorphism may not be sufficient to assess the role of VDR in osteoporosis. Indeed, although a low BMD is a major determinant of increased fracture risk, about half of patients with incident fractures have baseline BMD above the WHO diagnostic threshold of osteoporosis (T score ≤ -2.5) (16–19). Bone fragility also depends on the morphology, the architecture, and remodeling of bone as well as on the quality (properties) of the bone matrix. Some of these BMD-independent factors may be assessed to a certain extent by biochemical markers of bone turnover, bone ultrasound properties and hip geometry for which a genetic contribution has been reported (20–23). Interestingly, a recent twin study performed in 6570 women showed that the genes involved in the genetic influence of wrist fracture are probably, for a vast majority, different from those implicated in the determination of forearm BMD (24). Thus, analyzing gene polymorphisms directly in relation to fracture risk may be more clinically relevant than studying the genetic influence on BMD.

A few studies have analyzed the associations between VDR *Bsm*I genotypes and fracture. Most studies were cross-sectional, comparing the genotype distribution between women with prevalent vertebral or hip fracture and controls. They usually included a limited number of fracture cases and, like the BMD studies, have generated conflicting results (25–29). The association between VDR genotypes and the risk of incident fractures was investigated in three prospective case control or population studies and also yielded conflicting results (30–32). The potential mechanisms by which VDR genotypes may influence fracture risk, including bone turnover, rate of BMD changes, and circulating hormones were not investigated in these studies.

The aim of our study was to investigate the relationships between VDR *Bsm*I polymorphisms and the risk of incident vertebral and nonvertebral fractures in postmenopausal women followed prospectively for up to 12 yr. We also analyzed the potential influence of BMD, rate of BMD change, bone turnover, circulating hormones, and clinical risk factors for fracture on this relationship.

Subjects and Methods

We investigated the postmenopausal women of the Os des Femmes de Lyon (OFELY) cohort, a prospective study of the determinants of bone loss that has been previously described in detail (33). The cohort of this study comprises 1039 Caucasian women, 31–89 yr of age, including 671 postmenopausal women, recruited between February 1992 and December 1993 from the regional section of a large health insurance company (Mutuelle Générale de l'Education Nationale, Lyon, France) of the Rhône district (*i.e.* Lyon and its surroundings in France). Written informed consent was obtained from each woman, and the study was approved by the local ethical committee. We followed the women from this cohort during a median of 11 yr. In this analysis we investigated the 671 subjects who were menopausal at the baseline visit. DNA was not available for 82 women, resulting in a final group of 589 women.

Fracture assessment

Nonvertebral fractures. Prior fractures were those that occurred after the age of 40 yr, identified by self-reporting during the baseline questionnaire. Incident fractures were reported during each annual follow-up. For women who did not come to the clinical center, a letter was sent every year to identify the occurrence of fractures. All peripheral fractures were confirmed by radiographs or by a surgical report. Only low-trauma fractures, *i.e.* those occurring with falls from standing height or less, were taken into account, and we excluded fractures of fingers, toes, skull, and face.

Vertebral fractures. Lateral x-ray films of the thoracic and lumbar spine were obtained at baseline for 97% of women, and on average after 7.5 yr $\pm$ 1.6 (from 2.8–10.3) of follow-up for 86% of them. All prevalent and incident vertebral fractures were identified by the semiquantitative method of Genant *et al.* (34) by a trained rheumatologist. A vertebra was classified as fractured on the baseline radiograph if any vertical height (anterior, middle, and/or posterior) was reduced by more than 20%. An incident fracture was defined by a decrease of 20% or more and at least 4 mm in any vertebral height of one or more thoracic or lumbar vertebrae between follow-up and baseline x-ray films. We excluded vertebral fractures that occurred because of major trauma and vertebral deformities due to other causes than osteoporosis such as osteoarthritis and Scheuermann's disease.

Clinical evaluation and physical examination

Women completed a questionnaire at the initial screening visit and at each annual follow-up as described previously (35). It included medical history, medication use such as hormone replacement therapy, calcium and vitamin D supplementation, calcium intake, reproductive characteristics, age of menopause, and family history of fragility fracture. The occurrence of falls during the past 12 months was recorded. Physical activity was expressed by a score calculated from sport or recreation and job and home activities as described previously (35). The grip strength was measured at baseline by a hand dynamometer (Vigorimeter; Martin, Tuttlingen, Germany) on the left and right hand, using the maximum of two readings for each hand.

Bone densitometry

BMD was measured at the lumbar spine, total hip, whole body (BMD and bone mineral content), and mid, distal, and ultradistal radius. The mid area is composed mainly (~95%) of cortical bone, the distal area comprises both cortical and trabecular bone (~25%), and the ultradistal contains more trabecular bone. Subsequent BMD measurements at the radius were performed at yr 2 and then annually. Forearm BMD was measured by pencil beam dual-energy x-ray absorptiometry (DXA) with a QDR 2000 device between 1992 and 1999 and with a QDR 1000+ between 1999 and 2003 (Hologic, Waltham, MA). A cross-calibration between the two scanners has been done. A control phantom was scanned every day, and all DXA measurements were performed by the same experienced operator. The *in vitro* long-term precision error of DXA over the 11-yr study was 0.41%.

A valid assessment of the rate of BMD change at the other skeletal sites (hip, spine, whole body), measured with a multidetector mode (fan beam) was not obtained in this cohort because of technical problems that specifically affected this mode. A drift in phantom values was observed over time that could not be reliably corrected.

Biochemistry

Blood samples were collected between 0800 and 0930 h after an overnight fast at baseline. Serum samples were stored frozen at −70 C until assayed.

Bone formation was evaluated with human RIA for serum-intact osteocalcin, bone-specific alkaline phosphatase, and intact procollagen type I N-terminal propeptide as previously described (36). The intra- and interassay coefficients of variation for all three assays are less than 10%. Bone resorption was evaluated by measuring serum β isomerized C-terminal cross-linking telopeptide of type I collagen (βCTX) and urinary N-terminal cross-linking telopeptide of type I collagen by ELISA (36). Intra- and interassay coefficients of variation are lower than 10% for all assays. Urinary α and β isomerized CTX were also measured to derive the urinary α to β CTX ratio (37).

Serum total estradiol, estrone, testosterone, and dehydroepiandros-

terone (DHEA) sulfate were measured by tritiated RIA after diethylether extraction or dilution (for DHEA sulfate) as previously described (36). The total assay precision is below 10% for all assays, and the limit of detection of estradiol is 3 pg/ml. SHBG was measured by RIA, serum-intact PTH by immunochemiluminometric assay, and 25 hydroxyvitamin D [25 (OH) D] level was determined by a radio-binding assay as previously described (36). Serum IGF-1 was measured by RIA after acid ethanol extraction.

VDR genotyping

Genomic DNA was extracted from whole blood according to standard methods and used for PCR of the sequences flanking the *Bsm*I, *Taq*I, *and Apa*I restriction sites with a Corbett FTS-1 Thermal sequencer (Corbett Research, Mortlake, New South Wales, Australia). The PCR products were then digested by *Bsm*I (New England Biolabs, Inc., Beverly, MA) or *Apa*I or *Taq*I (Promega Inc., Madison, WI), and genotypes were analyzed by agarose gel electrophoresis as previously described (33).

Statistical analyses

Differences between genotype groups in baseline clinical, biochemical, and BMD parameters were assessed by ANOVA (continuous variables) or Pearson χ^2 (categorical variables) after logarithmic transformation of bone marker and hormone levels. Radius BMD changes were calculated from the mean of the individual slopes divided by the intercept and were expressed in percentage per year. Odds ratios (OR) with 95% confidence interval were obtained by logistic regression analyses to estimate the relative risk of fractures by genotypes of the risk allele, with no copies of the risk allele as the reference group. First, we calculated crude ORs and then, secondly we adjusted for potential confounding factors (age, BMD, prior fractures, bone markers, hormones). The population attributable risk (PAR), which corresponds to the proportion of women with incident fracture in the population that is attributable to the risk factor assuming a causal relationship, was calculated as population attributable risk = $p * (OR - 1)/[1 + p * (OR - 1)]$ where p is prevalence of the risk factor. All statistical analyses were performed on SAS software (version 8; SAS Institute, Cary, NC).

Results

Baseline characteristics

VDR genotype distribution was in Hardy-Weinberg equilibrium, with no overrepresentation of the BB genotypes that represented 15% of the population. The *Bsm*I, *Taq*I, and *Apa*I genotypes were highly associated as previously reported (33). Indeed, the genotype TT was found with bb in 98% of cases, and genotype tt was associated with genotype BB in 97% of cases. Similarly, genotype aa was found with bb in 60% of women and genotype AA was found with BB in 99% of cases. As shown in Table 1, women in the three *Bsm*I genotype groups were similar in age, time since menopause, height, weight, hormone replacement use, and calcium intake. There were also no significant differences in these parameters between *Taq*I and *Apa*I genotype groups (data not shown).

During a median (interquartile) 11 (1.1) yr follow-up, first incident fractures were recorded in 120 women, including 34 women with vertebral fractures and 86 with nonvertebral fractures.

Association between VDR genotypes and incident fracture

When women were grouped according to VDR *Bsm*I genotypes, all fragility fractures were significantly overrepresented in women carrying the B allele (Table 2). Allele B was dose dependently associated with increased risk of all fragility fractures; women with genotype bB had a relative risk of fracture (95% confidence interval) of 1.50 (0.95–2.40) compared with women in the bb genotype, and those carrying the BB genotype had a relative risk of fracture of 2.10 (1.16–3.79). When nonvertebral and vertebral fractures were analyzed separately, VDR genotypes were significantly associated with an increased risk of nonvertebral fractures, but not with incident vertebral fractures (Table 2). When all fractures, *i.e.* not only those resulting from low trauma, were analyzed (n = 136), VDR genotypes were also associated with fracture risk, women with bB and BB genotype having a relative risk of fracture (95% confidence interval) of 1.44 (0.83–3.15) and 2.48 (1.34–4.57), respectively compared with women with genotype bb.

As expected from the high association between *Taq*I and *Bsm*I polymorphisms, *Taq*I genotypes were also associated with fracture risk with 15.9, 21.5, and 27.6% of all incident fragility fractures occurring in genotype TT, Tt, and tt, respectively (P $\chi^2 = 0.06$). In contrast, there was no significant association between *Apa*I polymorphisms and incidence of fracture (P $\chi^2 = 0.45$).

We then performed haplotype analysis by combining genotypes for *Bsm*I, *Apa*I, and *Taq*I polymorphisms. Among the different combinations, genotypes bbAATT (0.85%), BbAATT (0.17%), BBAATT (0.17%), BBAATt (0.34%), BbAaTT (0.51%), bbAaTt (0.85%), BBAatt (0.17%), and BbaaTT (0.17%) accounted each for less than 1% of the population. Thus, we excluded from this haplotype analysis the 19 women containing one of these genotypes. Table 3 shows the distribution of fractures in women grouped according to the remaining common genotypes. Because there

TABLE 1. Baseline characteristics of postmenopausal women in relation to VDR *Bsm*I genotypes

Characteristic	VDR genotype			*P*
	bb (n = 213)	bB (n = 286)	BB (n = 90)	
Age (yr)	61.3 ± 8.6	62.0 ± 8.3	62.0 ± 8.3	0.61
Weight (kg)	60.2 ± 8.9	60.5 ± 9.1	59.4 ± 9.3	0.59
Height (cm)	158.7 ± 5.6	158.3 ± 5.8	158.7 ± 7.2	0.68
Body mass index (kg/m^2)	23.9 ± 3.3	24.1 ± 3.3	23.6 ± 3.6	0.38
Hormone replacement (%)	26	19	23	0.17
Daily calcium intake (mg)	820 ± 291	801 ± 298	882 ± 338	0.09
Physical activity score	14.3 ± 3.8	13.9 ± 3.8	14.1 ± 3.8	0.56
Left grip strength (bar)	0.68 ± 0.16	0.67 ± 0.16	0.68 ± 0.16	0.85
Prior fracture (%)	12.2	11.2	12.1	0.70
Maternal history of fragility fracture (%)	26	29	33	0.45
Fallers in the past year (%)	30	32	28	0.52

Data are shown as mean ± 1 SD.

TABLE 2. Number of women with incident fractures according to VDR *Bsm*I genotypes

VDR genotypes	No. of women with fracture/total number (%)		
	All fractures	Nonvertebral fractures	Vertebral fractures
bb	33/213 (15.5)	20/213 (9.4)	13/213 (6.1)
bB	62/286 (21.7)	46/286 (16.1)	16/286 (5.6)
BB	25/90 (27.8)	20/90 (22.2)	5/90 (5.6)
P (χ^2)	0.04	0.01	NS

NS, Not significant.

was an overrepresentation of fractures in women carrying the "BAt" haplotype, we grouped women according to carrier status for this VDR haplotype as homozygous carriers (BBAAtt) and heterozygous carriers (including genotypes BbAaTt and BbAATt) of the risk haplotype and compared with women not carrying the haplotype (including genotypes bbaaTT and bbAaTT). There was no significant difference in the variables shown on Table 1 between women grouped according to these VDR genotypes (data not shown). Women homozygous for the risk haplotype had a relative risk (95% confidence interval) of all fragility fractures of 2.0 (1.1–3.7) compared with women not carrying the haplotype, whereas heterozygous carriers had a relative risk of 1.4 (0.8–2.4).

Because this haplotype analysis gave association with fracture risk that was similar to that obtained with the *Bsm*I polymorphism only, in the subsequent analyses, only *Bsm*I genotypes were investigated.

VDR genotypes and risk factors for fractures

We investigated whether the relationships between VDR genotypes and fracture risk could be mediated by other risk factors for fracture that we identified in the OFELY cohort.

As previously reported (33), VDR genotypes were not associated with BMD at any of the skeletal sites investigated, including the total hip (Table 4). We thus investigated differences between VDR genotype groups in non-BMD risk factors for fractures.

Clinical factors

Six BMD-independent risk factors for all fractures have been previously identified in the OFELY cohort (35) including older age, number of falls in the previous year, lower grip strength, low physical activity, maternal history of fracture, and personal history of fractures. As shown on Table 1, there was no significant difference in any of these clinical risk factors between the VDR genotype groups.

TABLE 3. Number of postmenopausal women with fracture according to VDR *Bsm*I, *Apa*I, and *Taq*I genotypes

VDR genotype	No. of women with fracture/total number (%)
BBAAtt	24/86 (27.9)
BbAaTt	52/225 (23.1)
BbAATt	9/55 (16.4)
bbaaTT	22/127 (17.3)
bbAaTT	11/77 (14.3)
P (χ^2)	0.13

Skeletal-related factors

In the OFELY cohort, we previously identified increased bone turnover, and more specifically, increased serum CTX and bone-specific alkaline phosphatase, as BMD-independent factors for all fractures (36). There was no significant difference in any of the biochemical markers of bone turnover investigated between VDR genotype groups (Table 4). We also previously reported that an increased urinary α/β CTX was associated with an increased risk of all fractures, independently of BMD and of bone turnover rate (37). In this study, we found no difference in this ratio between VDR genotype groups (Table 4). As shown on Table 4, there was no significant difference in the rate of BMD change at the mid, distal, and ultradistal radius between VDR genotype groups.

Endogenous hormones

Among the various hormones we measured in the OFELY cohort at baseline, decreased serum levels of estradiol, DHEA sulfate, and IGF-I and increased concentrations of SHBG and PTH were associated with increased risk of all fractures independently of BMD (36). As shown on Table 4, there was no significant difference in the levels of these hormones and VDR genotypes, except for slightly higher PTH values in the bB and BB genotypes. Serum calcium and 25 (OH) D levels were also similar in the three genotype groups.

Multivariable model for the prediction of fracture risk

In multivariable logistic regression, none of the risk factors investigated above, taken individually or in combination, substantially altered the association between VDR genotypes and the risk of all fractures (data not shown). Because most of the variables above were associated with BMD and/or age and only PTH was significantly associated with VDR genotypes, we then performed a forward multiple variable model including age, hip BMD, and prevalent fractures as key established independent risk factors, and PTH and VDR polymorphisms. As shown on Table 5, the risk of fracture associated with genotype BB was of similar magnitude to that of low BMD and prior fractures, whereas serum PTH did not contribute significantly to the predictive model. As shown on Fig. 1, among women with a total hip BMD T-score more than −2.5 and genotype bb, only 11% had incident fracture, whereas 24% of women in the same BMD category, but carrying the BB genotype, had fracture. The highest fracture rate was observed in women with a BMD T-score less than −2.5 and carrying the BB genotype.

Discussion

In our study, we found that VDR genotypes were strongly associated with an increased risk of fractures independent of BMD levels, rate of radius BMD loss, and clinical and biochemical variables that have previously been shown to be associated with fracture risk.

Although VDR genotypes have been suggested previously to be associated with BMD or postmenopausal BMD loss, most recent metaanalyses indicated that, if any, the influence is limited to the spine and is very modest (15). In our population of postmenopausal women, we confirmed that VDR

TABLE 4. Risk factors for fractures in postmenopausal women in relation to VDR *Bsm*I genotypes

Parameters	VDR genotype			*P*
	bb (n = 213)	bB (n = 286)	BB (n = 90)	
Total hip BMD (g/cm^2)	0.811 ± 0.122	0.806 ± 0.119	0.795 ± 0.107	0.57
Radius BMD change (% per year)				
Mid	−0.40 ± 0.66	−0.37 ± 0.60	−0.36 ± 0.59	0.53
Distal	−0.62 ± 0.73	−0.61 ± 0.76	−0.52 ± 0.70	0.47
Ultradistal	−0.46 ± 1.04	−0.48 ± 1.03	−0.43 ± 1.25	0.41
Serum βCTX (pg/ml)	436 ± 227	450 ± 230	422 ± 272	0.84
Serum bone ALP (ng/ml)	11.5 ± 1.4	11.8 ± 1.4	10.9 ± 1.5	0.32
Urinary α/β CTX	0.281 ± 0.164	0.272 ± 0.103	0.285 ± 0.134	0.62
Serum estradiol (pg/ml)	47.5 ± 79.2	42.4 ± 74.6	38.9 ± 68.0	0.65
Serum DHEA sulfate (μg/ml)	0.657 ± 0.39	0.607 ± 0.40	0.611 ± 0.40	0.52
Serum SHBG (ng/ml)	16.1 ± 6.6	15.9 ± 7.9	17.8 ± 6.8	0.36
Serum IGF-I (ng/ml)	225 ± 71	226 ± 67	217 ± 52	0.71
Serum intact PTH (pg/ml)	30.5 ± 13.7	33.9 ± 13.1	33.3 ± 18.8	0.024
Serum 25 (OH) D (ng/ml)	36.6 ± 17.4	36.1 ± 14.5	36.0 ± 15.4	0.93
Serum calcium (mmol/liter)	2.32 ± 0.18	2.32 ± 0.08	2.34 ± 0.12	0.95

Data are shown as mean ± 1 SD.

genotypes were not associated with BMD level at any skeletal sites including the spine, the hip, the forearm, and the whole body. The power of previous studies to analyze the association between VDR genotypes and rate of BMD changes was somewhat impaired because of the imprecision in the estimate of individual BMD loss, resulting from the short follow up that did not exceed 5 yr (15, 38, 39). To our knowledge, our study is the longest that has examined associations between VDR genotypes and rate of BMD changes measured over 11 yr at a very precise site, the radius. Thus, our data strongly suggest that VDR genotypes do not influence postmenopausal bone loss at the radius.

We found an association between VDR genotype and nonvertebral fracture, but not vertebral fractures. That might be a statistical power issue as there were relatively few incident vertebral fractures (n = 34) compared with nonvertebral ones (n = 86). Conversely, the difference in prediction might be related to the mechanism underlying the association between VDR genotype and nonvertebral fracture risk that is unclear as this stage. Two previous prospective case-control studies in postmenopausal women have reported conflicting results on the association between VDR genotypes and fracture risk. In the Nurses' Health Study including postmenopausal women from 43–69 yr of age, VDR BB genotype was associated with a 2-fold increased risk of hip, but not distal radius fractures, which was not dose dependent (30). Conversely in the larger but older (>65 yr of age) population of the Study of Osteoporotic Fractures (31), no association was found between VDR genotypes and the risk of hip, vertebral, nonhip, and nonvertebral fractures. Finally, in a random sample of 1004 women aged 55–80 yr from the Rotterdam study, it was reported a significant dose-dependent relationship between VDR gene haplotypes and the risk of incident nonvertebral fractures occurring during a mean 3.8-yr follow-up (32). This relationship was independent of age, weight, and BMD. In a longer 7-yr follow-up of the same population, the authors subsequently reported a borderline significant association with incident vertebral fractures, but in contrast to their first report, no significant relationship with incident nonvertebral fractures (40). However, it should be mentioned that in the Rotterdam study, *bat* haplotypes constructed from direct analysis of *Bsm*I, *Apa*I, and *Taq*I RFLPs were analyzed, and it was suggested by the authors that this may have improved the sensitivity to find significant association, although this has not been formally demonstrated. In our study, combining VDR *Bsm*I, *Apa*I, and *Taq*I polymorphisms gave very similar results as the *Bsm*I genotype only, although this analysis was not based on direct haplotyping. As reported in previous studies (15, 30), we found that B was the risk allele whereas others (32) reported that women carrying the b allele were at risk for fracture. These discrepancies

TABLE 5. Multiple variable logistic regression model for the prediction of all fractures

Risk factor	Cut-off	Relative risk (95% CI)	Population attributable risk (%)
Age	>65 yr	2.65 (1.68–4.20)	36.1
Total hip BMD	T-score < −2.5	2.20 (1.37–3.55)	22.1
Prior fracture	Yes	2.53 (1.40–4.56)	16.8
VDR genotype	bB *vs.* bb	1.62 (0.97–2.70)	23.2
	BB *vs.* bb	2.46 (1.27–4.74)	18.2

CI, Confidence interval.

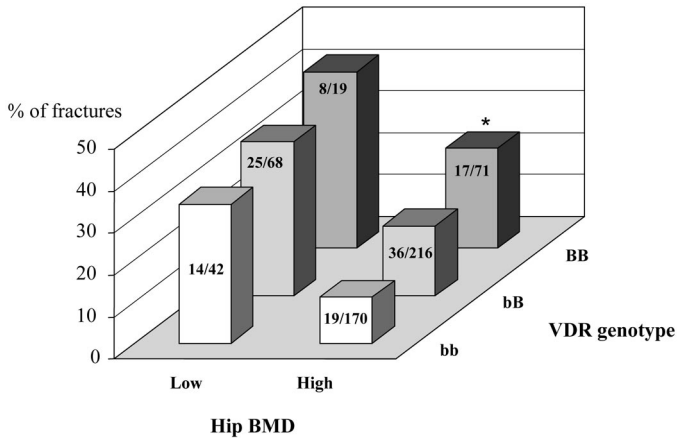

FIG. 1. Percentage of incident fractures according to total hip BMD levels and VDR *Bsm*I genotypes. Low BMD was defined as a BMD T-score less than −2.5. *Figures in the bars* represent the number of incident fractures on the total number of women. *, $P < 0.05$ for allele dose association of VDR genotypes with incident fractures.

may be due to technical errors leading to potential difference in genotype determination—although this is unlikely—or differences in population characteristics including interactions with environmental factors. Haplotyping of multiple VDR polymorphisms combined with functional analyses is likely to help in understanding these differences.

Our findings strongly suggest that VDR genotypes act directly and/or indirectly on bone fragility by mechanisms that are not reflected by the measurements of BMD or bone turnover. The originality and major strength of our study relates to the extensive clinical and biochemical investigation we performed on these women, which allowed us to adjust our analyses for several risk factors for fracture. Among the clinical parameters, grip strength and the number of falls in the previous years have been shown to be predictive of fractures independently of BMD in the OFELY cohort (35) and may also be associated with VDR polymorphisms as vitamin D regulates neuromuscular functions (41). Physical activity and calcium intake have also been suggested to interact with VDR genotypes on their effects on BMD or BMD changes (39,42). We found no significant difference between genotypes in these physical, dietary, and lifestyle factors, and adjustment for these parameters did not weaken the association between VDR genotypes and fractures, suggesting that they are not likely to be involved in mediating the observed effect.

One of the major BMD-independent risk factors that has emerged in the recent years is an increased bone turnover which can be detected by sensitive biochemical markers. Among circulating hormones, low 17-β estradiol, high SHBG, low DHEA sulfate, or low IGF-I have been shown to be associated with increased risk of hip, vertebral, and all fractures (36, 44) and genetic studies have reported a significant interaction between VDR and estrogen receptor α polymorphisms on BMD (45) or fracture risk (40). In our study, there was no significant association between VDR genotypes, biochemical markers of bone turnover, and the circulating levels of all these hormones. In addition, their inclusion in a multivariable model did not modify the effect of VDR polymorphisms on fracture risk.

An obvious potential mechanism by which VDR genotypes may influence bone fragility is through the calcium regulating system and the close relationships between the effects of vitamin D and PTH on bone metabolism. Although VDR alleles have been shown to be associated with differences in intestinal calcium absorption (46) and bone metabolism response to 1.25 $(OH)_2$ D_3 (47), the relationships between VDR genotypes and circulating levels of PTH, 25 (OH) D, or calcium have also been inconclusive both in healthy postmenopausal women and hemodialysis patients (48). In our study, we found significantly higher levels of serum-intact PTH in patients carrying the B allele, but no differences in serum 25 (OH) D and total calcium, in agreement with some, but not all studies (48). However, the difference between genotypes was very small, not dose dependent, and adjustment for circulating PTH levels did not modify the relationships between VDR genotypes and fracture risk. Consequently the biological relevance of this association between VDR genotypes and serum-intact PTH is unclear and could have resulted from chance findings.

Our study has strengths and some limitations. This is one of the largest and the longest prospective study on the relationships between VDR genotypes and fracture risk in postmenopausal women. As mentioned above, we were able to investigate most of the risk factors that could have confounded the association between VDR genotypes and fractures risk. However, we did not analyze the potential influence of VDR on bone geometry and bone matrix quality, two important determinants of skeletal fragility that may be genetically determined. It has been suggested that the effect of VDR on fracture risk may in part be mediated by its interaction with collagen I gene polymorphisms (32), which influence bone fragility mainly by changes in bone matrix quality rather than on BMD (49). However, we did not find any relationship between VDR genotypes and the urinary ratio of native to isomerized CTX, which has been proposed as an index of bone collagen matrix quality (37). In addition, no relationships between VDR genotypes and hip axis length or ultrasound parameters could be found in a twin study (50). We could not assess rate of BMD changes at the spine or the hip. We measured circulating levels of hormones that may not adequately reflect their local bone environment concentrations. We performed a single measurement of markers of bone turnover and hormones, which may not accurately reflect the bone turnover and hormone status because of the variability in the measurements. We did not perform direct haplotyping of *Bsm*I, *Apa*I, and *Taq*I VDR RFLPs. The VDR polymorphisms we have investigated are probably nonfunctional and thus are unlikely to be directly involved in their association with fracture risk. However, they could be in linkage disequilibrium with one or more functional polymorphisms yet to be identified elsewhere in the VDR gene, which may explain the relationships we observed (43). Finally, VDR genotypes were not available for 82 women of the initial cohort. The baseline demographic characteristics of these women were similar and not significantly different from the rest of the cohort, except for an older age (66 *vs.* 62 yr), and consequently a slightly lower physical activity and higher prevalence of prior fracture. However, because of the absence of relationships between VDR genotypes and these parameters, this is not likely to have influenced our findings.

In summary, we found that VDR gene polymorphisms were dose dependently associated with the risk of all fractures independently of BMD, rate of postmenopausal BMD loss, and circulating levels of several biochemical variables. The biological mechanisms by which VDR genotypes influence bone strength remain to be determined.

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