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Vitamin D and Estrogen Receptor Allelic Variants in Italian Postmenopausal Women: Evidence of Multiple Gene Contribution to Bone Mineral Density

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ABSTRACT

Bone mass and bone turnover are under genetic control. Restriction fragment length polymorphisms (RFLPs) at the vitamin D receptor (VDR) gene locus have been recently correlated to bone mineral density (BMD) and rate of bone loss. However, agreement on this relationship is not universal. The existence of ethnical and environmental differences between populations, a health-based selection bias in several previous studies, and the involvement of other genes could explain these discordant findings. In this study, we examined the relationship of VDR and estrogen receptor (ER) gene RFLPs with lumbar spine and upper femur BMD in 426 Italian postmenopausal women, 57.7 ± 0.4 yr old (144 normal, 106 osteopenic, and 176 osteoporotic). VDR gene RFLPs for *Apa*I, *Bsm* I, and *Taq*I restriction endonucleases and ER RFLPs for *Pvu*II and *Xba*I restriction endonucleases were assessed by Southern blotting analysis and were indicated, respectively, as A-a, B-b, T-t, P-p, and X-x (uppercase letters signifying the absence and lowercase letters the presence of the restriction site). After correcting for potential confounding factors (age, height, weight, age since menopause, osteophytosis, and facet joint osteoarthritis), a statistically significant VDR genotype effect on lumbar BMD ($P = 0.01$, analysis of covariance), but not on femoral BMD, was detected, with subjects in AABBtt genotype showing a 13% lower BMD than those with aabbTT genotype ($P < 0.05$, Tukey's test). Moreover, a statistically significant prevalence of AABBtt genotype in osteoporotics, and of AabbTT and aabbTT genotypes in nonosteoporotics, were detected. Conversely, there was no significant relationship of ER genotype to either lumbar or femoral BMD, even though a trend for higher BMD values in women with the ER PP genotype (with respect to those with ER pp genotype) was detected. When mean lumbar BMD was calculated for women grouped by ER and VDR genotype, we observed a significant difference between those within the 2 opposite associations AABBtt-PPXX and aabbTT-ppxx (0.71 ± 0.05 *vs.* 0.97 ± 0.03 g/cm^2, $P < 0.05$ Tukey's test). These results are consistent with a segregation of the VDR AABBtt genotype with a higher risk of developing osteoporosis, in the Italian female population. The introduction of another variable, the ER genotype, in the analysis of VDR genetic determination of BMD, may represent a useful model in the identification of patients at risk of developing a multigenic disorder like osteoporosis. (*J Clin Endocrinol Metab* **83:** 939–944, 1998)

BONE mineral density (BMD), the major determinant of osteoporotic fracture risk, is a quantitative trait determined by the interaction of genetic, metabolic, and environmental factors. In the last years, both twin and family studies have suggested a major genetic contribution in bone mass determination and in the development of osteoporosis (1–4). The number of candidate genes is large, ranging from those regulating calcium homeostasis to the several locally involved in bone cell recruitment and activity. The vitamin D receptor (VDR) gene has been shown to be a major locus for genetic influences on bone mass, and polymorphisms in this gene seemed to predict spinal and femoral BMD in an Australian population (5). The subjects homozygous for the presence of the Bsm I restriction endonuclease site were reported to have a bone mass about 15% higher than that of subjects homozygous for the absence of the site (5). Successively, haplotype analysis, based on three restriction fragment length polymorphisms (RFLPs) at the VDR gene locus, showed the possibility to discriminate individuals at high and very low osteoporotic risk, with subjects homozygous for the abT haplotype showing lumbar BMD values 0.15 g/cm^2 greater than subjects homozygous for the ABt haplotype (6). However, agreement on this relationship is not universal among different populations, some finding positive associations (7–12), others reporting no significant effect (13–17).

The cause of the discrepancies remains to be determined, and in part, it may be caused by the limited sample size of many studies. Indeed, relationships between VDR gene alleles and osteoporotic risk, mediated through differences in BMD, are unlikely to be observed in relatively small samples. A potential confounder in all such studies may be given also from the health-based selection bias, with the tendency to exclude osteoporotic women. Currently, the few association studies, in which the prevalence of VDR genotypes in osteoporotic and nonosteoporotic patients are compared, have been carried out in small samples, with limited statistical power (12, 15, 16, 18). Heterogeneity also is likely, with different major genes segregating in different patient sam-

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ples. In this regard, other polymorphic genes, such as those encoding for estrogen receptor (ER), collagen type I α1, and interleuchin 6 recently have been linked to variation in BMD (19–21). These genes could either positively or negatively modulate VDR gene effect, with a different power, which needs to be individually evaluated. In addition, environmental factors also could reciprocally interact with genetic factors. Recently, dietary calcium intake has been reported to contribute to the expression of the VDR gene effect, both on BMD (10, 11) and on intestinal calcium absorption (22). Finally, linkage disequilibrium with another bone metabolism-related gene on chromosome 12 (*i.e.* Collagen 2 A1 and retinoic acid receptor genes) cannot be excluded.

Being aware of the constant interaction between genetic and environmental factors in bone mass determination, the present study was performed to evaluate, for the first time, in a large and ethnically homogeneous group of postmenopausal women of Italian descent (stratified for BMD in normal, osteopenic, and osteoporotic patients): 1) the relationship of VDR and ER gene polymorphisms with BMD, after controlling for multiple confounders; and 2) the possibility of an interaction between VDR and ER genotypes with bone mass, assuming that variation of BMD is influenced by multiple genes.

Subjects and Methods

Subjects

Patients eligible for the study were selected among 1500 consecutive postmenopausal women who, in 1995, attended the metabolic bone diseases outpatient clinics in Siena and Florence for osteoporotic risk evaluation. For all subjects, a detailed medical history was obtained, and dietary calcium intake was assessed by a sequential self-questionnaire, including foods that account for the majority of calcium in the diet. Among this group of women, 866 had associated conditions known to affect bone metabolism and were excluded from analysis. These were diseases known to influence bone mass (98 women), use of bone active drugs (303 estrogen replacement therapy, 198 vitamin D metabolite, 96 bisphosphonate, 59 calcitonin, and 7 fluoride) or use of drugs that could potentially affect bone metabolism (55 glucocorticoid, 34 thyroid hormone, and 16 antacid). Eighty-nine women also were excluded because of their different ethnical origin. Blood was available for DNA isolation in 426 of the remaining 545 subjects. The age range of the studied women was 47–76 yr, with a mean (±SEM) age of 57.7 ± 0.43 yr. On the basis of BMD measurements and according to WHO criteria (23), 40% of the 426 subjects had osteoporosis, 26% had osteopenia, and 34% were normal. General characteristics of the population are presented in Table 1.

Genotyping

Genomic DNA was isolated from EDTA blood samples by a standard phenol-chloroform extraction procedure, and 8 μg of DNA were digested for 6 h in a vol of 50 μL with, respectively, 40 U of *Apa*I, Bsm I, *Taq*I, *Pvu*II, and *Xba*I restriction endonucleases (Boehringer Mannheim, Milan, Italy) at temperatures recommended by the manufacturer. The digested DNA was size-fractionated using 0.7–1% agarose gel electrophoresis and transferred to nylon-based filters (GeneScreen Plus, NEN Research Products, Boston, MA) by standard techniques (24). Filter membranes were prehybridized, then hybridized with the ^{32}P-labeled probe for 18 h at 65 C, washed, and autoradiographed for 24–48 h at −80 C in intensifying screen. For identifying the RFLPs, we used, as probes, a 2.1-kilobase complementary DNA (cDNA) coding region of the human VDR (25) and a 1.8-kilobase cDNA coding region of the human ER (26). The probes were radiolabeled with [α^{32}P]deoxycycidine triphosphate using a random priming labeling kit (Boehringer Mannheim, Milan, Italy). The RFLPs were coded as B-b (*Bsm* I), A-a (*Apa*I), T-t (*Taq*I), P-p (*Pvu*II), and X-x (*Xba*I), uppercase letters signifying the absence and lowercase letters the presence of the restriction site.

Bone densitometry

Lumbar BMD (L2-L4), measured by dual-energy x-ray absorptiometry (Hologic QDR 1000/W) was available for all the 426 studied women. The long term *in vitro* precision at this site measured on spinal phantom was 0.4% in Siena and 0.6% in Florence; the *in vivo* precision was 0.9% in both centers. Dual-energy x-ray absorptiometry BMD scans at the upper femur were available for 230 of the 426 women (113 osteoporotic, 58 osteopenic, and 59 normal), with *in vivo* coefficients of variations of 1.1% in Florence and 0.9% in Siena. Cross-calibration studies on the precision of measurements between the 2 centers were previously performed both *in vitro* (using a single anthropomorphic lumbar spine phantom) and *in vivo*, on 50 patients, covering most of the clinically observed spinal density range. A correction factor was not considered necessary.

Because of the influence of extravertebral calcification on spinal bone mass measures, each woman underwent a lateral lumbar spine x-ray examination to be scored for spinal osteophytosis (SPO), according to Orwoll (27), and for facet joint osteoarthritis (FOA) on a four-point scale (0 = none, 1 = mild, 2 = moderate, and 3 = severe), according to Masud (28). Vascular calcifications were not evaluated for their reported limited impact on spinal density measurements (27–29).

Statistical analysis

Data were expressed as mean ± SEM, with $P < 0.05$ accepted as the value of significance. The frequency distribution of VDR genotypes in osteoporotic, osteopenic, and normal groups were compared using cross-tabulation and standard χ^2 tests. Differences in antropometric characteristic, spinal BMD, and femoral BMD among the different VDR and ER genotypes were tested using ANOVA. Similar comparisons were done after adjusting mean BMD values for potential confounding factors such as age, height, weight, years since menopause (YSM), SPO, and

TABLE 1. General characteristics of the study group patients

	Normal	Osteopenic	Osteoporotic	Total
n	144	106	176	426
Age (yr)	57.1 ± 0.7	57.9 ± 0.8	58.2 ± 0.6	57.7 ± 0.4
YSM	8.5 ± 0.9	9.0 ± 0.9	10.1 ± 0.7	9.3 ± 0.5
Height (cm)	163.9 ± 0.8[a]	161.6 ± 0.7	159.4 ± 0.6	161.4 ± 0.4
Weight (kg)	64.1 ± 1.3	64.5 ± 1.2	62.9 ± 1.2	63.7 ± 0.6
Ca intake (mg/day)	542 ± 142	568 ± 164	587 ± 128	567 ± 102
Lumbar BMD (g/cm^2)	1.05 ± 0.01[a]	0.88 ± 0.01[a]	0.69 ± 0.01	0.86 ± 0.01
SPO score	0.79 ± 0.07	0.73 ± 0.09	0.63 ± 0.10	0.70 ± 0.05
FOA score	0.63 ± 0.06	0.57 ± 0.08	0.57 ± 0.09	0.59 ± 0.04
Corrected BMD (g/cm^2)[b]	1.03 ± 0.01[a]	0.85 ± 0.01[a]	0.67 ± 0.01	0.83 ± 0.01

Values are expressed as mean ± SEM.
[a] $P < 0.05$ ANOVA *vs.* osteoporotic group.
[b] Lumbar spine BMD values, corrected for age, height, weight, YSM, SPO, and FOA scores.

FOA scores, using analysis of covariance. Between groups, differences among genotype groups were tested using Tukey's test. All statistical analyses were performed using STATGRAPHICS (Manugistic Inc., Rockville, Maryland) and Statistica 5.1 (Statsoft Inc., Tulsa, Oklahoma).

Results

VDR RFLPs

No significant departures from Hardy-Weinberg equilibrium were observed for *Apa*I, Bsm I, and *Taq*I RFLPs. The distribution of genotypes was similar to that of other studies, in Caucasians. When the 3 RFLPs at the VDR gene locus were considered together in all subjects analyzed, 7 major genotypes were recognized: AaBbTt (n = 157), AABBtt (n = 71), aabbTT (n = 56). AABbTt (n = 49), AabbTT (n = 47), AAbbTT (n = 17), and AaBbTT (n = 13).

Clinical characteristics of patients, in relation to the VDR genotype, are shown in Table 2. Sixteen women having rare genotypes (n < 5) were excluded from analysis. Results indicated that subjects in the seven most common genotypes were well matched for age and did not significantly differ for YSM, height, weight, and dietary calcium intake. After correcting for potential confounding factors, a statistically significant segregation of VDR genotypes with lumbar BMD was detected ($P = 0.01$, analysis of covariance), with mean corrected BMD values at the lumbar spine significantly higher in women with aabbTT genotype, compared with those with either AABBtt or AaBbTt genotype ($P < 0.05$, Tukey's test). A similar, but not significant, trend was observed for femoral neck BMD, with a 6% higher BMD value in aabbTT than in AABBtt genotype (data not shown).

When Bsm I and *Taq*I polymorphisms were analyzed separately, we observed a significantly increased lumbar BMD in women with the bb or the TT genotypes than in those with BB and tt genotypes, respectively (0.87 ± 0.01 *vs.* 0.78 ± 0.02, bb *vs.* BB, $P < 0.05$, Tukey's test; and 0.86 ± 0.01 *vs.* 0.78 ± 0.02, TT *vs.* tt, $P < 0.05$, Tukey's test). Conversely, no differences in mean BMD levels for different *Apa*I genotypes were detected (data not shown).

Genotype determinations for osteoporotic, osteopenic, and normal groups are summarized in Table 3. We observed a significantly increased prevalence of AABBtt genotype in osteoporotic and osteopenic patients, compared with nonosteoporotic ($\chi^2 = 14.7$; $P = 0.0006$). On the contrary, aabbTT and AabbTT genotypes were significantly overrepresented in nonosteoporotic *vs.* osteoporotic women ($\chi^2 = 8.75$, df = 1, $P = 0.003$ for aabbTT genotype; and $\chi^2 = 6.05$, df = 1, $P = 0.04$ for AabbTT genotype). Cross-tabulation testing resulted statistically significant ($\chi^2 = 41.9$, df = 14, $P = 0.0004$).

ER RFLPs

*Pvu*II and *Xba*I RFLPs allele frequencies in this Italian population followed Hardy-Weinberg equilibrium. When we combined the 2 RFLPs we recognized six genotypes: PpXx (n = 174), ppxx (n = 126), PPXX (n = 73), Ppxx (n = 25), PPXx (n = 17), and PpXX (n = 11). Three genotypes (PPxx, ppXX, and ppXx) were not detected in the population examined in this study.

The clinical characteristics, by ER genotype, of the 426 studied women are given in Table 4. There were no significant differences in age, weight, height, YSM, and dietary calcium intake across genotypes. Analysis of the ER genotypes, in relation to adjusted BMD values, did not reveal any significant effect.

Single RFLP analysis revealed a trend for a 0.05 g/cm^2 higher lumbar BMD value in women with pp genotype than in those with PP genotype (data not shown). On the contrary, women with the xx genotype showed a 0.02 g/cm^2 lower lumbar BMD than those with XX genotype (data not shown). However, none of these differences reached statistical significance.

χ^2 analysis on the genotypic frequencies revealed no significant increased prevalence of any of the six ER genotypes

TABLE 3. VDR Genotypes for normal, osteopenic, and osteoporotic patients

Genotype	Patients			χ^2	P value
	Normal	Osteopenic	Osteoporotic		
AABBtt	11 (7.6%)	29 (18.9%)	40 (23,8%)	14.7	0.0006
AABbTt	15 (10.4%)	11 (10.4%)	23 (13.7%)	1.0	0.6
AaBbTt	56 (38.9%)	37 (34.9%)	64 (38.1%)	0.5	0.7
AAbbTT	8 (5.6%)	4 (3.8%)	5 (3.0%)	1.3	0.5
AaBbTT	5 (3.5%)	3 (2.8%)	5 (3.0%)	0.1	0.9
AabbTT	23 (16%)	12 (11.3%)	12 (7.1%)	6.0	0.04
aabbTT	26 (18%)	19 (17.9%)	11 (6.5%)	11.3	0.003

TABLE 2. Clinical features of the women in relation to VDR genotype; 16 of the 426 women exhibiting rare genotypes (n < 5) were excluded from analysis

	Genotype						
	AABBtt	AABbTt	AaBbTt	AAbbTT	AaBbTT	AabbTT	aabbTT
n	71	49	157	17	13	47	56
Age	55.8 ± 1.0	58.9 ± 1.4	57.4 ± 0.7	59.0 ± 2.7	58.8 ± 2.7	56.8 ± 1.3	57.2 ± 1.1
YSM	8.3 ± 1.6	9.7 ± 1.6	9.6 ± 0.8	7.9 ± 3.0	10.5 ± 3.3	9.8 ± 2.2	9.0 ± 1.4
Height (cm)	160.6 ± 0.9	161.5 ± 1.2	160.4 ± 0.7	163.6 ± 2.6	163.8 ± 2.6	163.1 ± 1.2	162.3 ± 1.0
Weight (kg)	63.4 ± 1.5	64.6 ± 2.0	63.2 ± 1.3	62.6 ± 4.1	63.1 ± 4.2	63.6 ± 1.9	65.0 ± 1.7
Ca intake (mg/day)	588 ± 162	607 ± 186	565 ± 132	549 ± 221	578 ± 227	551 ± 196	560 ± 189
FOA score	0.54 ± 0.12	0.70 ± 0.14	0.60 ± 0.08	0.47 ± 0.22	0.74 ± 0.30	0.63 ± 0.14	0.65 ± 0.12
SPO score	0.76 ± 0.12	0.61 ± 0.15	0.63 ± 0.09	0.58 ± 0.21	0.81 ± 0.27	0.70 ± 0.15	0.75 ± 0.13
Lumbar BMD	0.81 ± 0.03[a]	0.84 ± 0.03	0.85 ± 0.02[a]	0.87 ± 0.06	0.83 ± 0.07	0.89 ± 0.03	0.91 ± 0.03
Corrected BMD[b]	0.78 ± 0.02[a]	0.85 ± 0.03	0.82 ± 0.01[a]	0.85 ± 0.06	0.80 ± 0.07	0.87 ± 0.03	0.89 ± 0.02

Values are expressed as mean ± SEM.
[a] $P < 0.05$ Tukey's test *vs.* aabbTT genotype.
[b] Lumbar BMD values, corrected for age, height, weight, YSM, FOA, and SPO scores.

TABLE 4. Clinical features of the 426 women in relation to ER genotype

	Genotype					
	PPXX	PPXx	PpXX	PpXx	Ppxx	ppxx
n	73	17	11	174	25	126
Age	56.5 ± 1.0	58.2 ± 1.9	59.0 ± 2.6	57.6 ± 0.7	59.0 ± 1.7	58.1 ± 0.8
YSM	8.1 ± 1.2	9.5 ± 2.3	10.3 ± 3.1	9.3 ± 0.8	10.2 ± 2.0	9.7 ± 1.0
Height (cm)	161.5 ± 1.1	161.2 ± 2.3	163.7 ± 2.9	162.4 ± 0.7	158.3 ± 1.8	160.4 ± 0.8
Weight (kg)	62.6 ± 1.7	60.5 ± 3,2	65.4 ± 4.1	63.7 ± 1.1	63.3 ± 2.9	64.7 ± 1.3
Ca intake (mg/day)	554 ± 168	593 ± 221	556 ± 202	566 ± 121	601 ± 198	574 ± 138
FOA score	0.58 ± 0.12	0.49 ± 0.24	0.80 ± 0.30	0.43 ± 0.10	0.91 ± 0.18	0.76 ± 0.12
SPO score	0.85 ± 0.13	0.85 ± 0.27	0.90 ± 0.32	0.59 ± 0.10	0.83 ± 0.20	0.69 ± 0.11
Lumbar BMD	0.856 ± 0.02	0.872 ± 0.04	0.890 ± 0.04	0.850 ± 0.01	0.861 ± 0.03	0.873 ± 0.01
Corrected BMD[a]	0.825 ± 0.02	0.844 ± 0.04	0.866 ± 0.06	0.821 ± 0.01	0.833 ± 0.05	0.855 ± 0.01

Values are espressed as mean ± SEM.
[a] Lumbar spine BMD values, corrected for age, height, weight, YSM, SPO, and FOA scores.

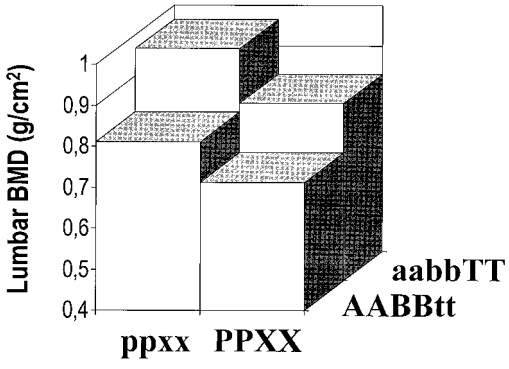

FIG. 1. Mean corrected lumbar BMD values, adjusted for potential confounding factors among women grouped by opposite VDR (AABBtt, aabbTT) and ER (PPXX, ppxx) genotypes.

in osteoporotic, osteopenic and nonosteoporotic groups (data not shown).

Combined effect of VDR and ER genes RFLPs

Sixteen major association groups were detected combining VDR and ER genotypes: AABBtt-PPXX (n = 9), AABBtt-PpXx (n = 29), AABBtt-ppxx(n = 25), AaBbTt-PPXX (n = 31), AaBbTt-PpXx (n = 70), AaBbTt-ppxx (n = 43), aabbTT-PPXX (n = 8), aabbTT-PpXx (n = 27), aabbTT-ppxx (n = 14), AaBbTt-Ppxx (n = 10), AABbTt-PPXX (n = 14), AABbTt-ppxx (n = 12), AABbTt-PpXx (n = 18), AabbTT-PPXX (n = 12), AabbTT-ppxx (n = 8), and AabbTT-PpXx (n = 23).

As shown in Fig. 1, when mean adjusted lumbar BMD values were calculated among women grouped by ER and VDR genotypes, we observed a statistically significant difference of approximately 0.26 g/cm^2 between the two opposite association AABBtt-PPXX and aabbTT-ppxx (0.713 ± 0.05 *vs.* 0.970 ± 0.03 g/cm^2, $P < 0.05$, Tukey's test). Furthermore, a trend for higher BMD values was detected between ppxx and PPXX subjects with the same VDR genotype ($P = 0.08$, AABBtt-PPXX *vs.* AABBtt-ppxx; $P = 0.07$, aabbTT-PPXX *vs.* aabbTT-ppxx).

Discussion

Evidence from epidemiological and twin studies clearly demonstrates that osteoporosis is a multifactorial disease with a strong genetic component. BMD, the major factor determining bone strength and consequently osteoporotic fracture risk, can be considered a quantitative polygenic trait. Because the description that the genetic component responsible for bone mass could be largely ascribed to a simple allelic change in the VDR gene (5), several conflicting reports on the relationship between VDR genotypes and BMD have been published (6–18, 22, 30). The VDR gene, however, is only one of a large group of candidate genes, ranging from those encoding cytokines and growth factors involved in local control of bone metabolism to those encoding collagenic and noncollagenic matrix components and those encoding calciotropic hormones and their receptors. Therefore, even though the VDR gene could represent a major gene in the determination of bone mass, other intrinsic and environmental influences certainly interact with it, and these interactions need to be quantified, to understand more of the causal spectrum (31).

Present data from a large and ethnically homogeneous population showed a significant segregation of VDR genotypes with lumbar BMD, in the Italian population, as previously shown in Australian women (5–6). Women in the AABBtt genotype group showed a spinal BMD 13% less than those with aabbTT genotype. Such a difference persisted even after adjustment of BMD values for the potential influence of SPO and FOA. The magnitude of this effect between extreme homozygotes was approximately 0.1 g/cm^2, slightly lower than that reported by Morrison *et al.* (5–6). A similar trend was observed for femoral neck BMD, but with no statistically significant difference between genotypes. How can we reconcile these evidences with the lack of segregation of VDR alleles with BMD in several patient samples (13–17)? One possible cause of discrepancy could be related to the relatively small samples of many other studies being not powerful enough to adequately assess the VDR gene allele effect on bone mass. This explanation does not apply to two recent studies that have looked specifically at the relationship between VDR genotypes and BMD in large samples of postmenopausal women (17, 30). The first study (30), of Uitterlinden and co-workers, reported a weak association between low bone mass and a particular VDR genotype, AAbbTT, which is different from the one reported to be associated with low BMD, by our and other groups (4–12). In the 426 analyzed Italian women, individuals with AAbbTT genotype showed BMD values that were interme-

diate, between those with aabbTT and AABBtt genotypes. However, Uitterlinden *et al.* limited their analysis to the femoral neck BMD, a site with predominantly cortical bone, whereas the genetic effect on bone mass seems to be stronger at sites with higher proportions of trabecular bone (2–5). In the second study (17), no significant relationships between VDR genotypes and BMD (measured at the spine, hip, and forearm) were detected in 268 French postmenopausal women, with frequency distribution of VDR genotypes quite different from that previously reported both by the same authors (14) and in other Caucasian populations of European ancestry (30, 32). Moreover, the contribution of VDR polymorphisms, both in Dutch and French studies, may have been masked by the relatively high calcium intake (17, 30). Indeed, environmental factors, such as calcium intake, are known to differ widely between populations and have been shown to contribute to the genetic influence of VDR genotypes on BMD (10–11), rates of bone loss (10–11), and intestinal calcium absorption (22). Consistent with the work of Dawson-Hughes *et al.* (22), we recently observed a significantly reduced fractional absorption capacity of strontium in a group of calcium-depleted women with AABBtt genotype, with respect to those with aabbTT genotype, suggesting a segregation of the VDR AABBtt genotype with a lower intestinal calcium absorption efficiency (32). For all these reasons, it is possible that the influence of VDR genotypes on bone metabolism could be observed only among populations with a relatively low calcium intake, as in the Italian population (33). Interestingly, in this sample of Italian postmenopausal women, we also observed a statistically significant increase in prevalence of both AABBtt genotype in the osteoporotic group and aabbTT and AabbTT genotypes in the nonosteoporotic group, in agreement with a possible segregation of the ABt homozygous haplotype with a higher risk of developing osteoporosis. Similarly, 2 other studies showed a disproportionate representation of the B allele in osteoporotic subjects, compared with a control group; but in both of them, these differences did not reach statistical significance (12, 18). By contrast, Looney and co-workers referred no large overrepresentation of the BB genotype in a group of North American severely osteoporotic women, compared with age-matched controls (15). However, all these previous studies were conducted in a limited number of subjects, with a consequent limited statistical power to test the hypothesis of a prevalence of a given genotype in osteoporotic subjects (12, 15, 16, 18).

The present study extends observations relating VDR genotypes and demonstrates that the addition of ER genotype to VDR genotype determination may provide a tool to identify, more precisely, individuals with a reduced bone mass. The magnitude of the effect of combining both VDR and ER genotype determination on lumbar BMD reaches approximately 2 SD and is significantly greater than that obtained from the analysis of the single ER or VDR gene effect. From this study, it also seems that the ER genotype PPXX confers some reduction in spinal BMD only when combined with VDR AABBtt genotype, without showing a segregation with BMD values by itself. Kobayashi *et al.* recently reported a statistically significant association of ER Px haplotype with a lower BMD in postmenopausal Japanese women, independent of VDR genotype (19). It is possible that these differences are related to the relative differential distribution of VDR and ER genotypes between populations of European and Asiatic ancestry. In fact, the PPxx genotype, representative of 8% of the Japanese population, was not detected in any of the 426 women examined in this study, whereas VDR genotype AABBtt, which in our Italian population is associated with the lowest BMD values, is extremely rare in the Japanese population (8, 16, 34). For this reason, a hypothetical segregation of BMD with polymorphisms at the ER gene locus could be more easily detectable in Asiatic women (where just 2 VDR genotypes, AabbTT and aabbTT, account for almost 80% of the total population) than in Caucasian populations of European ancestry, which exhibit high heterogeneity in VDR gene polymorphisms.

The epistatic effect between the ER and the VDR genes on BMD determination may be biologically fundamental, supporting a relevant role of the ER gene locus on BMD. Analysis of larger sample populations will make it possible to ascribe the ER gene locus either to the major gene family or to the polygenic aggregate, whose members are recognized among loci whose genetic effect is individually small. This approach, however, may be considered useful in future complex models of segregation analysis for osteoporotic risk.

In conclusion, in this homogeneous population of Italian postmenopausal women with a relatively low calcium intake, the allelic changes at the VDR gene locus are responsible for an important portion of the genetic component of spinal BMD. The results from the association analysis of ER and VDR genotypes effect on lumbar BMD suggest that the ER RFLPs could play a role, as well, exerting an additional contribution to bone mass determination. Other polymorphic genes and environmental factors could further modulate the expression of ER and VDR allelic effect on bone mass, making the picture as complicated as it is in nature.

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Erratum

In the announcement of drugs newly approved by the FDA on page 360 in the February issue of *The Journal of Clinical Endocrinology and Metabolism* [83(2):360], two entries were mistakenly conflated. The announcement should have read:

Repaglinide, trade name Prandin, made by Novonordisk.
Sibutramine, trade name Meridia, made by Knoll.
Raloxifene, trade name Evista, made by Lilly.

The Editors regret the confusion caused by this error.