A novel T/C polymorphism (ATG to ACG) at the translation initiation site of the vitamin D receptor (VDR) gene, defined by FokI restriction endonuclease, has been recently associated with variation in bone mineral density (BMD) and rates of bone loss in a group of postmenopausal Mexican-American women. The presence of the restriction site, designated as f, allows protein translation to initiate from the first ATG, while the allele lacking the site, indicated as F, initiates translation at a second ATG. In this study, we investigated the role of FokI polymorphism in a group of 400 postmenopausal women of Italian descent stratified for BMD into osteoporotic (n = 164), osteopenic (n = 117), and normal (n = 119) groups. There were 159 (41%) FF homozygotes, 55 (14%) ff homozygotes, and 186 (45%) Ff heterozygotes. In the whole population, we observed a weak association between FokI polymorphism and lumbar BMD (p = 0.06, analysis of covariance [ANCOVA]) but not with femoral neck BMD (p = 0.5, ANCOVA). Interestingly, the effect of FokI genotypes on lumbar BMD was influenced by the years since menopause such that differences in BMD related to different VDR allelic variants were greater among women in the first 5 years of menopause (p = 0.04, ANCOVA), progressively declining afterward. In addition, a significantly higher prevalence of ff genotype in osteoporotic than in osteopenic and normal women was observed (p = 0.04, Chi-square test). Finally, ff genotype resulted significantly over-represented in the group of women with a vertebral fracture as compared with controls (p = 0.003, Chi-square test), equivalent to a relative risk of 2.58 (95% confidence intervals 1.36–4.91). We conclude that in this population, FokI polymorphism at the VDR gene locus accounts for a part of the heritable component of BMD at the lumbar spine. (J Bone Miner Res 1999;14:1379–1386)
has been recently associated with variation in BMD and rates of bone loss in a group of postmenopausal Mexican-American women.\(^{(14)}\) Subjects homozygous for the presence of the restriction site were reported to have a 12.8% lower lumbar BMD than subjects homozygous for the absence of the site.\(^{(14)}\) This polymorphism is a T/C polymorphism (ATG to ACG) at the first initiation codon (ATG), and is located in exon 2 of the VDR gene, three codons proximal to a second start site downstream. Thus, the VDR gene from individuals with the T variant, homozygotes for the presence of the restriction site, possess two potential initiation codon. While the translation of the VDR mRNA from the T allele can initiate from the first initiation site, the translation of mRNA from the C allele must initiate from the second initiation site. Such a difference may provide a structural change that could potentially alter the function of the VDR protein. Interestingly, this T-C transition has been recently shown to result in the synthesis of a 3 amino acids smaller protein, with increased biological activity.\(^{(15)}\) Although two subsequent studies on Japanese\(^{(15)}\) and North American\(^{(16)}\) premenopausal women appear to be in agreement with the data provided by Gross et al. on Mexican-American women,\(^{(14)}\) two other studies in French\(^{(17)}\) and Swiss\(^{(18)}\) populations recently failed to detect a significant segregation of FokI polymorphism with BMD values at multiple skeletal sites. Larger studies performed in other populations are necessary before any conclusion can be drawn regarding the association between FokI polymorphism and bone mass.

In this study, we examined the relationship of FokI restriction fragment length polymorphism with BMD and with the occurrence of osteoporotic fractures, in a group of 400 postmenopausal women of Italian descent stratified for BMD into normal, osteopenic, and osteoporotic groups.

### MATERIALS AND METHODS

#### Subjects

Patients eligible for the study were selected among women who attended in 1995–1996 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. Women with a history of bone disease other than primary osteoporosis or which had used bone active drugs or drugs that could potentially affect bone metabolism were excluded from analysis. Subjects were also excluded if their parents or grandparents were not of Italian descent. Blood samples from 400 subjects were available for DNA isolation. The age range of the studied women was 45–72 years, with a mean (± SEM) age of 57.7 ± 7.6 years. On the basis of BMD measurements and according to World Health Organization criteria \(^{(19)}\) 41% of the 400 subjects had osteoporosis, 29% had osteopenia, and 30% were normal. General characteristics of the population are presented in Table 1.

#### Bone densitometry and fracture assessment

Lumbar BMD (L2–L4), measured by dual-energy X-ray absorptiometry (DXA; Hologic QDR 1000/W, Waltham, MA, U.S.A.) was available for all the 400 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. DXA BMD scans at the upper femur were available for 220 of the 400 studied women, 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 two centers were previously performed.\(^{(19)}\) A correction factor was not considered necessary.

The documentation of vertebral fractures was based entirely upon spine radiographs, following the method of McCloskey,\(^{(20)}\) with a 3 SD cut-off value for each vertebral level. Nonspine fractures were identified by self-report during the recruitment interview. All self-reported nonvertebral fractures were successively confirmed by the collection of respective hospital discharge summary, listing the fracture as discharge diagnosis, and/or by retrospective examination of the correspondent radiological film. Only nonvio-to...
lent fractures were further evaluated for the presence of osteophy-
tosis (SPO) and facet joint osteoarthritis (FOA) according to, respectively, the methods of Orwoll(21) and Masud.(22)

Genotyping

Genomic DNA was isolated from EDTA blood samples by a standard phenol-chloroform extraction procedure. The 265-bp fragment of genomic DNA containing the polymorphic portion of exon 2 was amplified by polymerase chain reaction, as described by Gross et al.(14) Polymerase chain reaction products were digested with \textit{Fok} \textsubscript{I} restriction endonuclease (New England Biolabs, Beverly, MA, USA.) at 37°C for 4 h and then electrophoresed through a 3% low melting point agarose gel, containing ethidium bromide. The presence of the restriction site, that generates two fragments of 196 bp and 69 bp, was indicated with \textit{f}, while its absence, resulting in a single uncut 265 bp fragment, was indicated with \textit{F}. Subjects were scored as \textit{ff} homozygotes, \textit{Ff} heterozygotes, and \textit{FF} homozygotes according to the digestion pattern.

All the subjects had been previously genotyped for \textit{Apa} \textsubscript{I} and \textit{Taq} \textsubscript{I} polymorphisms at the 3' end of the VDR gene.(9)

Statistical analysis

Data were evaluated by analysis of variance (ANOVA) and analysis of covariance (ANCOVA), with Fisher’s protected least significant difference (LSD) post hoc test, and presented as means ± SEM. \(p < 0.05\) was accepted as the value of significance. The following covariates were considered for the ANCOVA analysis: age, weight, years since menopause (YSM), calcium intake, and smoking status. The frequency distribution of genotypes in osteoporotic, osteopenic, and normal groups were compared using cross-tabulation and standard Chi-squared tests. The latter was also used to compare observed genotypes frequency with those expected under Hardy–Weinberg equilibrium.(23) Odds ratios (with 95% confidence intervals) were calculated by logistic regression analysis to estimate the relative risk of osteoporotic fracture. All statistical analyses were performed by using Statgraphics (Manugistic Inc., Rockville, MA, U.S.A.) and Statistica 5.1 (Statsoft, Inc., Tulsa, OK, U.S.A.).

Given the different sample sizes \((n = 400)\) for lumbar spine BMD and \((n = 220)\) for femoral neck BMD) and an alpha level of 0.05, this study had 90% statistical power to detect BMD differences between homozygous groups of about 6% at the lumbar spine and 8.5% at the femoral neck.

RESULTS

The prevalence of each genotype in the study population was 41% FF, 45% Ff, and 14% ff. The distribution of genotypes was very similar to what previously described in Mexican-American,(14) Japanese,(15) white North American,(16) French,(17) and Swiss(18) populations while it was significantly different from that described in a black North American population.(16)

Clinical characteristics of patients, in relation to \textit{Fok} \textsubscript{I} genotype are presented in Table 2. There were no significant differences in age, weight, height, YSM, dietary calcium intake, smoking status, SPO, and FOA scores distribution across genotypes. As shown in Table 3, BMD analysis after adjustments for potential confounding factors (i.e., age, YSM, height, weight, and Ca intake) revealed a relationship between lumbar BMD and \textit{Fok} \textsubscript{I} genotypes, approaching statistical significance \((p = 0.06, \text{ANCOVA})\). Particularly, women with FF genotype showed 6% higher mean spinal BMD values than those with ff genotype \((p = 0.06, \text{LSD test})\). A similar trend was also observed for femoral BMD, with differences ranging from 8% (Ward’s triangle) to 2% (neck) between FF and ff homozygotes (Table 3). However, none of these differences reached statistical significance. We next determined whether the role of \textit{Fok} \textsubscript{I} genotypes on lumbar BMD was influenced by the effect of menopause. Figure 1 shows the results of analyzing lumbar BMD by genotype according to three menopausal age

\begin{table}[h]
\centering
\caption{General Characteristics of Women in Relation to \textit{Fok} \textsubscript{I} Genotype}
\begin{tabular}{lcccc}
\hline
Characteristics & \textit{FF} & \textit{Ff} & \textit{ff} & \textit{p} \\
\hline
Subjects \((n)\) & 159 & 186 & 55 & \\
Age (years) & 57.7 ± 0.6 & 57.4 ± 0.6 & 58.8 ± 1.1 & 0.5* \\
Years since menopause & 9.8 ± 0.7 & 9.4 ± 0.6 & 10.8 ± 1.2 & 0.6* \\
Height (cm) & 161.2 ± 0.6 & 160.8 ± 0.5 & 161.1 ± 1.0 & 0.8* \\
Weight (kg) & 61.9 ± 0.7 & 62.9 ± 0.9 & 64.8 ± 1.9 & 0.2* \\
Calcium intake (mg/day) & 558 ± 151 & 593 ± 134 & 566 ± 194 & 0.8* \\
Smokers/nonsmokers \((n)\) & 37/122 & 44/142 & 16/39 & 0.7† \\
SPO score 0/1/2/3 \((n)\) & 70/67/18/4 & 79/77/26/4 & 25/16/9/5 & 0.2† \\
FOA score 0/1/2/3 \((n)\) & 79/57/18/5 & 91/68/19/8 & 21/26/5/3 & 0.7† \\
\hline
\end{tabular}
\end{table}

Values are expressed as means ± SEM.

* \(p\) value from ANOVA.

† \(p\) value from Chi-squared test.

SPO, spinal osteophytosis; FOA, facet joint osteoarthritis.
groups (YSM < 5, n = 155; 5 < YSM < 10, n = 125; and YSM > 10, n = 130). According to the number and distribution of genotypes in the three groups, there was a 90% power to detect a 8.5% lumbar spine BMD difference between alternate homozygotes. We observed a statistically significant segregation of FokI genotypes with lumbar BMD only in women in the first 5 years of menopause (p = 0.04, ANCOVA), with a difference of 9% between women <5 YSM grouped in FF and ff genotypes (0.950 ± 0.01 g/cm² vs. 0.867 ± 0.02 g/cm², FF vs. ff, p = 0.009, Fisher’s LSD test). Such a difference importantly decreased when we considered women >5 YSM (p = 0.06, ANCOVA) and was not yet detectable in women >10 YSM (p = 0.35, ANCOVA). When women were stratified by age, instead of by menopausal age, a statistically significant effect (p = 0.04, ANCOVA) was observed in the group of women less than 60 years of age (Fig. 2). Conversely, no statistically significant effect was observed after the age of 60 (Fig. 2). The analysis of Z scores values instead of adjusted BMD values did not change the above results.

Genotype determinations for osteoporotic, osteopenic, and normal groups are summarized in Table 4. We observed a statistically significant 2-fold increased prevalence of ff genotype in osteoporotic than nonosteoporotic patients (χ² = 4.7, d.f. = 1, p = 0.09).

A total of 55 nonspine fractures were observed in 50 of the 400 recruited women, including 16 wrist fractures, 14 hip fractures, 11 rib fractures, and 9 foot fractures. After spine radiograph examination, 68 of the 400 women showed a vertebral fracture. The low BMD associated genotype “ff” was significantly over-represented in patients with osteoporotic vertebral fracture (χ² = 7.4, d.f. = 1, p = 0.007, Chi-squared test), equivalent to a relative risk of 2.58 (95% confidence intervals 1.36–4.91) in “ff” homozygotes and of 1.59 (95% confidence intervals 0.91–2.78) in individuals who carry the “f” allele (Table 5). Conversely, there was no significantly different distribution of genotypes in subjects with a nonspine fracture, as compared with those without fractures (data not shown).

The joint distribution of FokI and, respectively, TaqI or ApaI genotypes is shown in Table 6. Linkage disequilibrium analysis revealed a significant relationship between FokI polymorphism and TaqI polymorphisms (χ² = 12.5, p = 0.01, Chi-squared test). In particular, a higher than expected number of FF women were TT homozygotes and a higher than expected number of ff women were tt homozygotes. On the contrary, the statistical test for independence of FokI and ApaI genotypes was not significant (χ² = 4.9, p = 0.30, Chi-squared test). After correcting for

---

### Table 3. Femoral and Lumbar BMD Values in Relation to FokI Genotype

<table>
<thead>
<tr>
<th>BMD (g/cm²)</th>
<th>FF</th>
<th>Ff</th>
<th>ff</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lumbar BMD (L₂–L₄)</td>
<td>0.863 ± 0.01 (159)</td>
<td>0.840 ± 0.01 (186)</td>
<td>0.812 ± 0.02 (55)</td>
<td>0.06</td>
</tr>
<tr>
<td>Femoral BMD (neck)</td>
<td>0.672 ± 0.01 (92)</td>
<td>0.667 ± 0.01 (93)</td>
<td>0.658 ± 0.02 (35)</td>
<td>0.5</td>
</tr>
<tr>
<td>Femoral BMD (Ward’s triangle)</td>
<td>0.505 ± 0.01 (92)</td>
<td>0.485 ± 0.01 (93)</td>
<td>0.460 ± 0.02 (35)</td>
<td>0.09</td>
</tr>
<tr>
<td>Femoral BMD (trochanter)</td>
<td>0.569 ± 0.01 (92)</td>
<td>0.556 ± 0.01 (93)</td>
<td>0.538 ± 0.02 (35)</td>
<td>0.1</td>
</tr>
<tr>
<td>Femoral BMD (intertrochanter)</td>
<td>0.940 ± 0.01 (92)</td>
<td>0.930 ± 0.01 (93)</td>
<td>0.938 ± 0.02 (35)</td>
<td>0.6</td>
</tr>
<tr>
<td>Femoral BMD (total)</td>
<td>0.766 ± 0.01 (92)</td>
<td>0.759 ± 0.01 (93)</td>
<td>0.742 ± 0.02 (35)</td>
<td>0.3</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SEM (n).  
* p value from analysis of covariance, adjusting BMD values for age, height, weight, years since menopause, and dietary calcium intake.
potential confounding factors, a statistical significant segregation of ApaI and TaqI genotypes with lumbar BMD was detected, with mean corrected BMD values significantly higher in women with aaTT genotype as compared with those with AAtt genotype (0.869 ± 0.01 g/cm² vs. 0.790 ± 0.01 g/cm², p = 0.01, Fisher’s LSD test). Analysis of the FokI polymorphism in combination with ApaI and TaqI polymorphisms did not alter these associations, with a difference in lumbar BMD of 7% between opposite homozygotes (0.868 ± 0.02 g/cm² vs. 0.808 ± 0.02 g/cm², aaTTff vs. AAttFF).

**DISCUSSION**

Vitamin D is a key hormone in bone metabolism and its function is required for normal mineralization of bone, absorption of calcium through the gut, control of calcium and phosphate homeostasis, and regulation of parathyroid hormone secretion. Given the centrality of this hormone in bone and calcium balance, the gene encoding its receptor, the VDR gene, has been recently postulated as a major genetic determinant of BMD and consequently of osteoporotic fracture risk. The possibility that polymorphisms in intron 8 (BsmI and TaqI) are associated with differences in BMD has received great attention. Since the first report showing a significant segregation of these polymorphisms with BMD values at multiple sites, studies reached divergent conclusions, some being confirmatory, others showing lack of segregation. These polymorphisms in intron 8 (BsmI and ApaI) and exon 9 (TaqI) do not change the amino acid sequence of the VDR protein and the causal molecular mechanism(s) to explain differences in bone mass and calcium homeostasis remain still uncertain. By contrast, the polymorphism detected by the restriction endonuclease FokI at the translation initiation site in exon 2 of the VDR gene results in a 3 amino acids difference between the protein encoded by the F and f alleles, providing a more obvious theoretical mechanism to affect VDR function and consequently bone and calcium homeostasis. In this regard, it has been recently demonstrated that the shorter form of the VDR (originated from the F allele), gives ~1.7-fold greater transcriptional activation in transfected HeLa cells than the longer form (originated from the f allele), suggesting the existence of a difference in the biological activity of the two VDR isoforms.

---

**Table 4. Distribution of FokI Genotypes in Osteoporotic, Osteopenic, and Nonosteoporotic Subjects**

<table>
<thead>
<tr>
<th>FokI genotype</th>
<th>Osteoporotic</th>
<th>Osteopenic</th>
<th>Nonosteoporotic</th>
<th>χ²</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FF</td>
<td>60 (37%)</td>
<td>46 (39%)</td>
<td>53 (38%)</td>
<td>1.83</td>
<td>0.40</td>
</tr>
<tr>
<td>Ff</td>
<td>73 (44%)</td>
<td>58 (50%)</td>
<td>55 (44%)</td>
<td>0.71</td>
<td>0.70</td>
</tr>
<tr>
<td>ff</td>
<td>31 (19%)</td>
<td>13 (11%)</td>
<td>11 (18%)</td>
<td>6.40</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Cross-tabulation test: χ² = 7.00, d.f. = 4, p = 0.13.

**Table 5. Distribution of Vertebral Fractures in Relation to FokI Genotype**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Vertebral fracture</th>
<th>Control</th>
<th>χ²</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FF</td>
<td>21 (31%)</td>
<td>138 (42%)</td>
<td>2.690</td>
<td>0.1</td>
</tr>
<tr>
<td>Ff</td>
<td>30 (44%)</td>
<td>156 (47%)</td>
<td>0.187</td>
<td>0.7</td>
</tr>
<tr>
<td>ff</td>
<td>17 (25%)</td>
<td>38 (11%)</td>
<td>8.743</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Cross-tabulation test: χ² = 9.26, d.f. = 2, p = 0.010.

**Table 6. Distribution of FokI and Respectively TaqI or ApaI Genotypes**

<table>
<thead>
<tr>
<th>FokI</th>
<th>FF</th>
<th>Ff</th>
<th>ff</th>
</tr>
</thead>
<tbody>
<tr>
<td>TaqI*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>67 (53)</td>
<td>48 (61)</td>
<td>16 (18)</td>
</tr>
<tr>
<td>Tt</td>
<td>71 (81)</td>
<td>110 (95)</td>
<td>26 (28)</td>
</tr>
<tr>
<td>tt</td>
<td>21 (25)</td>
<td>28 (30)</td>
<td>13 (9)</td>
</tr>
<tr>
<td>ApaI†</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aa</td>
<td>61 (64)</td>
<td>72 (71)</td>
<td>26 (22)</td>
</tr>
<tr>
<td>Aa</td>
<td>68 (71)</td>
<td>91 (87)</td>
<td>23 (26)</td>
</tr>
<tr>
<td>aa</td>
<td>30 (24)</td>
<td>23 (27)</td>
<td>6 (8)</td>
</tr>
</tbody>
</table>

Results are expressed as number of observed subjects and in parentheses the number expected under independence.

* p = 0.008, d.f. = 4, χ² = 13.9, Chi-squared test, FokI versus TaqI genotypes.
† p = 0.30, d.f. = 4, χ² = 4.9, Chi-squared test, FokI versus ApaI genotypes.

In our study population of 400 postmenopausal women, taken as a whole, we just observed a weak association of FokI polymorphism with lumbar BMD. The magnitude of difference in spinal bone mass between genotypes we described is lower than that originally reported by Gross et al. in a cohort of postmenopausal Mexican-American women. Possibly, differences in environmental influences as well as a different genetic background may have led to an enhanced FokI genotype effect in their population or may have diluted it in our population. Interestingly, after adjusting for covariates, we were able to detect a significant segregation of FokI allelic variant with BMD at the lumbar spine in early but not late postmenopause. The reason for this is not entirely clear. It is generally assumed that the genetic effect is mainly contributing to peak adult bone density but it is still not well understood whether and to
what extent age- and menopause-related changes in bone density are also genetically influenced.\(^{(126)}\) The strong interaction with age and particularly with YSM observed in this study suggests that differences in BMD between FokI genotypes are greatest before and immediately after menopause, with relative differences progressively declining afterward. Possibly the differences in BMD due to different FokI VDR genotypes may be progressively diluted by cumulative environmental influences, by the metabolic changes following the menopause, and/or by interactions with other genetic determinants of BMD acting with a different power at various ages in females. Larger sample size and prospective longitudinal follow-up studies are needed to definitely support a major role of FokI allelic variants on peak adult bone density.

Recently, three studies have looked specifically at relationships between FokI polymorphism and bone mass in premenopausal women. In a population of Japanese premenopausal women, data obtained supported a role of FokI polymorphism in determination of vertebral bone mass before menopause.\(^{(15)}\) A second study, conducted in a group of premenopausal American black and white women, showed a statistically significant difference in BMD among genotypes at the femoral neck but not at the lumbar spine.\(^{(16)}\) The latter data contrast with our observations in the 220 women who underwent a femoral DXA scan, where we observed a smaller and not significant difference in femoral bone mass, with a value approaching statistical significance only in femoral regions with the highest proportion of trabecular bone. This observation, together with the observed FokI genotype effect on lumbar BMD, is in agreement with the concept of genetic effect being more pronounced at sites with a predominant trabecular bone content.\(^{(2,3,27)}\) Moreover, due to the limited number of femoral DXA scans performed in the selected population, our study may be lacking sufficient statistical power to detect a small BMD difference at this site. In the third study, Eccleshall et al. failed to observe any association between FokI polymorphism and BMD at different skeletal sites.\(^{(17)}\)

However, in their sample of premenopausal French women, they found increased levels of bone resorption markers in ff women as compared with FF women, suggesting a possible role of this polymorphism on bone resorption.\(^{(17)}\) Taken together, these findings suggest that the effects of FokI allelic variants on BMD may vary depending on ethnicity, age, and menopausal status and may differ by skeletal site. Additionally, environmental factors, such as calcium intake, may also modulate the effects of FokI genotypes on bone mass, in a similar way as it has been previously demonstrated about VDR 3’-end region gene polymorphisms.\(^{(26-31)}\)

Interestingly, in our population we observed a statistically significant increase in the prevalence of the low bone density “ff” genotype in the osteoporotic group, in agreement with a possible segregation of this genotype with a higher risk of developing osteoporosis. The latter hypothesis is further supported by the 2- to 3-fold increased risk of a vertebral fracture in subjects bearing the “ff” allelic variant. More than 25% of vertebral fractures occurred in the homozygous ff genotype group. It is possible that the presence of the ff allelic variant, whether due to a decreased peak bone mass or to an increased bone turnover in the first 5–10 years of menopause, may confer an additional risk for osteoporotic vertebral fractures. Even though we have no data about bone turnover of the 400 postmenopausal women analyzed in this study, the recent study of Eccleshall et al. seems to confirm the possibility of an increased bone resorption rate in women with the homozygous ff genotype.\(^{(17)}\) A 5–11% prevalence of vertebral deformity has been recently described in European women aged 50–54 years.\(^{(32)}\) Given the presence of a significantly decreased lumbar BMD in ff women with respect to FF women, during the first 5 years of menopause, it is possible that a vertebral osteoporotic fracture occurs earlier in women bearing ff genotype. Unfortunately, we just assessed the presence of a vertebral fracture at the time of the recruitment by direct spinal X-ray examination, while we do not exactly know the age at which that fracture occurred. However, it is interesting to note that the mean ± SEM age of women with a vertebral fracture was significantly lower in those with ff genotype (63.5 ± 2.0) than in those with FF genotype (65.6 ± 1.5). By contrast, we did not observe any segregation of FokI genotypes with hip fractures, in agreement with a major VDR genetic effect at skeletal sites with a prevalent content of trabecular bone. Moreover, hip fractures typically occurs later in life, when the role of FokI polymorphism in BMD decreases and even disappears, at least in our population. Future studies in larger population samples of older women will attempt to obtain a convincing evidence of the role of FokI VDR polymorphism in hip fracture risk.

Bone mass, the major determinant of osteoporotic fracture risk, has been considered a quantitative polygenic trait.\(^{(33)}\) In this homogeneous population of Italian postmenopausal women, with a relatively low calcium intake, the allelic changes at both the 3’ end (ApaI, BsmI, and TaqI) and the translation initiation site in exon 2 (FokI) of the VDR gene locus account for a part of the heritable component of bone density at the lumbar spine. As it happens, in general in association studies, in which a polymorphism in a candidate gene is analyzed in unrelated affected and unaffected individuals from a population, this positive association implies nothing about whether the allele is necessary to confer osteoporotic phenotype and further implies nothing about any physical connection between the trait and such polymorphic marker. In fact, a positive association can arise for three reasons: the allele is effectively the cause of the disease; the allele does not cause the trait but is in linkage disequilibrium with the actual cause; and as an artifact of population admixture. In the present study, we can only exclude the latter cause, being all subjects of confirmed Italian descent. The possibility of linkage disequilibrium between VDR allelic variants and mutations in another bone related gene on chromosome 12, cannot be excluded. Moreover, other polymorphic genes as well as a myriad of environmental and metabolic influences may interact with the VDR-linked genetic potential, offering an explanation to the frequency of conflicting findings. Based
on current evidence, it further appears likely that the differences in bone mass among women grouped in different FokI genotypes, even though obtained earlier in life, rather than due to age-related bone loss, may confer an additional risk for osteoporotic vertebral fractures. Studies of the association of FokI genotypes with peak bone mass in younger individuals as well as longitudinal prospective studies on BMD and bone turnover rates will be necessary to clarify this issue.

ACKNOWLEDGMENTS

We are grateful to Dr. Carlo Gennari for the careful reading of the manuscript and for his continuing support and encouragement. We also thank Pasquale Imperiale for expert technical assistance in the statistical analyses.

REFERENCES


Address reprint requests to:
Maria Luisa Brandi, M.D., Ph.D.
Endocrine Unit
Department of Clinical Physiopathology
University of Florence
Viale Pieraccini 6
50139 Florence, Italy

Received in original form April 1, 1998; in revised form March 12, 1999; accepted April 13, 1999.