The effect of \textit{FokI} vitamin D receptor polymorphism on bone mineral density in Jordanian perimenopausal women

Raed M. Kanan
Department of Biotechnology and Genetic Engineering, Philadelphia University, Amman, Jordan

\textbf{CONTEXT:} Osteoporosis is a polygenic, multifactorial disease that is characterized by demineralization of bone, and thus presented with decreasing bone mineral mass. Vitamin D receptor (VDR) gene polymorphisms in the 3’-end region (as determined by the enzymes \textit{BsmI} and \textit{ApaI}) have been inconsistently associated with bone mineral mass. Another important VDR start codon polymorphism (as determined by the enzyme \textit{FokI}) has been found to be related to adult bone mineral density (BMD) in pre-and post-menopausal American women.

\textbf{AIMS:} This study aims to investigate the prevalence of the \textit{FokI} VDR gene polymorphism in Jordanian perimenopausal women and study its relationship with bone mineral density.

\textbf{MATERIALS AND METHODS:} DNA was isolated from 90 controls (Mean age = 50.41 \pm 1.29 y), and 120 patients with symptomatic vertebral fractures (Mean age = 49.14 \pm 3.19 y). Restriction Fragment Length Polymorphism (RFLP) analysis of \textit{FokI} was performed on DNA samples.

\textbf{STATISTICAL ANALYSIS:} Data was analyzed using SPSS v19 and Microsoft Excel 2007.

\textbf{RESULTS:} The results showed that in controls, the FF (−0.70 \pm 0.51) genotype is associated with high lumbar spine BMD Z-score as compared to Ff (−1.25 \pm 0.26) and ff (−1.66 \pm 0.47) genotypes ($P = 0.0095$). In patients, the ff genotype was associated with lower lumbar spine BMD in T-score (−2.31 \pm 0.17) and Z-score (−1.56 \pm 0.09) genotypes ($P = 0.031$). No significant association was seen in the femoral neck BMD.

\textbf{CONCLUSION:} \textit{FokI} polymorphism may be associated with low BMD in our studied population; however, further studies including other polymorphisms and large sample number are needed.

\textbf{Key words:} dbSNP rs10735810, Jordan, osteoporosis, vitamin D receptor

Osteoporotic bone is characterized by a skeletal defect that leads to deterioration of bone micro-architecture and decrease in bone mineral density (BMD). It is now well-established that familial and genetic factors are determinant of the peak bone mass. Studies in twin models have shown a strong genetic effect on the bone mass at forearm, lumbar spine, and femoral neck.\textsuperscript{[1]} Heredity has been shown to have an important role in determining the peak bone mass, with up to 80\% of the variance in BMD being attributed to genetic factors.\textsuperscript{[2-4]} In view of the important role of 1,25-dihydroxyvitamin D3 in calcium homeostasis, an initial particular emphasis has been placed on establishing a possible association between vitamin D receptor (VDR) gene polymorphisms and BMD.\textsuperscript{[5-8]} Interest in the area of the genetics of bone density and osteoporosis was proposed initially by an inspiring but controversial study of the relationship between common polymorphisms in the VDR gene (\textit{BsmI}, \textit{ApaI} and \textit{TaqI}) and BMD.\textsuperscript{[9]} The association between these VDR gene polymorphisms and BMD remains controversial since other studies showed no significant association and in some cases the opposite effect.\textsuperscript{[9,10]} Two meta-analyses of the largest and most relevant association studies have reported a weak relationship between VDR gene polymorphisms and BMD.\textsuperscript{[11,12]}
Since the above mentioned polymorphisms have no known effect on the function of the VDR gene, the attention was turned into the functional mutation located at exon 2 of the VDR gene, which can be detected by the *FokI* restriction endonuclease enzyme. This polymorphism is a transition from T to C (ATG to ACG) at the first initiation codon (ATG), and is located three codons proximal to a second start site downstream. The presence of *FokI* restriction site (denoted f) results in the expression of the longer M1 isoform of VDR protein. However, the shorter M4 isoform (424-residue) is produced in the absence of *FokI* site (denoted F). In transient transfection assays, employing vitamin D response element-linked reporter gene in a mammalian cell line, M4 isoform (F) was found to have higher transcriptional activity than the M1 form of VDR (f). This polymorphism has been firstly associated with variation in BMD and rates of bone loss in a group of postmenopausal Mexican-American women, where subjects carrying the ff genotype have a 12.8% lower lumbar BMD than the wild type subjects (FF). Following this study, two studies on Japanese and North American premenopausal women appear to be in agreement with it. However, two other studies in French and Swiss populations failed to detect a significant segregation of *FokI* polymorphism with BMD values at multiple skeletal sites.

It has been noted that the effect on *FokI* VDR polymorphism on bone mass could be indirect, since the FF genotype appears to facilitate a down-regulation of both vitamin D and parathyroid hormone (PTH). Indeed, PTH levels are reported to be higher in individuals carrying the ff genotype. It is also found that the individuals with the FF genotype have better insulin sensitivity, lower fat mass, and lower lean mass, than others. It is therefore not surprising that lower bone density has been associated with the ff genotype in some studies involving French, British, Czech, and Korean subjects.

In Arabs, studies on the association of the VDR genotypes and BMD are rare. In fact, out of 22 Arab countries only Jordan, Lebanon, Emirates, and Saudi Arabia have investigated the association between VDR polymorphism and BMD. Of these studies, the association of *FokI* with low BMD was studied only in Jordanian post-menopausal women. Our present study is the first to investigate the association between VDR gene *FokI* polymorphism and bone density in Jordanian perimenopausal women who had a wide range of bone density measurements.

### Materials and Methods

#### Subjects

The study was carried out on 120 Jordanian women referred to bone clinics with a mean age of 49 (range 41-52 years), with or without symptomatic vertebral fractures, and with a wide range of BMD from osteopenic to osteoporotic and 90 control subjects (mean age 50, range 48-52 years). The subjects were originally recruited but not used in the previous cohort study. All subjects gave their formal consent and were evaluated to exclude secondary causes of osteoporosis by clinical history, diet habit, hormonal and biochemical tests. Perimenopausal status was assessed by clinical history. Body mass index (BMI) calculations were determined according to the formula (BMI = Weight [Kg]/[Height (m) × Height (m)]). Data was collected in the period extending between 2008 and 2009.

#### Bone density measurement

The anterior and posterior measurement of BMD of the lumbar spine (L2-L4) was performed using dual energy X-ray absorptiometry (Hologic 2000W; QDR, Waltham, MA). The coefficient of variation for the repeated measurements *in vivo* with this system at the lumbar spine and the femur is 1-1.5%. BMD measurements were obtained as a real density in g/cm², and were also expressed as a Z-score (number of standard deviations [SDs] above or below the age-related mean value) and T-score (number of [SDs] from the mean value for young adults).

#### Genotyping

The *FokI* VDR gene polymorphism (dbSNP rs10735810) was genotyped and digested as described before (22). Briefly, samples were isolated from peripheral blood using the Wizard DNA Purification Kit (Promega, Madison, WI), then polymerase chain reaction (PCR) was performed and PCR products were digested with the *FokI* enzyme. The digested pattern was scored as
FF homozygotes for absence, Ff heterozygotes, or ff homozygotes for the presence of the polymorphism.

**Statistical analysis**

Results were presented as mean ± (SD). Data were analyzed by using appropriate statistical packages (SPSS Statistics Desktop for Windows, V19.0 and Microsoft Office Excel 2007). Significant differences in anthropometric measurements and BMD were determined using Student’s unpaired t-test, ANOVA and Mann-Whitney test.

**Results**

Anthropometric measurements showed no differences between controls and patients with symptomatic vertebral fractures in the studied population with similar age, weight, height, and BMI. Analyses of the spinal BMD (SNBMD) showed high significant differences at the T-score (P<0.001) between controls (−0.06 ± 1.03) and patients (−2.26 ± 0.53). Some significance was also observed between controls and patients at total SNBMD and Z-score (P < 0.05). No significance differences were seen at the femoral neck BMD, T-score or Z-score between controls and patients in the studied group (P > 0.05) [Table 1].

Genotype frequencies were following Hardy-Weinberg equilibrium and have shown no significant differences between controls and patients in the three genotypes (P > 0.05). The genotype frequencies in controls were 31.67% for FF, 53.33% for Ff, and 15% for ff. Similar frequencies were seen in patients with a distribution of 33.33% for FF, 52% for Ff, and 14.67% for ff [Table 1].

Table 2 shows that the genotypes distribution in controls at lumbar spine BMD was higher in the FF (−0.70 ± 0.51) genotype when adjusted to Z-score, compared to Ff (−1.25 ± 0.26) and ff (−1.66 ± 0.47) genotypes (P<0.01).

In patients, the ff genotype was associated with lower lumbar spine BMD after adjustment to T-score (−2.31 ± 0.17) and Z-score (−1.56 ± 0.09). The Ff genotype was associated with intermediate BMD in patients, when adjusted to T-score (−1.95 ± 0.24) and Z-score (−1.18 ± 0.01), with P < 0.05 against FF and P < 0.01 against ff. The wild genotype FF was associated with high BMD in patients, when adjusted to T-score (−1.50 ± 0.53) and Z-score (1.01 ± 0.43) against Ff (P < 0.05) and against ff (P < 0.001) [Figures 1 and 2].

The association with femoral neck BMD was insignificant in both controls and patients (P > 0.05) using ANOVA (student’s t-test) [Table 3].

**Discussion**

The prevalence of the start codon polymorphism (SCP) in our subjects was similar to previous study in Jordanian (post-menopausal women)[25] and

---

**Table 1: The distribution of anthropometric measurements and spine, femoral, T-and Z-score BMD in controls and patients. Results are expressed as means±SD**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Controls (n=90)</th>
<th>Patients (n=120)</th>
<th>P value (t-test, ANOVA, MW, χ²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>50.41±1.29</td>
<td>49.14±3.19</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.54±0.08</td>
<td>1.55±0.06</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>79.89±13.01</td>
<td>85.18±16.04</td>
<td>=0.059</td>
</tr>
<tr>
<td>Mean BMI (kg/m²)</td>
<td>33.7±2.03</td>
<td>35.4±4.0</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Spine BMD (g/cm²)</td>
<td>1.15±0.15</td>
<td>0.90±0.14</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Spine BMD (T-score)</td>
<td>−0.06±0.03</td>
<td>−2.26±0.53</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Spine BMD (Z-score)</td>
<td>−0.58±1.16</td>
<td>−1.58±0.76</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Femoral BMD (g/cm²)</td>
<td>0.95±0.15</td>
<td>0.89±0.12</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Femoral BMD (T-score)</td>
<td>−0.46±1.13</td>
<td>−0.64±1.53</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Femoral BMD (Z-score)</td>
<td>−0.30±1.11</td>
<td>−0.25±0.77</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>FF (count, frequency)</td>
<td>29, 31.67%</td>
<td>40, 33.33%</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Ff (count, frequency)</td>
<td>48, 53.33%</td>
<td>62, 52%</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>ff (count, frequency)</td>
<td>13, 15%</td>
<td>18, 14.67%</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

---

**Table 2: The distribution of FokI genotypes at the spinal BMD including T-and Z-scores in controls and patients**

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Controls (n=90)</th>
<th>Patients (n=120)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Spinal BMD (g/cm²)</td>
<td>T-score (g/cm²)</td>
</tr>
<tr>
<td>FF</td>
<td>1.20±0.23</td>
<td>−0.08±1.15</td>
</tr>
<tr>
<td>Ff</td>
<td>1.15±0.14</td>
<td>−1.00±0.19</td>
</tr>
<tr>
<td>ff</td>
<td>1.06±0.17</td>
<td>−1.04±0.60</td>
</tr>
</tbody>
</table>

Results are expressed as means±SD and student t-test was used to calculate the P value. *P<0.01 versus Ff and ff, †P<0.05 versus Ff and P<0.001 versus Ff, ‡P<0.001 versus Ff; BMD: Bone mineral density
The genotype frequencies reported for French, Caucasian-Americans, and Mexican-American women were in close range (37-40% FF, 44-48% Ff, and 15-18% ff). However, our results were markedly different from African-American women (65% FF and 4% ff). The present study is the first of its kind on perimenopausal Jordanian women with a wide range of BMD. It confirms our previous report on Jordanian post-menopausal women and some of the other reports on healthy post-menopausal and premenopausal women showing association between VDR FokI polymorphism and bone density.

The present study is the first of its kind on perimenopausal Jordanian women with a wide range of BMD. It confirms our previous report on Jordanian post-menopausal women and some of the other reports on healthy post-menopausal and premenopausal women showing association between VDR FokI polymorphism and bone density. However, our results were markedly different from African-American women (65% FF and 4% ff). The present study is the first of its kind on perimenopausal Jordanian women with a wide range of BMD. It confirms our previous report on Jordanian post-menopausal women and some of the other reports on healthy post-menopausal and premenopausal women showing association between VDR FokI polymorphism and bone density.

Similar to earlier studies on Mexican-American and Japanese women, the FF genotype was associated with higher spine BMD compared to Ff and ff genotypes, whereas the ff genotype was associated with lowest bone density. The influence on spinal and femoral BMD was evident in some earlier studies, while either femoral or spinal BMD alone was affected in others.

There was no association between FokI genotypes and absolute BMD in perimenopausal women in our study. One possible explanation may be that perimenopausal women have other underlying factors that mask a small but significant contribution of the VDR gene to the overall peak BMD. In view of the wide range of heritable factors involved in the development and maintenance of bone density, it is not altogether surprising that a given genetic component has a relatively minor but significant effect. Therefore, the majority of the patients with symptomatic vertebral fractures could have adverse inherited factors that are more dominant in reducing BMD than VDR FokI genotype. Indeed, some studies have identified a range of genetic factors that influence BMD.

Another possible explanation for discordant effect of the three genotypes on BMD compared with the published data could be that dietary calcium intake modulates the phenotypic effect of VDR FokI genotype. In fact, it was previously suggested that dietary calcium intake could also account for variability in the association of SCP genotype with BMD in different populations.

Moreover, pedigree analysis has shown that a major gene accounts for 30-50% of the genetic variation in BMD, thus, VDR FokI polymorphism could have a small influence on BMD. In fact, a recent review explained that although genome wide association studies have
identified nine major polymorphisms associated with low BMD (not including VDR polymorphisms), some polymorphisms could be missed because of their true small contribution to the studied effect (BMD) and small sample size of individuals with affected alleles.

Although recent reports have found no association between VDR polymorphisms and BMD, the effect has not been negated by other reports. On the contrary, a very recent study on Turkish population (which is in close ethnic and geographical proximity to our population) has shown association of the FokI polymorphism with BMD.

**Conclusion**

Although our study is limited in sample size, it sheds some lights on the effect of FokI polymorphism on our studied population.

**Acknowledgment**

The author would like to express his gratitude for the generous grant provided by Philadelphia University, Jordan. The author is also thankful to the contribution of Zein tahboub, Rabab Abu Layla, and Duha Abdulrahman.

**References**

22. Kanam RM, Varanasi SS, Francis RM, Parker L, Datta HK.


Source of Support: A generous grant provided by Philadelphia University, Jordan. Conflict of Interest: None declared.