

Vitamin D receptor alleles and bone mineral density in a normal premenopausal Brazilian female population

M. Lazaretti-Castro¹,
M.A. Duarte-de-Oliveira²,
E.M.K. Russo² and
J.G.H. Vieira^{1,2}

¹Disciplina de Endocrinologia, Universidade Federal de São Paulo, 04034-970 São Paulo, SP, Brasil

²Seção de Biologia Molecular, Laboratório Fleury, 01333-910 São Paulo, SP, Brasil

Abstract

Correspondence

J.G.H. Vieira
Disciplina de Endocrinologia
EPM/UNIFESP
Caixa Postal 20266
04034-970 São Paulo, SP
Brasil

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Studies on the association between vitamin D receptor (VDR) polymorphism and bone mineral density (BMD) in different populations have produced conflicting results probably due to ethnic differences in the populations studied. The Brazilian population is characterized by a very broad genetic background and a high degree of miscegenation. Of an initial group of 164, we studied 127 women from the city of São Paulo, aged 20 to 47 years (median, 31 years), with normal menses, a normal diet and no history of diseases or use of any medication that could alter BMD. VDR genotype was assessed by PCR amplification followed by *BsmI* digestion of DNA isolated from peripheral leukocytes. BMD was measured using dual energy X-ray absorptiometry (Lunar DPX) at the lumbar site (L2-L4) and femoral neck. Most of the women (77.6%) were considered to be of predominantly European ancestry (20.6% of them reported also native American ancestry), 12.8% were of African-Brazilian ancestry and 9.6% of Asian ancestry, 41.0% (52) were classified as bb, 48.8% (62) as Bb and 10.2% (13) as BB. The BB, Bb and bb groups did not differ in age, height, weight, body mass index or age at menarche. Lumbar spine BMD was significantly higher in the bb group ($1.22 \pm 0.16 \text{ g/cm}^2$) than in the BB group (1.08 ± 0.14 ; $P < 0.05$), and the Bb group presented an intermediate value (1.17 ± 0.15). Femoral neck BMD was higher in the bb group ($0.99 \pm 0.11 \text{ g/cm}^2$) compared to Bb (0.93 ± 0.12) and BB (0.90 ± 0.09) ($P < 0.05$). These data indicate that there is a significant correlation between the VDR *BsmI* genotype and BMD in healthy Brazilian premenopausal females.

Key words

- Vitamin D receptor genotype
 - Bone mineral density
 - Brazilian population
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Low bone mass is one of the main reasons for the high incidence of fractures that occur in older persons. The worldwide aging of the population and the serious consequences of fracture in old people have turned osteoporosis a public health top priority in developed countries and a potential addi-

tional burden to the health systems in developing ones. No treatment is universally effective in reversing bone loss, and the existing therapeutic alternatives, besides being usually expensive, show positive effects only on a long-term basis. Prevention seems to be the most cost-effective and reliable measure

available. Together with the application of general measures, information about segments of the population more prone to osteoporosis would be extremely useful for more rigorous measures of prevention, ideally starting at young ages. Peak bone mass, one of the major determinants of future bone density, has been shown to be strongly inherited (1,2). Recently, Morrison et al. (3,4) showed that polymorphism of the vitamin D receptor (VDR) gene could be a marker of this inheritance and that genotyping may provide a clue to predisposition to low bone mass.

Since the pioneering studies of Morrison et al. (3,4) several similar studies have been developed in different countries encompassing populations of diverse genetic background (5-10). These findings were extremely controversial, since in some groups the data reported by Morrison et al. (3,4) were confirmed (5,6,8,9) while in others no correlation between VDR genotype and bone mass was found (7,10). All of these studies were well conducted, and the main difference between them is related to the genetic and environmental background of the populations studied. Moreover, there are some findings that are difficult to explain only on a genetic background basis, such as the discrepant results described for Asian populations (9,10), the positive correlation described for white and black North American populations (5), and the absence of correlation in a Northern European population (7).

A very broad genetic background and a high degree of miscegenation characterize the Brazilian population, a phenomenon especially evident in the city of São Paulo. European (mainly Mediterranean), native American, African and Asian populations are the major ethnic groups represented in the city, with a significant degree of miscegenation. In order to study the distribution of VDR genotypes and its possible correlation with bone mineral density (BMD), we studied a group of 127 normal premenopausal

adult females from the city of São Paulo. This population was derived from an initial group of 164 employees of the Laboratório Fleury. The women, aged 20 to 47 years (median, 31 years), had normal menses, a normal diet and no history of fractures, diseases or use of any medication that could alter BMD. All subjects responded to an extensive questionnaire that included questions on family history of osteoporosis or fractures, diet, exercise and a self-reference on ethnic background. The answers provided the basis for the selection of the group of 127 women that was further studied. Based on the responses, 77.6% of the women were considered to be of predominantly European ancestry, and 20.6% of them reported also native American ancestry; 12.8% classified themselves as African-Brazilians and 9.6% as Asians, the latter reporting Japanese ancestry. Informed consent was obtained from all participants.

VDR genotype was assessed by PCR amplification followed by digestion with the restriction enzyme *BsmI* of DNA isolated from peripheral blood leukocytes. Primer sequences were derived from the study of Morrison et al. (3). After digestion, the products were submitted to gel electrophoresis and classified as BB when no digestion took place (800-bp band), as bb when complete digestion occurred (650- and 150-bp bands) and as Bb when partial digestion occurred (800-, 650- and 150-bp bands). BMD was measured by dual energy X-ray absorptiometry with a Lunar DPX bone densitometer at the lumbar site (L2-L4) and at the femoral neck. The unpaired *t*-test was used to compare the BMD results.

Genotyping showed that 41.0% were classified as bb, 48.8% as Bb and 10.2% as BB. The B allele was found in only 16.6% of the Asian group, in contrast to 60.8% for Europeans and 74.9% for African-Brazilians. The BB, Bb and bb groups did not differ in age, height, weight, body mass index or age at menarche. They also did not show any sig-

nificant difference in calcium intake, fracture index or family history of osteoporosis. Lumbar spine BMD was significantly higher in the bb group (1.22 ± 0.16 g/cm²; mean \pm SD) than in the BB group (1.08 ± 0.14 , $P < 0.05$; Figure 1A), and the Bb group presented an intermediate value (1.17 ± 0.15). Femoral neck BMD was higher in the bb group (0.99 ± 0.11 g/cm²) compared to Bb (0.93 ± 0.12) and BB (0.90 ± 0.09) ($P < 0.05$; Figure 1B).

Our data indicate a significant correlation between the VDR *BsmI* genotype and BMD in healthy Brazilian premenopausal females. The heterogeneous background of the population studied indicates that race probably had no strong impact on our findings. Peak bone mass is one of the major determinants of bone mass in older ages, which has a major impact on the incidence of osteoporosis. The controversial findings described in the recent literature may have alternative explanations. One is that osteoporosis, like hypertension and other highly prevalent diseases, is a polygenic disease and by genotyping VDR we are looking at a single marker that could be in linkage disequilibrium with a nearby locus related to peak bone mass. Another is the effect of the environment where the studied population lives, with strong influences such as sun exposure and calcium content of the regular diet. In conclusion, our findings that the

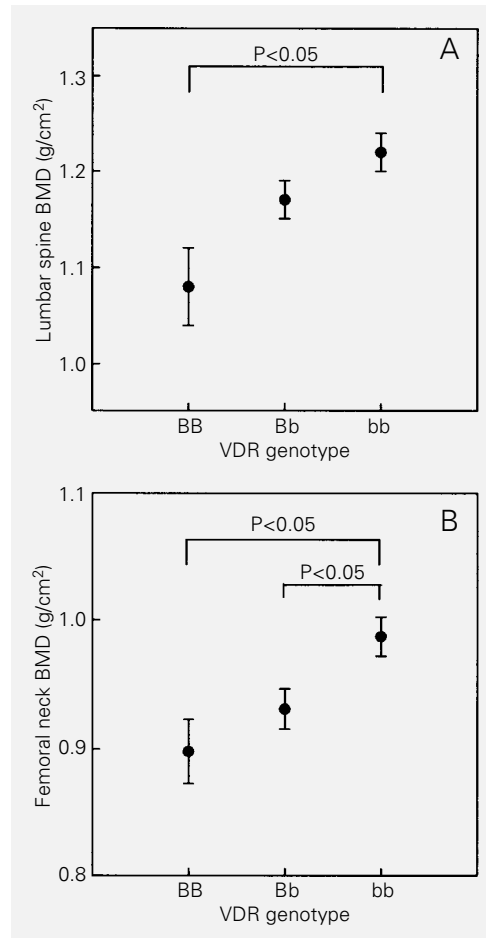


Figure 1 - Allele effect of the vitamin D receptor (VDR) genotype on lumbar spine (A) and femoral neck (B) bone mineral density (BMD) in premenopausal Brazilian women. Data are reported as mean \pm SEM for 127 women (13 BB, 62 Bb and 52 bb). Unpaired t-test was used for statistical analysis.

VDR genotype is correlated with peak bone mass in a Brazilian female population of very heterogeneous genetic background add new information to this highly controversial subject.

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