Vitamin D Receptor Alleles Do Not Correlate With Bone Mineral Density in Premenopausal Caucasian Women From the Southeastern United States

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Genetic factors are important in determining peak bone density. Recent studies indicate that polymorphisms of the vitamin D receptor (VDR) may account for much of the genetic contribution to bone density, and VDR genotype may be useful to predict the risk of developing osteoporosis. However, the association between VDR genotype and bone mineral density (BMD) has not been observed in all populations. We determined VDR genotype in 69 healthy premenopausal Caucasion women from the southeastern United States and measured BMD at the lumbar spine (anterior-posterior [AP] and lateral views) and proximal femur. We found no association between VDR genotype and BMD at any site. Our results indicate that in this population, VDR genotype does not predict peak bone density and should not be used to predict the risk of developing osteoporosis.

GENETIC FACTORS play an important role in determining bone mineral density (BMD), and much of the genetic contribution to differences in BMD may be due to differential accrual of bone. Understanding the genetic factors that influence peak BMD will lead to important insights into osteoporosis, and a genetic test that could determine whether an individual is at risk for osteoporosis would be useful clinically. Data reported by Morrison et al[1] indicate that vitamin D receptor (VDR) polymorphisms account for most of the genetic contributions to bone density. Indeed, individuals who were homozygous for the b allele of the VDR had 15% greater BMD at the lumbar spine than those who were homozygous for the B allele.[1] This difference in BMD is clinically significant and corresponds to a 10-year difference in age at which BMD would be less than 2 standard deviations of the mean BMD in young normal women. Several investigators have sought to repeat these findings by examining correlations between VDR polymorphisms and measurements of BMD at the lumbar spine and hip. Some studies have found a correlation between VDR polymorphisms and BMD.[2-4] However, others have not found a correlation.[5-8] We sought to determine if there is a clinically relevant correlation between VDR alleles and peak BMD in our population of healthy Caucasian women from the southeastern United States. Since recent studies by Myers et al[9] demonstrate that lateral imaging of the lumber spine is a more sensitive indicator of vertebral compressive strength than anterior-posterior (AP) scanning, we examined BMD of the lumber spine by lateral imaging and AP imaging. Our results indicate that in this population, there is no clinically relevant correlation between VDR polymorphisms and BMD.

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SUBJECTS AND METHODS

Subjects

We recruited healthy 18- to 45-year-old premenopausal Caucasian women by advertising in local newspapers. Before enrollment into the study, a detailed medical history was obtained from prospective study subjects. We excluded women who had conditions known to affect BMD or to cause artifactual readings of BMD. Such conditions include the presence of spinal deformity, diseases known to affect BMD, use of drugs known to affect BMD, intake of high doses of calcium or vitamin D, irregular menses, pregnancy, lactation, or a history of pregnancy or lactation within 1 year before the study. We did not exclude women taking oral contraceptives. All study subjects provided written informed consent, and the study was approved by the Duke University Medical Center Institutional Review Board.

BMD Measurement

BMD was measured by dual-energy x-ray absorptiometry using a Hologic QDR 2000 device (Hologic, Waltham, MA). All measurements were made on a single instrument, which was calibrated daily. BMD measurements were made of the AP view of the lumber spine (L1 to L4), the lateral view of the lumbar spine (L2 to L4), and the proximal right femur (total hip, neck, trochanter, intertrochanteric region, and Ward's triangle). Height and weight were recorded for all study subjects.

DNA Analysis

DNA was extracted from whole blood using standard methods.[10] Polymerase chain reaction (PCR) primers that flank the previously determined polymorphic *BsmI*/*BSa*MI restriction site were used to amplify DNA from each individual. The primer sequences are as follows: forward 5′-CAA CCA AGA CTA CAA GTA CCG CGT CAG TGA-3′ and reverse 5′-AAC CAG CGG GAA GAG GTC AAG GG-3′. Sixty nanograms of genomic DNA was amplified using a Perkin-Elmer (Norwalk, CT) 480 PCR apparatus. After a 1-minute denaturation at 94°C, 36 cycles of amplification were performed at 94°C for 1 minute, 60°C for 1 minute, and 72°C for 1 minute, followed by a 7-minute extension step at 72°C. The resulting PCR product was digested with the restriction enzyme *BSa*MI, an isoschizomer of the *Bsm*I enzyme. This enzyme was used because it is less expensive than *Bsm*I and more reliably digests DNA than *Bsm*I. The digested material was electrophoresed on a 1.5% agarose gel at 25 mA for 4.5 hours. Gels were stained with ethidium bromide, photographed, and scored. Genotype was defined as BB (absence of restriction site on both alleles), bb (presence of restriction site on both alleles), or Bb (heterozygous).

Statistical Analysis

We compared unadjusted BMD between BB, Bb, and bb groups at the various sites using ANOVA. We also age-adjusted BMD for the three groups using z scores that were calculated by the bone densitometer. We designed the study to have an 80% probability of detecting a 10% difference in BMD at a P value of .05.

RESULTS

Sixty-nine women met all exclusion criteria and were willing to participate in the study. The age of the women was 37 ± 6.5 years (mean ± SD) and ranged from 20 to 45. As dictated by the exclusion criteria, all women were premenopausal. Among the 69 women, 22 were homozygotes for the bb allele, 36 were heterozygotes Bb, and 11 were homozygotes for the BB allele. Thus, allele frequency for the b allele was 0.58 and for the B allele 0.42, a finding similar to allele frequencies in other Caucasian populations.[7,8] Genotype subgroups with corresponding anthropometric data and mean values for BMD are shown in Table 1. Height was similar between the three groups, as was age, although there was a trend toward older age in the bb group. There was also a trend for the BB group to weigh less than the other two groups, although this was not statistically significant. There were no significant differences in BMD measured by the lateral image of the lumbar spine between the three VDR genotypes. Similar results were obtained for AP measurements of the lumbar spine. There were no significant differences in BMD measurements at any location in the proximal femur. Overall, there were no trends to indicate improved bone density with the bb genotype at any site. Indeed, the only possible trend occurred at the total hip, which displayed a small nonsignificant decrease in BMD in individuals who had the bb genotype. When BMD measurements were age-adjusted by comparing z scores between groups, there were no significant differences at any site (data not shown).

DISCUSSION

In this study, we measured BMD of the lumbar spine using both AP and lateral images, as well as measuring BMD at the proximal femur. We found no association between VDR genotype and BMD at any of these sites in this population of healthy premenopausal Caucasian women from the southeastern United States. We used the restriction enzyme *BSa*MI, an isoschizomer of *Bsm*I, to identify polymorphisms, since polymorphisms at this restriction site were most highly correlated with BMD in studies that detected a correlation.[1] Although it is possible that our study did not have enough subjects to detect a small effect on bone density, it did have enough power to detect differences of the magnitude previously reported. Indeed, there were no trends toward increased BMD in bb homozygotes.

Table 1. Anthropometric and BMD Data by VDR Genotype

Characteristic	VDR Genotype			P
	BB	Bb	bb	
No. of subjects	11	36	22	
Height (cm)	164.6 ± 6.8	165.1 ± 5.1	165.0 ± 5.6	.97
Weight (kg)	56.8 ± 6.5	61.8 ± 10.0	62.7 ± 10.9	.25
Age (yr)	34 ± 6.4	37 ± 5.9	38 ± 7.4	.25
BMD				
Lumbar spine				
AP	1.010 ± 0.117	0.985 ± 0.111	0.998 ± 0.109	.81
Lateral	0.753 ± 0.089	0.741 ± 0.067	0.758 ± 0.079	.63
Total hip	0.888 ± 0.092	0.858 ± 0.100	0.833 ± 0.194	.49
Neck	0.817 ± 0.110	0.778 ± 0.103	0.803 ± 0.092	.48
Trochanter	0.687 ± 0.089	0.644 ± 0.091	0.655 ± 0.064	.21
Intertrochanteric region	1.016 ± 0.107	0.992 ± 0.114	1.001 ± 0.098	.72
Ward's triangle	0.766 ± 0.153	0.686 ± 0.131	0.725 ± 0.125	.19

NOTE. Values are the mean ± SD. BMD is expressed as g/cm².

Our results are in contrast to those of some previous studies[1-4,11] but support the results of others.[5-8,12,13] Morrison et al,[1] who studied Caucasian women from Australia, reported a strong relationship between VDR genotype and BMD. They reported that women with the bb genotype had a mean BMD that was 15% higher than that of women with the BB genotype. Yamagata et al[4] found similar results in Japan in 47 premenopausal women and 55 postmenopausal women. Several studies found an association between VDR allele and BMD in postmenopausal women.[3,4,14,15] However, in one of these studies,[14] the effect could only be demonstrated in women who were more than 10 years postmenopausal. In contrast, Riggs et al[15] detected an effect in premenopausal and early postmenopausal women, but not in late postmenopausal women. Of note, they were unable to detect a difference in allele frequency between osteoporotic and normal postmenopausal women, as would be expected if VDR genotype was a clinically important risk factor for osteoporosis.

Our study supports the findings of several investigators.[5-8] Hustmyer et al[7] demonstrated that there was no association between VDR genotype and BMD in monozygotic and dizygotic twin pairs. However, these investigators were able to demonstrate that genetic factors other than VDR genotype were important in the attainment of peak BMD. Garnero et al,[5] who studied unrelated premenopausal French women, found no association between VDR genotype and BMD. They also found no association between VDR genotype and biochemical markers of bone formation or resorption. These investigators have performed a similar study in unrelated postmenopausal French women and have again found no association between VDR genotype and either BMD or biochemical markers.[6] Additionally, several studies have compared VDR genotypes in osteoporotic women and controls. None of these studies have found an increased proportion of the BB genotype in the osteoporotic group, as would be expected if VDR alleles were clinically important determinants of bone density.[13,15]

There are several possible explanations for the discrepant findings in various studies. These include population admixture artifact, linkage dysequilibrium between the VDR gene and another gene, and environmental modification of the genotypic effect on BMD.

Population admixture can give rise to associations of no biological significance, because different populations will often have different allele frequencies for genetic markers. If there are differences between two populations in the biological variable under study, then associations can be found between a genetic marker and the biological trait by studying a heterogeneous population that contains members of two (or more) different

subpopulations. The association would not hold if the populations were studied individually. In the case of BMD, this would occur if one population has a high frequency of the b allele and also has relatively high bone density and another population has a high frequency of the B allele and lower bone density. If the two populations are studied together as one population, the b allele will be associated with higher BMD, whereas this association would not exist if the two populations were studied separately. Most studies that have found an association between VDR genotype and BMD have been performed in Caucasian populations that may consist of several different ethnic groups. However, some studies that have found an association between VDR genotype and BMD have been performed in homogeneous populations, including one study in Japanese women.[4] Thus, although population admixture artifact may account for some of the observed association between VDR genotype and BMD, it probably does not account for all of the observed association in studies that found such an association.

Another possible explanation for the different observations by various investigators is that VDR alleles may not directly influence BMD, but may be in linkage dysequilibrium with another gene that influences BMD. Such a concept is supported by recent observations made by Uitterlinden et al,[16] who reported an association between VDR genotype and BMD, but in their study, women with the BB genotype had a higher BMD, in contrast to several other studies[1-4] that found a higher BMD for the bb genotype. If the VDR is in linkage dysequilibrium with another gene that has direct effects on BMD, it is possible that associations between BMD and VDR polymorphisms are more easily observed in homogeneous populations than in highly heterogeneous populations such as those found in the United States.

Alternatively, it is possible that environmental factors such as calcium intake play a critical role in modifying the effect of genotype on BMD. In this regard, studies by Krall et al[14] were only able to demonstrate a difference in rates of postmenopausal bone loss between women of different VDR genotypes who had inadequate calcium intakes. More recent studies[17] demonstrate that although calcium absorption was similar on a high-calcium diet, individuals with the BB genotype had significantly lower calcium absorption on a low-calcium diet than individuals with the bb genotype. Thus, VDR genotype may only play an important role in determining BMD in women who have inadequate calcium intake.

In summary, VDR alleles have no clinically significant correlation to peak BMD in Caucasian women from the southeastern United States. VDR genotype should not be used to predict an individual's risk for osteoporosis.

REFERENCES

1. Morrison NA, Qi JC, Tokita A, et al: Prediction of bone density from vitamin D receptor alleles. Nature 367:284-287, 1994
2. Fleet JC, Harris SS, Wood RJ, et al: The BsmI vitamin D receptor restriction length polymorphism (BB) predicts low bone density in premenopausal black and white women. J Bone Miner Res 10:985-990, 1995
3. Spector TD, Keen RW, Arden NK, et al: Influence of vitamin D receptor genotype on bone mineral density in postmenopausal women: A twin study in Britain. Br Med J 310:1357-1360, 1995
4. Yamagata Z, Miyamura T, Lijima S, et al: Vitamin D receptor gene polymorphisms and bone mineral density in healthy Japanese women. Lancet 344:1027, 1994
5. Garnero P, Borel O, Sornay-Rendu E, et al: Vitamin D receptor gene polymorphisms do not predict bone turnover and bone mass in healthy premenopausal women. J Bone Miner Res 10:1283-1288, 1995
6. Garneno P, Borel O, Sornay-Rendu E, et al: Vitamin D receptor gene polymorphisms do not predict peak bone mass, postmenopausal bone loss and bone turnover: The OFELY Study. J Bone Miner Res 10:S161, 1995
7. Hustmyer FG, Peacock M, Hui S, et al: Bone mineral density in relation to polymorphisms at the vitamin D receptor gene locus. J Clin Invest 94:2130-2134, 1994
8. Melhus H, Kindmark A, Amer S, et al: Vitamin D receptor genotypes in osteoporosis. Lancet 344:949-950, 1994
9. Myers BS, Arbogast KB, Lobaugh B, et al: Improved assessment of lumbar vertebral body strength using supine lateral dual-energy x-ray absorptiometry. J Bone Miner Res 9:687-693, 1994
10. Aldridge J, Kunkel L, Bruns G, et al: A strategy to reveal high frequency RFLPs along the human X chromosome. Am J Hum Genet 36:546-564, 1984
11. Ferrari S, Rizzoli R, Chevalley T, et al: Vitamin D receptor gene polymorphisms and change in lumbar-spine bone mineral density. Lancet 345:423-424, 1995
12. Gallagher P, Goldgar D, Kinyamu H, et al: Vitamin D receptor genotypes in type I osteoporosis. J Bone Miner Res 9:S143, 1994
13. Looney JE, Yoon HK, Fischer M, et al: Lack of a high prevalence of the BB vitamin D receptor genotype in severely osteoporotic women. J Clin Endocrinol Metab 80:2158-2162, 1995
14. Krall EA, Parry P, Lichter JB, et al: Vitamin D receptor alleles and rates of bone loss: Influences of years since menopause and calcium intake. J Bone Miner Res 10:978-984, 1995
15. Riggs BL, Nguyen TV, Melton LJ III, et al: The contribution of vitamin D receptor gene alleles to the determination of bone mineral density in normal and osteoporotic women. J Bone Miner Res 10:991-996, 1995
16. Uitterlinden AG, Pols HAP, van Daele PLA, et al: Association between vitamin D receptor gene polymorphisms and bone mineral density. J Bone Miner Res 10:S161, 1995
17. Dawson-Hughes B, Harris SS, Finneran S: Calcium absorption on high and low calcium intakes in relation to vitamin D receptor genotype. J Bone Miner Res 10:S162, 1995 (abstr)