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Original Article

Common allelic variants of the vitamin receptor D gene rs7975232 (ApaI) do not influence bone mineral density figures in postmenopausal osteoporotic women

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Abstract: This study examined the association between bone mineral density (BMD) and the rs7975232 (ApaI) polymorphism of the vitamin receptor D (VDR) gene. The polymorphism was detected using the real-time PCR TaqMan method. The rs7975232 genotype was determined in 274 postmenopausal osteoporotic Spanish women who were 60.53±8.02 years old. The observed genotype frequencies were in agreement with Hardy-Weinberg equilibrium (χ^2=1.85, P=0.1736). There were no significant differences in the rs7975232 genotype groups in our total sample of osteoporotic women regarding age, years since menopause, height, weight, and BMD at femoral neck, femoral trochanter and lumbar spine. Significant differences were found in menarche age (aa vs Aa; P=0.008) and BMI (aa vs AA; P=0.029). We conclude that the VDR gene rs7975232 polymorphism is not related to figures of bone mineral density in postmenopausal osteoporotic Spanish women.

Keywords: Polymorphism, bone mineral density, ApaI, osteoporosis, postmenopausal

Introduction

Osteoporosis is a polygenic disorder that is determined by the effects of several genes, each with relatively modest effects on bone mass and other determinants of fracture risk. Osteoporosis is a disease of low bone mineral mass and microarchitectural deterioration of bone, which leads to increased fracture risk [1]. Bone mineral density (BMD) is usually used as a measure of bone strength, and diagnoses of osteoporosis are based on its analysis [1].

Previous studies in twins and families showed that genetic factors play an important role in the formation of BMD and that genetic influences can account for up to 85% of the bone mass, with the strongest effects in the axial skeleton [2-4]. Population-based studies and case-control studies have similarly identified polymorphisms in several candidate genes that have been associated with bone mass or osteoporotic fracture, including the vitamin D receptor (VDR) [5], the estrogen receptor [6], regulator genes of the synthesis of TGF-β1 [7] and the collagen type I α-1 gene [8]. Vitamin D, through its principal bioactive form 1,25-dihydroxyvitamin D3 (1,25-(OH)2D3), plays a crucial role in bone metabolism. In 1994, Morrison et al. reported a strong relationship between BMD and restriction fragment length polymorphisms (RFLPs) based on BsmI endonuclease digestion at the vitamin D receptor (VDR) gene locus in Caucasian women [5]; since then, significant associations between common polymorphisms of the VDR gene, including the ApaI polymorphism (rs7975232), and BMD in Caucasian women have been reported [9, 10]. However, there are still conflicting results.

This study aimed to investigate the relationship of commonly studied polymorphisms in the VDR gene, rs7975232, with the BMD figures in a cohort of Spanish postmenopausal women.

Table 1. Characteristics of the total sample

	n = 274
Age (years)	60.53 ± 8.02
Menarche age (years)	13.05 ± 1.47
YSM (years)	13.80 ± 8.23
Weigth (kg)	61.81 ± 10.76
Height (m)	1.53 ± 0.059
BMI (kg/m^2)	26.42 ± 4.90
Ad-SoS (m/s)	2004.280 ± 71.176
BMD FN (g/cm^2)	0.705 ± 0.094
BMD TR (g/cm^2)	0.556 ± 0.086
BMD L2 (g/cm^2)	0.755 ± 0.088
BMD L3 (g/cm^2)	0.748 ± 0.079
BMD L4 (g/cm^2)	0.726 ± 0.079
BMD L2-L4 (g/cm^2)	0.740 ± 0.072

Values are the mean ± SD.

Methods

This was an observational, cross-sectional study. The present study sample comprised 274 consecutive subjects included in the Caceres Database for the Diagnosis of Osteoporosis (CAFOR) study between 2010 and 2011. The study was performed in accordance with the Declaration of Helsinki and was approved by the Research Ethics Committee of the University of Extremadura. Written informed consent was obtained from all the subjects.

Densitometric study

Densitometric measurements were performed to determine the BMD in the femoral neck (FN), femoral trochanter (FT) and the lumbar spine at the L2, L3, L4 and L2-L4 levels. Additionally, body weight and height were measured to calculate body mass index (BMI). Densitometric tests were performed with the use of a NORLAND XR-800 (Norland Medical Systems, Inc.). BMD scores were expressed as grams per square centimeter.

Quantitative ultrasound study

We assessed ultrasound bone status using an ultrasound device, model DBM Sonic 1200® (Emsor, S.A., Madrid, Spain), which measured the amplitude-dependent speed of sound (Ad-SoS) in meters per second. We measured the phalanges (II-V) of the non-dominant hand and computed an average value. We achieved contact by means of standard ultrasound gel. Two 16-mm-diameter, 1.25-MHz transducers were assembled on a high-precision caliper that measured the distance between the probes. We positioned the probes on the mediolateral phalangeal surfaces using the phalanx head as a reference point. Positioning and repositioning the instrument was easy because it uses the prominences of the lower phalangeal epiphysis as a reference; the clip is placed just behind the prominences.

Statistical analysis

The allelic and genotypic frequencies were estimated by gene counting, and the goodness of fit of the genotype distribution for Hardy-Weinberg equilibrium (HWE) was tested using a chi-square (χ^2) test. Values of $P>0.05$ indicated HWE.

The statistical analysis of the results was performed with SPSS 20 for Windows. Normal distributions and homogeneity of variances were assessed using the Kolmogorov-Smirnov and Levene tests, respectively. An analysis of variance (ANOVA) followed by Bonferroni's post-hoc test was used to compare different genotypes in each SNP. An analysis of co-variance (ANCOVA) was used to compare the VDR genotypes adjusted for the co-variants age, BMI, years since menopause, height and weight.

Results

The frequency of occurrence of the polymorphisms of ApaI in the studied sample was as follows: aa, 23.4% (n=64); Aa, 45.6% (n=125); and AA, 31.0% (n=85). The observed genotype frequencies were in agreement with Hardy-Weinberg equilibrium (χ^2=1.85, P=0.1736). The characteristics of the total sample are shown in **Table 1**. No significant differences were found between the ApaI genotypes regarding age, years since menopause (YSM), weight and height (P>0.05 between groups) (**Table 2**). Significant differences were found between the aa and Aa groups regarding menarche age (P=0.008) and between the aa and AA groups regarding BMI (P=0.029) (**Table 2**). No significant differences were found in the crude BMD between the studied groups (**Table 3**) or after further adjustment for potential confounding factors (**Table 3**).

Table 2. Characteristics of the total sample in patients with the ApaI genotype

Group	aa (64)	Aa (125)	AA (85)	*P*-value	aa vs AA	aa vs Aa
Age (years)	59.48 ± 6.98	60.96 ± 8.25	60.68 ± 8.42	0.475		
Menarche age (years)	12.64 ± 1.57	13.32 ± 1.37	12.97 ± 1.48	0.009		0.008
YSM (years)	13.61 ± 8.42	14.04 ± 8.05	13.59 ± 8.42	0.908		
Weigth (kg)	59.27 ± 10.73	62.23 ± 10.02	63.11 ± 11.60	0.081		
Height (m)	1.54 ± 0.06	1.53 ± 0.06	1.52 ± 0.04	0.284		
BMI (kg/m^2)	25.02 ± 4.82	26.66 ± 4.63	27.12 ± 5.19	0.026	0.029	

Values are the mean ± SD. *P*-value by ANOVA.

Table 3. BMD according to the VDR ApaI genotypes

		aa (64)	Aa	AA	*P*-value			aa	Aa	AA	*P*-value
BMD1 (g/cm^2)	QUS	2004.211 ± 67.039	2001.43 ± 72.472	2007.125 ± 72.103	0.905	BMD1 (g/cm^2)	QUS	2003.288 ± 8.951	2002.138 ± 6.431	2006.782 ± 7.994	0.901
	FN	0.705 ± 0.100	0.717 ± 0.086	0.687 ± 0.995	0.082		FN	0.707 ± 0.012	0.718 ± 0.008	0.686 ± 0.010	0.058
	FT	0.549 ± 0.085	0.567 ± 0.092	0.545 ± 0.777	0.186		FT	0.552 ± 0.011	0.568 ± 0.008	0.543 ± 0.009	0.117
	L2	0.745 ± 0.093	0.754 ± 0.089	0.762 ± 0.084	0.501		L2	0.744 ± 0.011	0.756 ± 0.008	0.761 ± 0.010	0.491
	L3	0.745 ± 0.079	0.742 ± 0.083	0.760 ± 0.072	0.277		L3	0.745 ± 0.010	0.742 ± 0.007	0.744 ± 0.778	0.229
	L4	0.715 ± 0.083	0.726 ± 0.075	0732 ± 0080	0.423		L4	0.720 ± 0.010	0.726 ± 0.007	0.732 ± 0.715	0.675
	L2-L4	0.745 ± 0.075	0.735 ± 0.067	0.742 ± 0.077	0.63		L2-L4	0.748 ± 0.009	0.735 ± 0.007	0.743 ± 0.008	0.479

Data shown are mean ± SD, and *P* values obtained from ANOVA and ANCOVA. The BMD are denoted as BMD1 and BMD2, which belong to the raw BMD and BMD adjusted by age, YSM, height, weight and BMI.

Discussion

Osteoporosis is a worldwide health issue with a high prevalence of disease. The ability to predict and prevent osteoporotic-related fractures would be a major benefit to both patients and the health system. Thus, finding a genetic marker that could predict those at the greatest risk of developing osteoporosis or its evolution is an attractive proposition.

At present, our study evaluated the different bone mineral density figures according to the rs7975232 genotype in a group of unrelated postmenopausal osteoporotic Spanish women. The ApaI polymorphism is located in the non-coding region of the VDR gene and does not have an effect on the final protein product [11]. The A allele is correlated with enhanced mRNA stability or transcriptional activity and greater vitamin D activity [5]. Overall, this fact highlights the importance of understanding the mechanisms by which these polymorphisms affect VDR action. We found no association between the ApaI polymorphism genotype and BMD figures, which again contrasts with some of the earlier studies [12-14] but confirms other studies [15-18].

Data from a meta-analysis of Li and colleagues [13] suggest that the racial/ethnic genetic background plays a role. Indeed, in Caucasians, no associations with the ApaI polymorphism were found by a meta-analysis with either fracture risk [19] or BMD [20].

In Spain, an earlier study from Bustamante and colleagues [21] in a cohort of 719 postmenopausal women found no associations of BMD with the ApaI polymorphism; our results were more in accordance with this study.

In summary, our study suggests that BMD figures are not associated with the ApaI genotype in postmenopausal osteoporotic Spanish women and add to the hypothesis that the relevance of the ApaI genotype in osteoporosis should be questioned.

Disclosure of conflict of ineterest

None.

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