VITAMIN D RECEPTOR GENOTYPES AND BONE MINERAL DENSITY IN POSTMENOPAUSAL WOMEN: INTERACTION WITH PHYSICAL ACTIVITY

Running head: VDR genotype, physical acitivity level and BMD

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ABSTRACT

The present study investigated the association between vitamin D receptor (VDR) genotypes with bone mineral density (BMD) and its interaction with physical activity level (PAL). A sample of 192 volunteers (67.84 ± 5.23 years) underwent BMD evaluation and were genotyped for VDR ApaI, BsmI, FokI and TaqI polymorphisms. Haplotypes were reconstructed through expectation-maximization (EM) algorithm and regression based haplotype-specific association tests were performed with studied phenotypes. None of the polymorphisms were associated with BMD at any site; however, haplotype was associated with femoral neck and Ward's triangle BMD. Interaction between PAL and VDR genotypes was significant for the FokI polymorphism at femoral neck and Ward's triangle BMD. The FokI T/T genotype was associated with higher BMD in active women. It was concluded that VDR haplotypes, but not genotypes, are associated with femoral neck and Ward's triangle BMD in postmenopausal women. Moreover, the results suggest that VDR FokI polymorphism may be a potential determinant of BMD response to physical activity.

Key words: bone mineral density, physical activity, genetic association

INTRODUCTION

Osteoporosis is an age-related disorder characterized by low bone mineral density (BMD), deterioration of skeletal micro architecture, bone frailty and increased fracture risk (Cummings, Kelsey, Nevitt, & O'Dowd, 1985). Complications associated with osteoporosis are the major cause of death and disability among elders, representing a burden on public health costs as the life expectancy grows worldwide (Johnell & Kanis, 2004). Among the phenotypes that characterize bone frailty, BMD has been reported as the best predictor of fractures (Marshall, Johnell, & Wedel, 1996). Therefore, knowledge about the physiological mechanisms driving the age-associated BMD loss is mandatory to the development of efficient prophylactic and therapeutic interventions to reduce bone fractures (Nguyen & Eisman, 2006). Mechanisms leading to osteoporosis are multifactorial, and involve environmental and genetic factors, as well as their interaction (Cummings et al., 1985; Naganathan, MacGregor, & Sambrook, 2007; Peacock, Turner, Econs, & Foroud, 2002). There is a reasonable body of evidence, based on segregation analysis, showing that genetic factors play a major role in BMD determination. (Peacock et al., 2002). In that sense, it has been suggested that the existence of a major gene may be responsible for a relatively large proportion of the inter-individual BMD variation (Deng et al., 2002). Among the environmental factors, physical activity habits play a pivotal role in bone mass and structure characteristics (Puntila et al., 2001).

Although the heritability of BMD is well recognized, identification of specific allelic variants contributing to this phenotype requires more investigations. A pioneer study reporting an association between BMD and a gene suggested that polymorphisms

in the vitamin D receptor (VDR) gene could explain up to 75% of the total genetic variation of BMD among twins (Morrison et al., 1994). Since then, three adjacent polymorphisms at the 3'-end of the VDR gene (BsmI, ApaI and TaqI), became the most frequently studied with regard to their relation to BMD variation. In agreement with the findings of Morrison et al. (1994), some studies suggested that the A, T and C alleles of the BsmI, ApaI and TaqI sites, respectively, are the risk alleles associated with low BMD (Morrison, Yeoman, Kelly, & Eisman, 1992; Salamone et al., 1996; Vandevyver, Wylin, Cassiman, Raus, & Geusens, 1997). However, agreement on this relationship is not universal, and some studies have found no association (Bandres et al., 2005; Blanchet et al., 2002; Tsuritani et al., 1998; Zajickova, Zofkova, Bahbouh, & Krepelova, 2002; Zmuda, Cauley, Danielson, Wolf, & Ferrell, 1997) while others reported an association contrary to that initially proposed (Douroudis et al., 2003). The most likely explanation for the controversy is in the fact that the BsmI, ApaI and TaqI polymorphisms are not functional. BsmI and ApaI are located in intron 8 and do not affect any splicing site or transcription factor. The TaqI is present in exon 9, a coding sequence, but is a synonymous polymorphism, and both alleles are related to the same amino acid (isoleucine).

A functional polymorphism at the translation initiation site of the VDR gene, defined by FokI restriction endonuclease, has also been associated with variations in BMD. This polymorphism is characterized by a T to C substitution at exon 2 that eliminates the first ATG translation initiation site. Thus, two forms of the VDR protein can be translated, which differ by three amino acids resulting in proteins of 427 (T allele) and 424 (C allele) amino acids. Subjects homozygous for the presence of the restriction

site (T allele) were reported to have lower BMD than carriers of the C allele (Bandres et al., 2005; Falchetti et al., 2007; Zajickova et al., 2002), however, others have reported no significant effect (Rapuri, Gallagher, Knezetic, Kinyamu, & Ryschon, 2004; Zmuda et al., 1997).

Multiple Single Nucleotide Polymorphisms (SNPs) alleles when evaluated together as haplotypes or as interactions among themselves may increase the power to detect association (Clark, 2004). Despite the growing body of evidence showing this association, VDR haplotypic analysis in relation to BMD is not well documented in the literature. The few existing studies are basically restricted to 3' restriction fragment length polymorphisms (RFLPs) BsmI, ApaI and TaqI haplotypes and results are also contradictory (Kim et al., 2003; Thakkinstian, D'Este, & Attia, 2004; Uitterlinden, Fang, Van Meurs, Pols, & Van Leeuwen, 2004). Indeed, although the number of association studies between VDR gene and BMD is relatively large, almost all were carried out with the allelic variants analyzed individually.

The effect of gene-exercise relationships on health-related phenotypes, including BMD, has been gaining interest among researchers (Wolfarth et al., 2005). The studies analyzing the interaction between physical activity and VDR polymorphisms at the 3' untranslated region (UTR) on BMD have yielded conflicting results. While some studies have suggested a more favourable response to the ApaI C/C and TaqI T/T genotypes (Kitagawa, Kitagawa, Nagaya, & Tokudome, 2001), others report no difference between ApaI (Jarvinen et al., 1998) or BsmI polymorphisms (Rabon-Stith et al., 2005), with Blanchet et al. (2002) reporting an impaired response to the BsmI G/G genotype. Regarding FokI polymorphism, some authors suggested an impaired response in young

Japanese men carrying the T allele (Nakamura et al., 2002; Tajima et al., 2000). However, these studies are limited by the reduced number of subjects and the low frequency of the T/T genotype. In older men and women, Rabon-Stith et al. (2005) suggested that FokI genotype was related to femoral neck BMD response to strength training, but not to aerobic training, however, the difference among genotypes did not achieve significance. Therefore, the interaction between VDR gene polymorphisms and physical activity on BMD are still controversial.

The objectives of the present study were to investigate: 1) the association between BMD and the four most studied VDR SNPs (ApaI, BsmI, FokI and TaqI); 2) the interaction between these SNPs and physical activity level on BMD determination; and 3) the association between VDR haplotypes and BMD in postmenopausal women.

MATERIALS AND METHODS

Subjects

One hundred and ninety two postmenopausal women participated in the present study as volunteers. Subjects had no clinical disorder and were not using medications that could interfere with bone metabolism. Volunteers were able to walk without assistance and had no metallic prosthesis implant. A more detailed description of the participants' characteristics is given in Tables 1 and 2. Volunteers were recruited from a social project developed by the University department and were invited to participate in this study by phone calls. During the first visit to the laboratory, each subject signed a detailed consent form to take part in the study, answered two face-to-face questionnaires and provided venous blood samples for subsequent genotype analysis. One questionnaire addressed medical history, co-morbities, hormonal replacement, self referred skin color and lifestyle habits. The other questionnaire assessed volunteer's physical activity level (PAL). During the second visit, body composition was measured by dual-energy x-ray absortiometry. The study protocol was approved by the University Ethics Committee.

Anthropometry and bone densitometry

Body weight was measured to the nearest 0.1 Kg using a calibrated physician's beam scale (model 31, Filizola, São Paulo, Brazil) with women dressed in light T-shirt and shorts. Height was determined without shoes to the nearest 0.1 cm using a stadiometer (model 31, Filizola, São Paulo, Brazil), after a voluntary deep inspiration. Body mass index (BMI) was calculated as body weight divided by height squared (Kg/m^2).

Measurements of BMD (grams per square centimetres – g/cm^2) were performed at the femoral neck, Ward's triangle and lumbar spine (L2-L4) using dual-energy x-ray absortiometry (DEXA; DPX-L, Lunar Radiation Corporation, Madison, WI). The percent coefficient of variation for femoral neck, Ward's triangle and lumbar spine measurements were 2.4%, 2.2% and 1.3%, respectively. The DEXA scanner was calibrated daily against an aluminum spine phantom provided by the manufacturer.

Questionnaires

Physical activity level (PAL) was assessed using the shortened version of the International Physical Activity Questionnaire (IPAQ), which was administered face-to-face by two trained interviewers. The IPAQ development is supported by the World Health Organization and its measurement properties were considered satisfactory in various countries (Craig et al., 2003), including the studied population (Matsudo et al., 2001). Based on questionnaire results, all individuals were categorized as sedentary, insufficiently active, active, or very active. In accordance with previous recommendations from the US Surgeon General's Report (USDHHS, 1996), a dichotomised classification was conducted as follows: insufficient physically active (IA) individuals were defined by a score of less than 150 minutes/week of moderate and/or vigorous physical activity; and physically active (AT) if they scored above this threshold.

Genotyping

In order to make comprehension easier when referring to the VDR SNPs, the former designations of TaqI, ApaI, BsmI and FokI were used instead of the National

Center for Biothechnology Information (NCBI) dbSNP "rs" identification. Alternatively, when presenting genotypes and haplotypes the dbSNP reference alleles were used. The VDR polymorphisms were genotyped through a single base extension protocol as previously described (Lins et al., 2007). Briefly, high-molecular-weight genomic DNA was extracted using a modified salting-out method and then amplified by polymerase chain reaction (PCR). PCR products were treated and genotyping was performed by mini-sequecing, using the ABI Prism® SNaPshot™ Multiplex System (Applied Biosystems, Foster City, CA, USA) followed by capillary electrophoresis on an ABI Prism® 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). In addition, thirteen ancestry informative markers (AIMs) were genotyped by the same methodology and were identical to those previously described by Moreno Lima et al (2007).

Statistical analysis

Exploratory and descriptive statistical procedures were respectively used to search for outliers and to calculate means and standard deviation (SD) for all the presented variables. Exact test was used to verify if the observed genotype frequencies were in Hardy-Weinberg equilibrium. Individual maximum likelihood estimation of admixture proportion was carried out based on parental population (European, African and Amerindian) allele frequencies for the thirteen genotyped AIMs using the IAE3CI software. Genomic ancestry estimates were tested as potential covariates in subsequent analysis.

One-way analysis of variance (ANOVA) was conducted to search for differences in age, height, BMI, genomic ancestry estimate and percent body fat between genotype

groups. Analysis of covariance (ANCOVA) was performed to investigate genotype differences in each of the examined BMD sites. The effect of the interaction between VDR genotypes and PAL on BMD determination was also assessed by ANCOVA. Multiple comparisons with confidence interval adjustments by Bonferroni procedures were used for post hoc comparisons when necessary. All potential covariates were summed up in a stepwise multiple regression against each BMD site (femoral neck, Ward's triangle and lumbar spine) as dependent variables. Covariates with $p < 0.10$ were selected to enter in the ANCOVA models. Statistical significance was established at $p<0.05$ and the aforementioned analyses were performed using the SPSS software for Windows version 8.0 (SPSS, Chicago, IL).

Besides the four allelic variants used in the present analysis, previous genotyping data of the Cdx-2 polymorphism from the same study population (Gentil et al., 2007) was used for haplotype reconstruction. Haplotype estimates and regression based specific association tests were carried out using the Whap (Purcell, Daly, & Sham, 2007) software, which is based on the expectation-maximization (EM) algorithm. It uses the estimates of subsequent probabilities to account for the ambiguity of haplotype phase estimates on subsequent association tests using unrelated individuals. A likelihood ratio test on the regression based omnibus permutation step indicates whether there is significant influence of haplotypes on the phenotypes under investigation. The haplotype-specific regression based test implemented by the Whap software compares the haplotype effect against all other haplotypes and indicates if the effect observed on a specific haplotype is significant. This analysis package is able to handle quantitative traits and covariates for regression analysis. The haplotype association analyses were performed

considering BMD measures at the femoral neck, Ward's Triangle and lumbar spine as dependent variables.

RESULTS

Stepwise analyses revealed that age, BMI, hormone replacement and smoking were the most important predictors for femoral neck (r^2=0.268) and Ward's triangle BMD (r^2=0.256). The model that best predicted BMD at the lumbar spine included BMI and age (r^2=0.153). When entering the individual genetic ancestry estimate, the model for lumbar spine BMD included BMI, age and individual African ancestry (r^2=0.172). However, the models for femoral neck and Ward's triangle BMD remained unchanged. The variables included in the models were used as covariates in subsequent ANCOVAs.

Genotypes and BMD

Allele frequencies and genotype distribution are presented in Table 3. The observed and expected genotype frequencies are in accordance with Hardy-Weinberg equilibrium as calculated using exact tests. No significant differences were observed among VDR genotype groups for age, height, weight, BMI, ancestry levels, or percent body fat. When analyzed individually, none of the investigated polymorphims (ApaI, BsmI, FokI and TaqI), were associated with any of the evaluated BMD phenotypes.

Interaction between VDR genotypes and PAL on BMD determination

There were no interactions between ApaI, BsmI or TaqI polymorphisms and PAL for femoral neck, lumbar spine or Ward's triangle BMD. However, the interaction between FokI genotypes and PAL were significant for femoral neck (p=0.012) and Ward's triangle (p=0.002) BMD, but not for the lumbar spine (p=0.818). Comparisons

within FokI genotypes revealed that women bearing the T/T genotype classified as AT had higher BMD at the femoral neck and Ward's triangle than women bearing the same genotype classified as IA (Table 4). In addition, comparisons within PAL revealed significant differences between genotypes only in the AT group. Women with the T/T genotype classified as AT had significantly higher femoral neck and Ward's triangle BMD than women bearing the C/C genotype. Carriers of C/T genotype presented intermediate values of BMD, although it only reached significance for Ward's triangle BMD, in which the C/T carriers presented significantly lower values than the T/T women (Table 4).

Haplotype Analysis

Haplotype frequencies were estimated using the genotype data of the five SNPs (the four used in the present study plus previous Cdx-2 genotyping data) and revealed 15 common haplotypes with frequencies above 0.02 and a proportion of 0.964 haplotypes covered (Table 5). *Omnibus* permutation tests showed no significant haplotype association with lumbar spine BMD ($p>0.05$). For Ward's triangle and femoral neck BMD, significant association was found for some haplotypes, however, with the inclusion of BMI, age, African genomic ancestry estimate and PAL as covariates, significance was only observed for haplotype 7 ($P < 0.05$). The haplotype-specific regression tests were carried out within each significant *omnibus* permutation association tests, and Haplotype 7 was found to be associated with a significant decreased Ward's triangle and femoral neck BMD, regardless the inclusion of covariates correction.

DISCUSSION

The present study did not find differences in femoral neck, Ward's triangle or lumbar spine BMD among the polymorphisms at the 3'-UTR (BsmI, ApaI and TaqI). This observation does not corroborate previous findings involving postmenopausal women (Morrison et al., 1994; Vandevyver et al., 1997), however, it is in agreement with most of the reviewed literature (Tsuritani et al., 1998; Zajickova et al., 2002; Zmuda et al., 1997).

Previous studies assessing the interaction between the 3'-UTR polymorphisms and physical activity have yielded conflicting results. In a cross-sectional study, Blanchet et al. (2002) reported that active postmenopausal women bearing the G/G genotype presented lower lumbar spine BMD than active women with the A/A and G/A genotypes. On the other hand, Tsuritani et al. (1998) reported that after a one year walking program, only postmenopausal women carrying the G/G genotype increased lumbar spine BMD in relation to the control group. Kitagawa et al. (2001) studied the inter-relation between habitual physical activity and VDR polymorphisms on the determination of forearm BMD in young Japanese females and reported better response to the C/C ApaI and T/T TaqI genotypes in comparison to C/A and C/T, respectively.

In the present study, it was observed that the SNPs located at the 3' UTR do not interact with PAL for BMD determination. This is in agreement with the results of Jarvinen et al. (1998), who did not report differences in BMD gains among the different BsmI genotypes in Caucasian postmenopausal women that participated in an 18-month high impact physical activity program. Similarly, Rabon-Stith et al. (2005) did not report

differences in BMD responses among different BsmI genotypes in older persons involved in aerobic or resistance training. The absence of significant effects of the 3'-UTR polymorphisms may have physiological explanations. ApaI and BsmI polymorphisms occur in the intron that separates exons VIII and IX, therefore, none of them affect the structure of the VDR protein (Whitfield et al., 2001). On the other hand, the TaqI site is located in exon IX, however, this thymine to cytosine variant is expected to be silent, since both ATT and ATC codons are related to the amino acid isoleucine (Morrison et al., 1992).

The *Fok*I polymorphic site is located in the exon II of the VDR gene, and is characterized by an ATG to ACG transition with a consequent upstream initiation codon. In individuals with the C allele, translation is initiated at a second ATG start site leading to a protein three amino acids shorter than the full-length VDR protein (Arai et al., 1997). Individuals with the T allele synthesize the full-length VDR protein with 427 amino acids.

The VDR gene start codon for the FokI polymorphism also presented a lack of association with the studied BMD sites. This is in agreement with some studies evaluating postmenopausal women (Rapuri et al., 2004; Zmuda et al., 1997), but in conflict with others (Bandres et al., 2005; Falchetti et al., 2007; Zajickova et al., 2002). However, when stratifying participants into IA and AT according to FokI genotype, analysis revealed significant associations for femoral neck and Ward's triangle BMD. In this regard, results revealed significant differences only within the AT groups, with the T/T genotype associated with higher BMD than the C/T and C/C carriers. These results suggest an increased BMD response to physical activity in postmenopausal women

carrying the T/T genotype. This suggestion was reinforced by the observation that gene-PAL interaction analysis revealed that only the FokI T/T genotype presented significantly higher femoral neck and Ward's triangle BMD when comparing physically active and inactive individuals.

Literature does not provide conclusive evidences regarding the effect of the interaction between FokI polymorphism and physical activity on BMD and/or bone metabolism. Some authors have suggested an improved response for the C allele (Nakamura et al., 2002; Rabon-Stith et al., 2005; Tajima et al., 2000), however, a detailed analysis of the studies shows that the initial suggestions should be viewed with caution. Tajima et al. (2000) compared the effects of resistance training in Japanese young men bearing the FokI C/C genotype with those carrying the T allele and reported that serum B-ALP and osteocalcin were similarly increased in both groups. Later, Nakamura et al. (2002) compared highly trained young male athletes with age-matched controls and reported higher values for bone mineral content (BMC), but not BMD, in the C/C genotype when compared to C/T. Also, in the study by Nakamura et al. (2002) the T/T genotype was not included in the analysis given its low observed frequency. According to the results of Rabon-Stith et al. (2005), elderly persons bearing the C/T genotype who were involved in resistance training presented a trend, nearing significance (p=0.058), for higher responses in femoral neck BMD than carries of the T/T genotype. Heterogeneity of the age, gender, population under study and physical activity evaluation methods may explain part of the existing contradictory evidence.

Studies in humans reported a higher calcium absorption for the C/C genotype, despite the similarity in calcium deposition in bones among the genotypes (Abrams et al.,

2005), leading to the suggestion that VDR acts on the absorption of calcium in the intestine rather than in its mineralization. Moreover, animal studies suggested that VDR is not essential to bone mineralization (Amling et al., 1999), which brings the hypothesis that in the absence of VDR, others molecules are activated to maintain bone homeostasis.

A recent study suggested that VDR is an inhibitor of osteoblast activity and, therefore, may be a negative regulator of bone metabolism (Sooy, Sabbagh, & Demay, 2005). Moreover the more active C allele may impair bone adaptation to exercise, which could explain the results of the present study. Another hypothesis could be derived from the studies of Salamone et al. (1996) and Kitagawa et al. (2001). These authors suggested that VDR genotype and physical activity act in inverse directions, such that the genotypes related to low BMD at baseline are associated with better responses to physical activity. The analysis of the results shows a similar trend for the FokI polymorphisms as shown in Table 4. The question remaining is how the VDR protein structure difference affects BMD and its response to physical activity stimuli.

Although BMD itself was not associated with any genotype at any site, further analysis revealed that haplotype 7 was associated with reduced femoral neck and Ward's triangle BMD, regardless of age, BMI, PAL and genomic ancestry estimate. *Omnibus* permutation test using haplotype estimates only from TaqI, ApaI and BsmI polymorphisms have not shown significant association to any BMD traits described here, apart from the correction for covariates ($p > 0{,}998$) (results not presented). These findings reinforce the idea that FokI polymorphic site may have an important function in explaining the variation of BMD phenotypes. A redeeming feature of our findings is that TaqI-ApaI-BsmI haplotype TAG, also presented as bAT, was previously described to be

correlated with low BMD (Douroudis et al., 2003; Uitterlinden et al., 1996). As presented in this study, FokI T and Cdx2 G alleles combined with TAG haplotype explained low BMD at femoral neck and Ward's triangle of postmenopausal women.

It is important to note that a cross-sectional design was used in the present study, thus, a cause and effect relationship can not be established between variables. In addition, although there was an effect of physical activity favoring carriers of the FokI T/T genotype, this genotype is found in only a very small proportion of the population. Therefore, the effect of physical activity in the overall population may not be strongly dependent on VDR genotype.

CONCLUSION

Based on the observed results, it is concluded that the investigated VDR polymorphisms, when analysed individually, are not associated with BMD in postmenopausal women. When focusing on the interaction between VDR polymorphisms and PAL on BMD determination, this cross-sectional study demonstrated that femoral neck and Ward's triangle BMD may depend on the FokI polymorphism, with the T/T genotype related to higher BMD responsiveness to physical activity. Furthermore, the results showed that the haplotype 7 (TAGTG) is associated with low BMD at femoral neck and Ward's triangle, thus providing evidence that this combination of alleles for the TaqI, ApaI, BsmI, FokI, and Cdx2 polymorphisms, respectively, constitute a risk factor for development of osteopenic conditions. Future research concerning the role of VDR in the bone mechanotransduction system should be undertaken to elucidate the physiological interplay between physical activity and the VDR gene.

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Table 1. Characteristics of the study subjects for total sample and according to physical activity level.

Variables	Total (*n*=192)	IA (*n*=75)	AT (*n*=117)
Age (years)	67.8 ± 5.2	68.0 ± 5.3	67.7 ± 5.2
Body mass (kg)	64.6 ± 11.7	65.1 ± 12.4	64.2 ± 11.2
Height (cm)	152.0 ± 6.341	151.5 ± 5.6	152.3 ± 6.8
BMI (g/m^2)	27.9 ± 4.5	28.3 ± 4.7	27.6 ± 4.3
Body fat (%)	38.3 ± 6.3	39.0 ± 6.8	37.9 ± 6.0
BMD L2-L4 (g/cm^2)	0.978 ± 0.178	0.959 ± 0.170	0.989 ± 0.182
BMD FN (g/cm^2)	0.869 ± 0.136	0.862 ± 0.127	0.876 ± 0.121
BMD WT (g/cm^2)	0.684 ± 0.158	0.671 ± 0.143	0.690 ± 0.165

IA = Insufficiently actives; AT = actives; BMI = Body Mass Index; BMD = Bone Mineral Density; FN = Femoral Neck; WT = Ward's Triangle.

There were no difference between IA and AT for any variable

Table 2. Characteristics of smoking status, hormonal replacement therapy, calcium supplementation, prevalent chronic diseases and physical activity level of the volunteers.

Characteristics	*N* (%)
Smokers	
Yes	4 (2.1)
No	188 (97.9)
Obesity	
Yes (BMI > 30Kg/m^2)	55 (28.6)
No (BMI < 30Kg/m^2)	137 (71.4)
Hormonal Replacement Therapy	
Yes	33 (17,2)
No	159 (82,8)
Calcium Supplementation	
Yes	58 (30.2)
No	134 (69.8)
Prevalent Chronic Diseases	
Hypertension	127 (66.1)
Type II Diabetes	32 (16.7)
Physical Activity Level	
Sedentary	8 (4.1)
Insufficiently Active	67 (34.9)
Active	114 (59.4)
Very Active	3 (1.6)

Table 3. Allelic frequencies, genotype distribution and dbSNP numbers of the studied polymorphisms.

VDR Polymorphism	dbSNP number	Allelic frequency				Genotype distribution		
		Allele 1 (A1)		Allele 2 (A2)		A1/A1	A1/A2	A2/A2
FokI	rs10735810	C	0.652	T	0.348	41.6%	45.8%	12.6%
BsmI	rs1544410	G	0.681	A	0.319	48.9%	38.6%	12.5%
ApaI	rs7975232	A	0.588	C	0.412	32.4%	52.0%	15.6%
TaqI	rs731236	T	0.666	C	0.334	48.6%	36.3%	15.1%

dbSNP = single nucleotide polymorphism data bank (National Center for Biotechnology Information - NCBI); VDR = vitamin D receptor.

Table 4. Characteristics of the subjects in accordance with the FokI genotypes and physical activity level. Values are expressed as means ± Standard deviation.

	IA			AT			
FokI	C/C (*n*=29)	C/T (*n*=36)	T/T (*n*=10)	C/C (*n*=50)	C/T (*n*=51)	T/T (*n*=14)	p[a]
Age (years)	66.6 ± 3.7	66.7 ± 5.3	66.1 ± 6.5	66.6 ± 5.8	66.4 ± 4.8	67.3 ± 4.1	0.925
Body mass (kg)	61.0 ± 8.5	68.1 ± 13.0	67.0 ± 17.5	65.1 ± 12.0	62.7 ± 10.2	62.1 ± 7.8	0.171
Height (cm)	151.0 ± 5.4	152.2 ± 5.4	151.8 ± 8.1	153.3 ± 5.6	150.9 ± 7.4	152.0 ± 7.2	0.314
BMI (g/m^2)	26.8 ± 3.8	29.3 ± 4.8	28.9 ± 6.5	27.6 ± 4.3	27.5 ± 3.5	27.0 ± 4.0	0.415
Body fat (%)	38.07 ± 7.61	40.18 ± 6.20	38.09 ± 7.90	37.77 ± 6.24	37.94 ± 5.51	37.14 ± 5.71	0.830
BMD L2-L4 (g/cm^2)	0.978 ± 0.193	0.951 ± 0.164	0.943 ± 0.191	1.010 ± 0.180	0.984 ± 0.142	0.913 ± 0.298	0.839
BMD FN (g/cm^2)	0.874 ± 0.109	0.870 ± 0.137	0.829 ± 0.138	0.859 ± 0.130	0.880 ± 0.129	0.902 ± 0.187	**0.012***,†,#
BMD WT (g/cm^2)	0.696 ± 0.130	0.678 ± 0.152	0.641 ± 0.149	0.665 ± 0.141	0.693 ± 0.150	0.768 ± 0.225	**0.002***,#

IA = Insufficiently actives; AT = actives; BMI = body mass index; BMD = bone mineral density; FN = femoral neck; WT = Ward's triangle

[a] p-value for interaction between physical activity level and VDR polymorphism.

* T/T-AT > T/T-IA

† T/T-AT > C/C-AT

T/T-AT > C/T-AT and C/C-AT

Table 5. List of Phased Haplotypes Reconstructed Through EM Algorithm and Its Respective Frequencies. Haplotype follows the gene sequence of markers as TaqI, ApaI, BsmI, FokI, and Cdx2 polymorphisms.

ID	Haplotype	Frequency
1	CAACG	0.166
2	TCGCG	0.153
3	TCGCA	0.121
4	TCGTG	0.096
5	TAGCG	0.066
6	CAATG	0.065
7	TAGTG	0.056
8	CAATA	0.048
9	TAGCA	0.046
10	TCGTA	0.036
11	TAGTA	0.034
12	CAGCA	0.034
13	TAACA	0.033
14	TAACG	0.022
15	CAGTG	0.021

EM = expectation-maximization.