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ORIGINAL ARTICLE

Effect of age, gender and calciotropic hormones on the relationship between vitamin D receptor gene polymorphisms and bone mineral density

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Background/Objectives: Hypovitaminosis D is a major public health problem worldwide and unexpectedly more so in sunny countries. Vitamin D receptor (VDR) gene is associated with inter-individual variance in bone mineral density (BMD). Studies assessing the effect of VDR gene polymorphisms on BMD yielded conflicting results. The aim of this study was to assess the relationship between VDR polymorphisms and BMD in the Lebanese, across age groups and genders and to assess the effect of PTH and lean mass and vitamin D levels on such relationship.
Subjects/Methods: In total, 203 subjects aged 65–85 years and 336 children aged 10–17 years. Polymorphisms in the VDR gene were assessed with the restriction enzymes *Bsm*I, *Taq*I and *Apa*I. Bone mineral content, BMD and lean mass were measured using Dual-Energy X-ray Absorptiometry (DXA). The dominant hand strength was measured in children.
Results: Heterozygote genotype was the most frequent in both age groups. There was no difference in the frequency distribution of genotypes between the young and the elderly. No relationship between VDR genotypes and lean mass was found in either age group. Heterozygote boys had the lowest parathormone (PTH) and heterozygote elderly women had the highest BMD at the spine and forearm.
Conclusions: In the Lebanese, the relationship between VDR polymorphisms and BMD differs by age. Survival does not seem to differ by VDR genotype. However, further studies are needed to assess the effect of VDR gene polymorphisms on mortality *per se* and time to mortality, not evaluated in this study.
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Keywords: bone mineral density; gene polymorphisms; vitamin D; vitamin D receptor; survival

Introduction

Hypovitaminosis D is a major public health problem worldwide and unexpectedly more so in sunny countries such as ones in the Middle East (El-Hajj Fuleihan and Vieth, 2007; Lips, 2007; Kimball *et al*., 2008; Holick and Chen, 2008). The biological effects of vitamin D on its classic target organs, in particular muscle and bone, are mediated through the cytosolic vitamin D receptor (VDR).

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Studies assessing the association between VDR gene polymorphisms and bone mineral density (BMD) in different populations led to conflicting results. In adults, this relationship varied with gender, menopausal status, ethnic group and according to the skeletal site measured (Hustmyer *et al*, 1994; Morrison *et al*., 1994; Keen *et al*., 1997; McClure *et al*., 1997; Laaksonen *et al*., 2002; Pottelbergh *et al*., 2002; Duman *et al*., 2004; Macdonald *et al*., 2006; Saadi *et al*., 2006). In children, the results differed according to gender, pubertal status and to the restriction enzyme used (Ames *et al*., 1999; Laaksonen *et al*., 2004). It is not clear whether the role of VDR gene polymorphisms on bone mass, if any, is mediated through impairment in bone gain during childhood or through acceleration of bone loss in older age and therefore the effect of age on the relationship between VDR polymorphisms and bone mass is unknown. Furthermore, no

such studies have been conducted in the same population across genders.

Recently, our group showed that the deleterious effect of hypovitaminosis D on bone may in large part be mediated through lean mass and PTH (Arabi *et al*., 2006). VDR is widely expressed in muscle and parathyroid glands, but it is unclear whether the relationship between VDR genotypes and bone mass is mediated through an effect on lean mass, PTH, or both.

Some studies assessed the relationship between VDR genotypes and muscle strength and/or lean mass in humans (Guesens *et al*., 1997; Pottelbergh *et al*., 2002; Grundberg *et al*., 2004), and between VDR and PTH levels (McClure *et al*., 1997; Pottelbergh *et al*., 2002; Zofkova *et al*., 2003; Macdonald *et al*., 2006). The expression of VDR in muscle decreases significantly with aging (Bischoff-Ferrari *et al*., 2004). This may result in a decreased functional response of the muscle cells to vitamin D and may thus partially explain the lack of the relationship between VDR with muscle, bone or both in older age groups.

In the adolescent study, it has been noted that calcium intake modulates the relationship between VDR and bone mass (Ferrari *et al*., 1998) but it is unclear whether circulating 25 (OH) vitamin D (25-OHD) level also has a similar modulatory effect on the relationship between VDR and bone. Chronic modulation by lifestyle factors may bring out a relationship between VDR and bone mass with age, which may not be present in younger age groups. It is well known that peak bone mass is strongly influenced by heredity. Subsequently, BMD in children and young adults maybe strongly related to genetic factors more than environmental factors. As lifestyle may be cumulated throughout life, its effect on BMD may become dominant in the elderly and overcome the genetic effect.

Besides its calcemic activities, vitamin D has other important roles in human health (Nagpal and Rathnachalam, 2005; Staud, 2005). Vitamin D has potent immunomodulatory effects on many immune cell types. A very low serum vitamin D level was found to be a major risk factor for developing prostate cancer and colon cancer. Moreover, vitamin D supplementation has been shown to inhibit tumor progression in patients with breast cancer and to inhibit tumor growth of skin cancer (Nagpal and Rathnachalam, 2005; Staud, 2005). Circulating 25-OHD levels were also associated with improved survival in colorectal and lung cancer patients and vitamin D insufficiency was observed in various other diseases such as autoimmune, infectious, musculoskeletal, neurological and cardiovascular diseases (Pilz *et al*., 2009). In patients with chronic kidney diseases, serum 25-OHD levels <15 ng/ml had an increased risk for all-cause mortality when compared with those with levels over 30 ng/ml (Mehrotra *et al*., 2009).

Consistent with these effects, VDR is widely expressed in most immune cell types, normal prostate tissues, breast tissues, colon cells and hematological cells. Moreover, some diseases such as rheumatoid arthritis and some cancers show increased levels of VDR protein when compared with their normal counterparts (Nagpal and Rathnachalam, 2005; Staud, 2005). VDR genotypes may affect the incidence of cancer and/or autoimmune disease and ultimately mortality. Such difference would result in a change in VDR genotype distribution with aging. In a recent study in patients with non-small lung cancer, the T allele of the VDR FokI T polymorphism and the G-T-C (Cdx2-*Fok*I-*Bsm*I) haplotypes were associated with worse survival (Heist *et al*., 2008).

The objectives of this study were:

1. To investigate the relationship between bone mass and VDR gene polymorphisms in the Lebanese, across age groups and genders.
2. To investigate the relationship between bone mass and VDR polymorphisms, after adjustment for all other confounders such PTH and lean mass, calcium intake and vitamin D levels.
3. To explore the possibility that certain VDR genotypes may incur a survival benefit by studying such genotypes using three restriction enzymes in a population that includes both genders at extremes of lifecycle and of the same ethnicity.

Materials and methods

Study participants

Elderly population. In all, 460 elderly, aged 65–85 years (73 ± 5.2) participated in a population-based study aiming at assessing the prevalence of osteoporosis in the Lebanese elderly. The participants were randomly recruited from Beirut area, by a multilevel cluster technique using geographical maps. All participants were resident in the Beirut with both parents of Middle Eastern origin. The study was conducted in two different centers. Almost one-third of the subjects contacted refused to participate in the study. Thus, the rejection rate was $\sim$30%. VDR polymorphisms were determined in 203 subjects assessed at the first center, therefore only the data of these participants (132 women and 71 men) were used in the analyses.

Children and adolescents. Three hundred and sixty-three healthy school children (184 boys and 179 girls) between 10 and 17 years of age were enrolled in a randomized, double-blind, placebo-controlled trial evaluating the efficacy of vitamin D supplementation on skeletal health. Participants were recruited from four schools in Beirut with balanced representation from both socioeconomic statuses. VDR polymorphisms were determined in 336 (169 boys and 167 girls) children. Data obtained at baseline were used for the purpose of this study.

Subjects from both age groups were excluded if they had a medical condition or took medications likely to affect bone metabolism.

Informed consent

Both studies were approved by the Institutional Review Board of the American University of Beirut. Written

informed consent was obtained from all the elderly participants, and from all the children and/or one of their parents before participation in the study.

Assessments

Clinical assessment. In the elderly, physical examination included height (cm), weight (kg) and calcium intake (mg/day). The estimated time that the study subjects spent outdoors was assessed by asking them regarding the time spent outdoors during the week preceding the screening visit. Supplementation with calcium and/or vitamin D was assessed. Dairy calcium intake was assessed using a food frequency questionnaire that evaluated the consumption of calcium-enriched foods, mostly from dairy products.

Children underwent a physical examination including height (cm), weight (kg) and pubertal stage. Pubertal status was determined using pubic hair stages according to Tanner criteria. Exercise frequency (hours/week), calcium intake (mg/day) and sun exposure were assessed with questionnaires. Exercise frequency was assessed on the basis of a questionnaire inquiring regarding the average number of hours spent on sports per week. The dietary questionnaire used was derived from a more extensive and validated version that we developed in our unit, which showed that 80% of the calcium intake in adolescents is from dairy products. Therefore, the food frequency questionnaire used stressed the consumption of dairy products. Sun exposure was assessed as the average number of hours spent in the sun for weekdays and weekends and the prorated average was reported.

Biochemical assessments. Serum calcium, phosphorus, alkaline phosphatase were measured by standard calorimetric methods, using the Hitachi 912 analyzer (Mannheim, Germany). Serum 25-OHD (ng/ml) was measured by a competitive protein-binding assay (Diasorin Incstar, Diasorin, Saluggia, Italy). The normal range as reported in the kit was 10–60 ng/ml. Serum PTH was measured by ELSA-PTH immunoradiometric assay (CisBio International, Gif-Sur-Yvette, Cedex, France). The normal range as reported in the kit was 8–78 pg/ml. The intra-assay and the inter-assay variability for 25-OHD and PTH in our lab were below 10%.

Bone mineral density. Bone mineral content (BMC) of the total body (grams), BMD (g/cm^2) of the antero-posterior lumbar spine (L1–L4), the left femur, the left forearm (1/3 radius), as well as lean mass (grams) were measured by DXA, using a Hologic 4500A device (Hologic, Bedford, MA, USA).

Quality control of the densitometer. In all, 83 serial duplicate scans were performed *in vivo*. The mean ± s.d. for precision, expressed as the coefficient of variation (CV %) were calculated and were as follows: Lumbar spine = 0.90 ± 0.79%, total hip = 0.84 ± 0.70%, femoral neck = 1.35 ± 1.14%, trochanter = 1.08 ± 0.84% and forearm = 1.02 ± 0.72%.

VDR genotyping. DNA was extracted from whole blood and polymorphisms in the VDR gene were assessed by PCR amplification of the specific region of the gene, followed by digestion with the restriction enzymes *Bsm*I, *Taq*I and *Apa*I and fragment separation by agarose gel electrophoresis (Morrison *et al*., 1994). Alleles were designated according to the presence or absence of the specific restriction enzyme cleavage site, with capital letters denoting the absence and small letters denoting the presence of the restriction site.

Statistical analyses

Analyses assessing the effect of VDR genotypes on BMD and BMC were performed in the children and in the elderly separately.

Chi-square test was used to assess difference in VDR genotype distribution between genders or between age groups, as well as the difference in VDR genotype distribution between our study population and others. Independent *t*-tests were performed to assess the relationship between continuous variables and gender. One-way analyses of variance analysis of variance were performed to assess the relationship between VDR genotype and continuous outcomes such as BMD, BMC, PTH, 25-OHD, lean mass and muscle strength.

A series of multivariate analyses were performed to assess the effect of VDR genotype on BMD or BMC after adjustment for other covariates. These multivariate analyses were based on linear regression models using all enter method, in which BMD or BMC of a specific skeletal site was the outcome and the well-known predictors of bone density in children and elderly were the independent or confounding variables. In children, the confounders were age, height, lean mass, pubertal stage, socioeconomic status, exercise, 25-OHD, calcium intake, PTH and VDR genotype. In the elderly, the confounders were age, height, lean mass, 25-OHD, PTH and VDR genotype. The models were built with and without interaction between vitamin D and VDR and results of the best-fit model were reported. All the models were built for each restriction enzyme separately.

P-values below 0.05 were considered statistically significant for all statistical tests. Analyses were performed using STATA, version 7 (StataCorp, College Station, TX, USA).

Results

Findings in children and adolescents

Characteristics of the study population. The mean age was 12.8 ± 1.9 in boys and 13.0 ± 2.1 in girls. Boys had higher body mass index than girls (21.1 ± 4.2 versus 20.1 ± 3.5, $P = 0.01$). They had more sun exposure and spent more time in sport than girls (7.8 ± 6 versus 3.8 ± 4 h per week, $P < 0.001$). Daily calcium intake was 756.6 ± 352 mg in boys and 670.6 ± 359 mg in girls ($P = 0.01$). The mean serum calcium, phosphorus, alkaline phosphatase and PTH were within the

normal range in both genders. 25-OHD levels were low in both genders; they were lower in girls than in boys ($P=0.004$).

25-OHD correlated with BMC and BMD at multiple skeletal levels (data previously published) (El-Hajj Fuleihan *et al*., 2006).

Relationships between VDR genotype and classical determinants of bone density

VDR gene polymorphisms and anthropometrical and lifestyle parameters. there was no difference in mean age, height, weight, sun exposure, exercise and calcium intake between the three VDR genotypes using each of the enzymes *Bsm*I, *Apa*I or *Taq*I in either gender. Similarly, there was no significant relationship between VDR polymorphism frequency distribution and Tanner stages or socioeconomic status.

VDR gene polymorphisms and vitamin D, PTH and lean mass. there was no relationship between VDR polymorphisms, assessed by *Bsm*I restriction enzyme, and lean mass in either gender (Table 1). Similarly, there was no difference in mean 25-OHD between the different VDR genotypes in either gender (Table 1). The same results were obtained using *Taq*I and *Apa*I restriction enzymes (data not shown). Boys with the Bb genotype had lower PTH levels compared with homozygotes ($P=0.01$) (Table 1). Similar results were obtained using the *Taq*I enzyme ($P=0.004$).

Relationships between VDR genotype and bone mass. There was no significant difference in mean BMC or BMD at any skeletal site between different genotypes using the *Bsm* restriction enzyme in either gender (Table 1). There was no interaction between VDR and 25-OHD in the association between VDR genotypes and BMD/BMC in children. These findings were consistent with the *Taq*I and *Apa*I enzymes (data not shown).

The lack of relationship between BMC and BMD and VDR genotypes persisted after adjustment for age, height, pubertal stages, exercise, calcium intake, vitamin D and PTH (data not shown).

Findings in the elderly

Characteristics of the study population. The mean age was 73.6 ± 5.0 years in men and 74.5 ± 5.1 years in women. Men had lower body mass index than women (27.4 ± 4 versus 30.8 ± 6, $P<0.001$). Men spent more time outdoors than women (3.1 ± 4 versus 1.4 ± 2.1 h per week, $P=0.001$). The mean serum calcium, phosphorus, alkaline phosphatase and PTH were normal in both genders. 25-OHD were low in both genders, they were lower in women than in men (11.3 ± 3 versus 9.5 ± 3 $P=0.003$).

The relationship between 25-OHD and bone mass was previously published (Arabi *et al*., 2006). 25-OHD correlated with BMD at multiple skeletal levels (Arabi *et al*., 2006).

Relationships between VDR genotype and classical determinants of bone density

VDR gene polymorphisms and anthropometrical and lifestyle parameters. there was no difference in mean age, height, weight, time spent outdoors between the three VDR genotypes using *Bsm*I, *Apa*I or *Taq*I in either gender.

VDR gene polymorphisms and vitamin D, PTH and lean mass. no relationship was detected between VDR polymorphisms and lean mass or between 25-OHD levels and VDR genotypes in either gender (Table 2). Similarly, there was no relationship between PTH and VDR genotypes in men. In women, heterozygote subjects for *Bsm*I and *Apa*I tended to have lower PTH levels than homozygote subjects, but this difference did not reach statistical significance (Table 2).

Table 1 Musculoskeletal parameters according to the VDR genotype using *Bsm*I restriction enzyme in children[a]

	Boys			*Girls*		
	bb (N = *62*)	*Bb* (N = *81*)	*BB* (N = *24*)	*bb* (N = *48*)	*Bb* (N = *88*)	*BB* (N = *31*)
25-OHD (ng/ml)	15.3 ± 5.0	17.1 ± 7.2	16.2 ± 7.8	14.3 ± 9.4	14.0 ± 7.3	14.5 ± 8.6
PTH (pg/ml)	22.2 ± 25[b]	16.2 ± 18[b,c]	21.8 ± 18[c]	23.0 ± 34.6	17.8 ± 22.1	12.1 ± 13.2
Lean mass (grams)	35 876 ± 11 175	34 410 ± 10 329	35 616 ± 10 788	30 468 ± 6698	29 577 ± 5725	29 761 ± 5971
Lumbar spine BMC (grams)	37.21 ± 11.4	35.23 ± 12.2	37.80 ± 14.2	42.11 ± 12.6	37.33 ± 10.4	39.20 ± 12.3
Lumbar spine BMD (g/cm^2)	0.723 ± 0.13	0.689 ± 0.14	0.715 ± 0.14	0.820 ± 0.17	0.767 ± 0.14	0.789 ± 0.15
Total hip BMC (grams)	29.43 ± 9.2	26.90 ± 9.0	28.30 ± 10.5	23.81 ± 6.0	23.11 ± 5.1	22.91 ± 5.8
Total hip BMD (g/cm^2)	0.864 ± 0.12	0.828 ± 0.14	0.858 ± 0.15	0.790 ± 0.13	0.784 ± 0.11	0.782 ± 0.13
Femoral neck BMC (grams)	3.91 ± 0.8	3.70 ± 0.8	3.84 ± 1.0	3.30 ± 0.8	3.23 ± 0.6	3.31 ± 0.6
Femoral neck BMD (g/cm^2)	0.789 ± 0.11	0.770 ± 0.12	0.794 ± 0.14	0.739 ± 0.12	0.717 ± 0.10	0.718 ± 0.11
Radius BMC (grams)	1.42 ± 0.3	1.44 ± 0.3	1.40 ± 0.3	1.34 ± 0.2	1.25 ± 0.2	1.30 ± 0.2
Radius BMD (g/cm^2)	0.578 ± 0.07	0.566 ± 0.07	0.558 ± 0.07	0.584 ± 0.06	0.617 ± 0.08	0.574 ± 0.07
Subtotal body BMC (grams)	1281 ± 439	1170 ± 437	1235 ± 481	1199 ± 333	1102 ± 315	1146 ± 333

Abbreviations: BMC, bone mineral content; BMD, bone mineral density; VDR, vitamin D receptor.
[a]Similar results were obtained with *Taq*I restriction enzyme.
[b,c]Values with same superscript are significantly different from each others ($P<0.05$).

Table 2 Musculoskeletal parameters according to the VDR genotype using *Bsm*I restriction enzyme in the elderly[a]

	Men			*Women*		
	bb (N = *18*)	*Bb* (N = *40*)	*BB* (N = *13*)	*bb* (N = *44*)	*Bb* (N = *56*)	*BB* (N = *30*)
25-OHD (ng/ml)	11.0 ± 3.6	11.7 ± 4.1	10.5 ± 2.5	9.4 ± 2.6	9.6 ± 3.9	8.9 ± 2.8
PTH (pg/ml)	42.8 ± 22.8	59.6 ± 59.4	67.8 ± 95.8	48.9 ± 26.1	45.2 ± 22.5	55.7 ± 36.2
Lean mass (grams)	46 083 ± 6436	47 670 ± 6989	45 337 ± 4499	36 614 ± 5368	37 595 ± 8196	37 674 ± 031
Lumbar spine BMD (g/cm^2)	0.810 ± 0.08	0.897 ± 0.17	0.863 ± 0.07	0.733 ± 0.13[b]	0.804 ± 0.14[b,c]	0.740 ± 0.11[c]
Total hip BMD (g/cm^2)	0.816 ± 0.11	0.820 ± 0.14	0.857 ± 0.10	0.688 ± 0.10	0.725 ± 0.12	0.689 ± 0.13
Femoral neck BMD (g/cm^2)	0.649 ± 0.06	0.671 ± 0.13	0.680 ± 0.09	0.572 ± 0.08	0.612 ± 0.10	0.601 ± 0.10
Forearm BMD (g/cm^2)	0.638 ± 0.07	0.648 ± 0.10	0.665 ± 0.08	0.476 ± 0.08[b]	0.526 ± 0.08[b]	0.481 ± 0.08
Subtotal body BMC (grams)	1524 ± 196	1652 ± 421	1586 ± 270	1121 ± 285	1164 ± 238	1095 ± 221

Abbreviations: BMC, bone mineral content; BMD, bone mineral density; VDR, vitamin D receptor.
[a]Similar results were obtained with *Taq*I restriction enzyme.
[b,c]Values with same superscript are significantly different from each others ($P<0.05$).

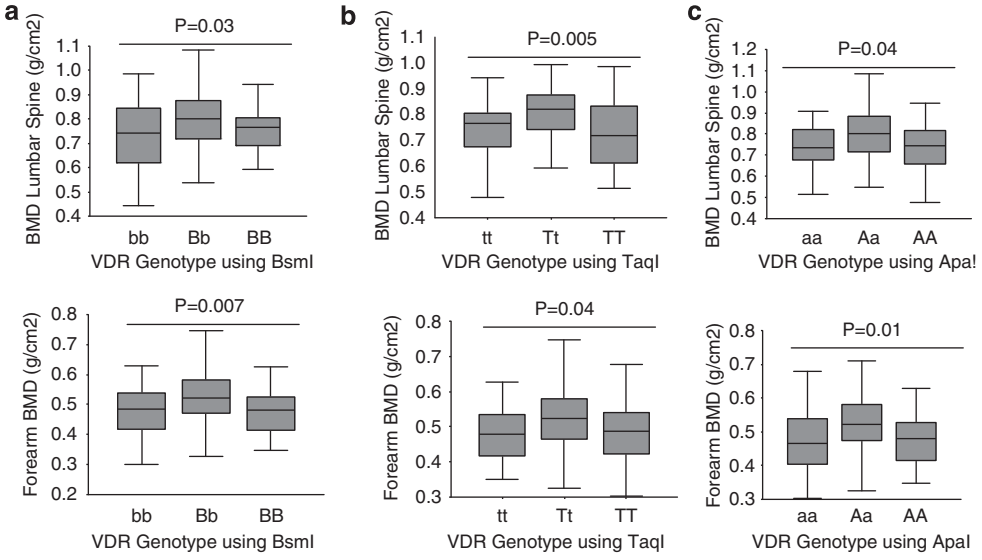

Figure 1 Box plots showing the median, 25th and 75th percentiles of BMD in women according to VDR genotypes. There was a significant difference between VDR genotypes using *Bsm*I enzyme in the lumbar spine BMD ($P=0.03$) and forearm BMD ($P=0.007$), by analysis of variance (ANOVA) (panel **a**). There was a significant difference between VDR genotypes using *Taq*I enzyme in the lumbar spine BMD ($P=0.005$) and forearm BMD ($P=0.04$) by ANOVA (panel **b**). There was a significant difference between VDR genotypes using *Apa*I enzyme in the lumbar spine BMD ($P=0.04$) and forearm BMD ($P=0.01$) by ANOVA (panel **c**). Only data showing a significant relationship between VDR genotypes and BMD are shown in the figures.

Relationships between VDR genotype and bone mass. Heterozygote women had higher BMD at the spine and the forearm compared with homozygotes (Figure 1). There was no interaction between 25-OHD and VDR genotypes at any skeletal site. The relationship between VDR genotypes and BMD was consistent with *Bsm*I, *Taq*I and *Apa*I and it persisted after adjustment for age, height, lean mass, 25-OHD and PTH (Table 3). Lean mass and VDR genotypes were independent predictors for lumbar spine and forearm BMD in multivariate analyses and this finding was consistent with *Bsm*I, *Apa*I and *Taq*I enzyme (Table 3). No similar relationship between VDR genotypes and BMD was observed in men.

Frequency distribution of VDR genotypes

The frequency distributions of VDR genotypes were in Hardy–Weinberg equilibrium. In both age groups, heterozygote genotypes were more prevalent within gender and in the overall population. There was no significant difference in the distribution of VDR genotypes for *Bsm*I, *Apa*I or *Taq*I between genders in either group. There was no difference in genotype distribution between children and elderly in either gender. In addition, there was no difference in the frequency distributions of genotypes with those reported in Western and other Arab populations. However, the frequency distributions differed from those reported in Taiwanese (Table 4).

Table 3 Multivariate analyses showing the adjusted relationship between VDR gene polymorphisms and BMD in women[a]

Enzyme	*Predictors*	*Spine*			*Forearm*		
		β-estimate (s.e.)	*95% CI*	P	*β-estimate (s.e.)*	*95% CI*	P
*Bsm*I							
	Age	−0.001 (0.002)	(−0.006; 0.003)	0.5	−0.002 (0.001)	(−0.004; 0.0007)	0.1
	Height	−0.001 (0.002)	(−0.006; 0.003)	0.5	0.0009 (0.001)	(−0.001; 0.003)	0.4
	Lean mass	0.000011 (2×10^{-6})	($6.6\times10-6$; 0.00001)	<0.001	7.4×10^{-6} (1.2×10^{-6})	($4.8\times10-6$; $9.9\times10-6$)	<0.001
	PTH	−0.0003 (0.004)	(−0.001; 0.0005)	0.4	−0.0004 (0.0002)	(−0.0009; 0.00001)	0.05
	25-OHD	0.003 (0.004)	(−0.004; 0.01)	0.4	0.01 (0.002)	(−0.002; 0.005)	0.4
	Bb	0.03 (0.02)	(0.01; 0.09)	0.01	0.034 (0.01)	(0.006; 0.06)	0.01
	BB	−0.01 (0.03)	(−0.08; 0.05)	0.7	−0.007 (0.016)	(−0.23; 0.69)	0.6
*Apa*I							
	Age	−0.002 (0.002)	(−0.006 ; 0.002)	0.4	−0.002 (0.001)	(−0.005 ; −0.0002)	0.02
	Height	−0.001 (0.002)	(−0.006 ; 0.0033)	0.5	0.0005 (0.001)	(−0.0018 ; 0.0029)	0.6
	Lean mass	0.0001 (2×10^{-6})	($7\times10-6$; 0.0001)	<0.001	7.7×10^{-6} (1.2×10^{-6})	($5.2\times10-6$; 0.00001)	<0.001
	PTH	−0.0003 (0.0004)	(−0.001 ; 0.0004)	0.3	−0.0003 (0.0002)	(−0.00084 ; 0.0001)	0.1
	25-OHD	0.002 (0.003)	(−0.005 ; 0.009)	0.5	0.0017 (0.0019)	(−0.002 ; 0.005)	0.3
	Aa	0.02 (0.03)	(0.01 ; 0.09)	0.05	0.042 (0.018)	(−0.006 ; 0.01)	0.02
	AA	−0.02 (0.4)	(−0.10 ; 0.05)	0.5	−0.006 (0.01)	(−0.11 ; 0.77)	0.7
*Taq*I							
	Age	0.0008 (0.002)	(−0.004; 0.005)	0.7	−0.002 (0.001)	(−0.005; −0.0001)	0.03
	Height	−0.001 (0.002)	(−0.005; 0.005)	0.6	0.0005 (0.001)	(−0.0019; 0.0029)	0.6
	Lean mass	0.000012 (2×10^{-6})	($8\times10-6$; 0.00001)	<0.001	7.6×10^{-6} (1.2×10^{-6})	($5.0\times10-6$; 0.00001)	<0.001
	PTH	−0.0006 (0.0004)	(−0.001; 0.0002)	0.1	−0.0004 (0.0002)	(−0.0009; 0.00007)	0.09
	25-OHD	0.03 (0.003)	(−0.005; 0.009)	0.5	0.05 (0.002)	(−0.001; 0.006)	0.250
	Tt	0.04 0.07 (0.03)	(0.008; 0.13)	0.02	0.04 (0.01)	(0.012; 0.07)	0.007
	TT	−0.002 (0.03)	(−0.06; 0.06)	0.9	0.01 (0.01)	(−0.02; 0.04)	0.5

Abbreviations: BMD, bone mineral density; CI, confidence interval; VDR, vitamin D receptor.
[a]References were bb for *Bsm*I, aa for *Apa*I and tt for *Taq*I.

Discussion

This study, based on a population that included different age groups and both genders, showed that heterozygote genotypes for *Bsm*I, *Apa*I and *Taq*I were more prevalent in children and in the elderly, without difference between genders or between age groups. Heterozygote subjects had the lowest PTH values in boys, with a similar trend in elderly women, who also had the highest BMD at the spine and the forearm.

The effect of vitamin D on human health does not seem to be limited to musculo-skeletal function. Indeed, vitamin D has recently emerged as an important risk factor for several health problems associated with increased mortality such as prostate, breast and colon cancer, diabetes, and cardiovascular diseases (Nagpal and Rathnachalam, 2005; Staud, 2005). It is possible that VDR polymorphisms modulate the relationship between vitamin D deficiency and health conditions with increased mortality. The assessment of such effects was beyond the aims of this study, however, the lack of difference in the frequency distribution of VDR genotypes between the children and the elderly within the same population excludes any major survival effect of specific VDR polymorphisms and renders the role of VDR as a major modulator of the relationship between vitamin D and conditions associated with high mortality less likely.

Ethnic differences in the VDR genotype frequencies have been reported. Studies of Asian populations showed that more than 90% of the subjects were classified as bb genotype (Lau *et al*., 1999; Chang *et al*., 2000). Conversely, most studies in Western populations showed that the heterozygous genotypes were the most common (Hustmyer *et al*., 1994; Garnero *et al*., 2005; Macdonald *et al*., 2006). Similarly to Western population, VDR genotype frequencies in Arab Emirati women showed that the Bb genotypes are the most common (Saadi *et al*., 2006), in accordance with our findings.

There was a relationship between VDR genotypes and BMD in women but not in men and in the elderly but not in children. The relationship between BMD and VDR genotypes has been previously studied and the results yielded controversial results, according to the age, gender, ethnic group studied and to the enzyme used. In a large meta-analyses (Uitterlinden *et al*., 2006), there was no relationship between BMD and VDR using *Cdx2, Fok*I, *Apa*I, *Taq*I and *Bsm*I polymorphisms. In this meta-analysis, the authors analyzed *Cdx2* ad *Fok*I as single-nucleotide polymorphisms separately but they analyzed *Apa*I, *Taq*I and *Bsm*I polymorphisms as haplotypes. In our study, we analyzed these three enzymes separately because the sample size does not allow haplotype analyses. This may explain why our results differ. Moreover, our populations' studies are from a homogeneous ethnic

Table 4 Frequency distribution of VDR genotype in Lebanese children and elderly and other populations[a]

	Lebanese						*Western*					
	Boys N = *169*	*Girls* N = *167*	*Overall* N = *336*	*Men*	*Women*	*Overall*	*British*[b] N = *3100*	*American*[c] N = *125*	*French*[d] N = *589*	*Arab Emirati*[e] N = *259*	*Asian Taiwani*[f] N = *268*
bb	62 (37%)	48 (29%)	110 (33%)	18 (25%)	44 (34%)	62 (31%)	34	35	36	20	93.1
Bb	81 (48%)	88 (53%)	169 (50%)	40 (56%)	56 (43%)	96 (48%)	48	50	49	44	6.5
BB	24 (15%)	31 (18%)	56 (17%)	13 (19%)	30 (23%)	43 (21%)	18	15	15	36	0.4
aa	31 (18%)	22 (13%)	53 (16%)	6 (8%)	17 (13%)	23 (12%)	19	25	—	—	52.4
Aa	79 (47%)	82 (49%)	161 (48%)	36 (51%)	66 (50%)	102 (50%)	50	47	—	—	42.3
AA	59 (35%)	63 (38%)	122 (36%)	29 (41%)	49 (37%)	78 (38%)	30	28	—	—	5.3
tt	23 (14%)	24 (15%)	47 (14%)	11 (16%)	27 (21%)	39 (20%)	16	14	—	—	0.4
Tt	81 (48%)	89 (53%)	170 (51%)	42 (59%)	57 (43%)	99 (48%)	48	47	—	—	5.6
TT	65 (38%)	54 (32%)	119 (35%)	18 (25%)	48 (36%)	66 (32%)	36	49	—	—	9.4

Abbreviation: VDR, vitamin D receptor.
[a]No significant difference between genders within the same age group; no significant difference between age group within the same gender; no significant difference between the children and the elderly in the overall group.
[b,c,d,e]No significant difference in VDR genotype distribution compared with this study.
[f]Significantly different VDR genotype distribution compared with this current study ($P < 0.05$).
References: (a) Macdonald *et al*. (2006), based on 3100 women aged 50–63 years; (b) Hustmyer *et al* (1994), based on 63 premenopausal pairs (mean age 35 years) and 43 postmenopausal pairs (mean age 35 years) of white female twins; (c) Garnero *et al*. (2005), based on 589 postmenopausal women (mean age, 62 years); (d) Saadi *et al*. (2006), based on 175 pre- and 84 postmenopausal; age range 20–85 years; (e) Chang *et al*. (2000), based on 248 healthy young subjects.

group, which may not be the case of the populations analyzed in the meta-analysis.

Higher BMD was observed in heterozygote women. In genetic research, linear models, recessive models or dominant models rather than heterozygote effects are usually observed. Reasons behind the high BMD values in heterozygote subjects in this study are not clear. One possible explanation may be 'heterozygote advantage' in which the presence of one mutant allele alone provides a 'survival' advantage over its absence or its presence in the homozygous state. Examples of heterozygote advantage have been described in the literature and help to explain the high frequency of some mutant alleles in specific populations. For example, carriers of β-thalassemia have an increased resistance to the malaria parasite as compared with homozygotes with two normal globin genes and to patients affected with this hemoglobin disorder, which explains the high frequency of the disease in the Mediterranean region, parts of Asia and Africa (Flint *et al*., 1993). Similarly, it is possible that heterozygotes for VDR polymorphisms have an advantage, through an unknown mechanism, over homozygote individuals. Higher BMD in heterozygote subjects has also been reported by a previous study using *Fok*I enzyme (Laaksonen *et al*., 2004).

The prevalence of vitamin D deficiency is increasing worldwide (Chapuy *et al*., 1997). People living in sunny countries have always been considered at lower risk of hypovitaminosis D. Nevertheless, a high prevalence of vitamin D deficiency has been reported in Lebanon (Gannage-Yared *et al*., 2000; El-Hajj Fuleihan *et al*., 2001; Arabi *et al*., 2006). Whether some VDR genotypes show resistance to cutaneous production of vitamin D and therefore have a role in this unexpected high prevalence of vitamin D deficiency has been analyzed in limted studies. Baroncelli *et al*.(2008) showed that the VDR B allele may predispose to vitamin D deficiency . Moreover, it has been reported that greater than usual doses of vitamin D are needed to cure rickets in children of the Middle East (Essawy *et al*., 1992; Abdullah *et al*., 2002). However, in view of the lack of difference of serum 25-OHD levels between different VDR genotypes in our study, we cannot conclude that vitamin D status in our population is regulated by VDR polymorphisms.

VDR is widely expressed in muscle (Bischoff *et al*., 2001; Bischoff-Ferrari *et al*., 2004). No study assessed the possible difference in VDR expression in muscles between VDR genotypes. Such difference, if present, would lead to a difference in functional response of muscle to vitamin D and therefore a difference in lean mass between VDR genotypes. There was no relationship between VDR polymorphisms and lean mass in either gender or age group in this study, similarly to what was found in previous studies (Grundberg *et al*., 2004; Garnero *et al*., 2005).

VDR is expressed in parathyroid glands. The difference in PTH levels between genotypes despite similar calcium intake and vitamin D levels suggests a possible difference in the

response of the parathyroid gland to vitamin D, or a difference in the intestinal absorption of calcium. Indeed, Howard *et al*. (1995) suggested a possible resistance to vitamin D action at the intestine and/or parathyroid glands in some VDR genotypes and Ames *et al*. (1999) showed a significant association of VDR genotypes with calcium absorption in children. Despite the difference in PTH levels between VDR genotypes, there was no effect of VDR genotype on bone density in children, even after adjustment for major determinants of bone density at this age. Interestingly, heterozygous children had the lowest PTH levels and heterozygous elderly tended to have the lowest PTH levels and had the highest BMD values. Thus, the relationship between VDR polymorphism and BMD in the elderly maybe mediated, at least in part, by the difference in PTH levels early in life. It is possible that some environmental exposures that affect both PTH levels and bone density such as chronic vitamin D deficiency status, chronic low calcium intake or absorption modulate this relationship, which explains the lack of relationship in children and its presence at an older age.

The study has some limitations. The possibility of type 1 error and the small sample size for a genetic study. However, VDR genotypes distributions were in Hardy–Weinberg equilibrium and the populations of both studies were representative of the relatively small Lebanese population, estimated to be 3 millions only. Although there were differences in BMD by genotype in the elderly and not the young, the cross-sectional nature of the study does not allow for inferences regarding bone loss rates because of VDR polymorphisms in the elderly.

In conclusion, there is no survival benefit of VDR gene polymorphisms. Heterozygote subjects, the most common genotype, had the lowest PTH levels in boys, with a similar trend in elderly women, who also had the highest BMD at the spine and forearm. The relationship between VDR and BMD varies by age and possibly lifestyle factors; this explains, at least in part, the conflicting results of previous studies assessing this relationship.

Conflict of interest

The authors declare no conflict of interest.

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