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Lack of association between vitamin D receptor gene polymorphism (BsmI) and osteomalacia

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Abstract Vitamin D receptor (VDR) gene polymorphism has been reported to be a determinant of bone formation and intestinal calcium absorption. We carried out this study to assess the role of VDR gene polymorphism in the pathogenesis of osteomalacia. We investigated BsmI polymorphisms in the gene encoding the 1,25 dihydroxyvitamin D receptor in 38 patients with osteomalacia and 31 healthy controls, along with examination of serum calcium, phosphorus, alkaline phosphatase, parathyroid hormone, and 25 hydroxyvitamin D levels. *VDR* allelic variants were: BB, 31.6%; Bb, 44.7%; and bb, 23.7% in the osteomalacia patients and BB, 19.4%; Bb, 61.3%; and bb, 19.4% in the controls. Although heterozygotes (Bb) were more frequent than other genotypes in both groups, the BB genotype was found to be more prevalent in osteomalacia than in controls. There was no statistical relationship between VDR genotype and osteomalacia. It is concluded that, in this small group of patients, there was no relationship between *VDR* allelic polymorphisms and osteomalacia.

Key words vitamin D · osteomalacia · vitamin D receptor gene · BsmI polymorphism

Introduction

1, 25-Dihydroxyvitamin D (1, 25 (OH) 2D), is the most important steroid hormone that plays a role in bone mineral metabolism. The precursor, vitamin D, which can be obtained from dietary sources or synthesized in the skin, is sequentially hydroxylated by liver and kidney to its active metabolite 1, 25 (OH)2D. The effects of 1, 25 (OH) 2D are mediated by the vitamin D receptor (VDR), which is a member of the steroid/thyroid hormone nuclear receptor family [1].

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The absence of vitamin D results from inadequate synthesis in the skin, lack of dietary intake, impaired vitamin D activation, or resistance to vitamin D action [1]. Regardless of the cause, the resultant clinical situation results in insufficient intestinal calcium absorption. Inadequate calcium intake influences bone loss and the risk of osteoporosis [1–3]. Genetic factors are also considered to be major determinants of bone mineral mass and bone metabolism and studies suggest that 75%–80% of the variance in adult bone mineral density (BMD) can be attributed to genetic influences [4–9]. In a recent study, genetic factors influencing BMD were examined, and it was found that *VDR* gene and parathyroid hormone (*PTH*) gene polymorphisms were associated with lumbar spine BMD in children [10]. The mechanism by which genetic factors affect bone mass remains to be elucidated. *VDR* gene polymorphisms have been reported to be a determinant of peak bone mass by interfering with intestinal calcium absorption [11–14]. In addition, several studies have shown associations of *VDR* genotypes with bone turnover, calcium intake, response to vitamin D therapy, and response to exercise [15].

Osteomalacia is widely prevalent in Turkey [16]. Several factors, in addition to inadequate dietary intake or sun exposure, could be instrumental in the occurrence of osteomalacia. In this study, the influence of *VDR* gene polymorphism, which has been previously reported to affect calcium absorption, on osteomalacia was investigated.

Subjects and methods

Subjects

Thirty-eight patients (33 female, 5 male; age, 41.92 ± 16.11 years) who met the clinical (bone pain worsening by activity, muscle weakness of the lower extremities,

walking difficulties), biochemical (low or low-normal serum calcium and phosphorus, low serum 25 hydroxyvitamin D [OHD], increased alkaline phosphatase and PTH levels), and radiological (pseudofractures) criteria of osteomalacia were included in this study. Patients with osteomalacia due to causes other than vitamin D depletion, such as hypophosphatemia and renal osteodystrophy were excluded from the study. Thirty-one sex- and age-matched healthy volunteers (29 female, 2 male; age, 39.26 ± 11.98 years) without any known disease of bone were included in the study as a control group. None of the subjects in the two groups were taking medicine affecting bone metabolism. Patients and healthy controls were not classified according to their calcium intake, which was determined to be above 800 mg per day, assessed by asking about 3-day calcium intake. The study was approved by the Ethics Committee of the Research Fund of Istanbul University. Informed consent was obtained from all subjects.

Biochemical analysis

Fasting blood samples were collected for the measurement of calcium (Ca), phosphorus (P), alkaline phosphatase (ALP), parathyroid hormone (PTH), and 25OHD. Serum Ca, P, and ALP were measured with a Roche Diagnostics (Mannheim, Germany) Modular System. Serum intact PTH was measured with a DSL-8000 Active Intact PTH IRMA kit from Diagnostic Systems Laboratories (Webster, TX, USA), with intra- and interassay coefficients of variation of 2.8% and 3.6% respectively. Serum 25OHD was measured with a Diasorin 25OHD RIA kit (Stillwater, MN, USA) with intra- and interassay coefficients of variation of 10.4% and 9.4%, respectively.

Genotype assignment

Genomic DNA was prepared from 10 ml of ethylenediamine tetraacctic acid (EDTA)-treated blood, by a simple salting out procedure [17]. Detection of the BsmI site, located in intron 8, was performed by polymerase chain reaction (PCR) amplification of a region carrying the BsmI site primers originating in exon 7 (primer 1, 5′-CAACCAAGACTACAAGTACCG CGTCAGTGA-3′) and intron 8 (primer 2, 5′-AACCAGCGGGAAGAGGTCAAGGG-3′) producing an 825-bp fragment [9]. PCR products were generated in a 30-μl reaction volume containing 1 μg of genomic DNA, 50 mM KCL, 10 mM Tris-HCl (pH 8.4), 0.01% gelatin, 1.5 mM $MgCl_2$, 200 μM of each dATP, dGTP, dCTP, dTTP, 0.2 μM of each primer, and 1 U of *Taq* DNA polymerase. The BsmI amplification was performed as follows: incubation for 3 min at 94°C, 35 cycles of incubation for 20 s at 94°C, 40 s at 62°C, and 1 min at 72°C, followed by an extension step of 6 min at 72°C [18]. To determine the presence of a restriction site within an amplified product, a 10-μl aliquot was digested with 5 U of BsmI endonuclease at 65°C. Digestion products were electrophoresed in a 2% agarose gel containing ethidium bromide (50 μg/ml). DNA fragments were visualized by ultraviolet illumination, and fragment sizes were estimated by comparison to a 50-bp ladder run on the same gel. The presence of the BsmI restriction site generates 175-bp and 650-bp fragments, whereas the absence of this site yields an 825-bp fragment. The presence of the restriction enzyme site is indicated by a lowercase letter (b) and the absence of the site by an uppercase letter (B).

Statistics

Statistical analysis was performed using a commercial statistical package (SPSS 7.5 for Windows; Chicago, IL, USA). Biochemical parameters in the patient and control groups were compared with standard two-sample *t*-tests; *VDR* genotype distribution in the two groups was assessed by the Pearson χ^2 test; *VDR* genotype distribution was compared in the patient and control groups by the Kruskal Wallis test; and two-way analysis of variance (ANOVA) was used to analyze the *VDR* genotype effect on biochemical parameters in the two groups.

Results

Biochemical parameters

The patient and control groups were similar with respect to age (41.92 ± 16.11 years vs 39.26 ± 11.98 years; $P = 0.169$). Serum calcium (2.14 ± 0.22 mM vs 2.33 ± 0.12 mM; $P < 0.05$), phosphorus (0.96 ± 0.24 mM vs 1.20 ± 0.14 mM; $P < 0.01$), and 25OHD (15.74 ± 10.15 nM vs 64.09 ± 43.55 nM; $P < 0.0001$) levels were lower, and serum ALP (364.34 ± 335.73 IU/l vs 58.75 ± 14.93 IU/l; $P < 0.0001$) and PTH (31.22 ± 27.37 pM vs 3.88 ± 2.22 pM; $P < 0.0001$) levels were higher in the patient group than those in the control group (Table 1). These findings were statistically significant.

VDR alleles

The distribution of *VDR* alleles in the patient and control groups (BB 31.6% vs 19.4%; Bb 44.7% vs 61.3%; bb 23.7% vs 19.4%, respectively) was not found to differ significantly. The influence of *VDR* genotypes on biochemical findings (Tables 2, 3) and the relationship between genotypes and osteomalacia were not statistically significant (Table 1). There was no correlation between the severity of osteomalacia and the *VDR* genotype.

Table 1. Comparison of findings, in osteomalacia and control groups, with vitamin D receptor (*VDR*) genotypes

			ANOVA		
Parameters	Patients	Controls	a	b	c
Age (years)	41.92 ± 16.11	39.26 ± 11.98	NS	NS	NS
Ca (mM)	2.14 ± 0.22	2.33 ± 0.12	$P < 0.05$	NS	NS
P (mM)	0.96 ± 0.24	1.20 ± 0.14	$P < 0.01$	NS	NS
ALP (IU/l)	364.34 ± 335.73	58.75 ± 14.93	$P < 0.0001$	NS	NS
25OHD (nM)	15.74 ± 10.15	64.09 ± 43.55	$P < 0.0001$	NS	NS
PTH (pM)	31.22 ± 27.37	3.88 ± 2.22	$P < 0.0001$	NS	NS

NS, not significant; ANOVA, analysis of variance; ALP, alkaline phosphatase; 25OHD, 25 hydroxyvitamin D; PTH, parathyroid hormone
a, comparison of the two groups; b, *P* value of genotype effect; c, interaction between genotype and osteomalacia

Table 2. *VDR* genotypes and biochemical findings in osteomalacia patients

	VDR genotypes			
Parameters	BB	Bb	bb	Kruskal-Wallis one-way analysis of variance
Age (years)	38.36 ± 16.85	47.82 ± 15.18	35.11 ± 14.43	χ^2, 4.244; df, 2; $P = 0.120$
Ca (mM)	2.08 ± 0.17	2.17 ± 0.28	2.17 ± 0.11	χ^2, 2.294; df, 2; $P = 0.318$
P (mM)	1.01 ± 0.19	1.01 ± 0.25	0.81 ± 0.23	χ^2, 4.661; df, 2; $P = 0.097$
ALP (IU/l)	342.82 ± 284.48	336.60 ± 380.03	436.89 ± 342.24	χ^2, 0.500; df, 2; $P = 0.779$
25OHD (nM)	13.95 ± 8.63	17.24 ± 12.87	15.32 ± 6.16	χ^2, 0.644; df, 2; $P = 0.725$
PTH (pM)	28.90 ± 18.79	30.50 ± 35.22	35.01 ± 22.28	χ^2, 1.697; df, 2; $P = 0.428$

df, degrees of freedom

Table 3. *VDR* genotypes and biochemical findings in control group

	VDR genotypes			
Parameters	BB	Bb	bb	Kruskal-Wallis one-way analysis of variance
Age (years)	45.17 ± 13.48	37.68 ± 11.75	38.33 ± 11.41	χ^2, 1.920; df, 2; $P = 0.383$
Ca (mM)	2.35 ± 0.18	2.30 ± 0.11	2.40 ± 0.03	χ^2, 3.455; df, 2; $P = 0.178$
P (mM)	1.21 ± 0.15	1.20 ± 0.15	1.17 ± 0.14	χ^2, 0.164; df, 2; $P = 0.921$
ALP (IU/l)	50.50 ± 26.56	60.57 ± 12.32	58.80 ± 13.88	χ^2, 0.025; df, 2; $P = 0.988$
25OHD (nM)	73.30 ± 73.53	61.25 ± 31.62	62.44 ± 41.38	χ^2, 0.175; df, 2; $P = 0.916$
PTH (pM)	3.13 ± 1.53	4.30 ± 2.07	3.42 ± 3.18	χ^2, 2.942; df, 2; $P = 0.230$

Discussion

The influence of allelic polymorphism in the *VDR* gene on bone formation and resorption has been established [4,5,9,12]. In contrast, many studies in several populations have failed to detect a significant association between bone mass and *VDR* gene alleles [4–6,19,20]. Recent studies also suggest a possible role of *VDR* gene variants in the development of other diseases, including breast and prostate cancer, osteoarthritis, atherosclerotic coronary artery disease, diabetes, primary hyperparathyroidism, infections, and psoriasis [14,21].

In addition to the well-known effect of *VDR* gene polymorphism on bone formation, there was some information linking mineral metabolism to *VDR* gene alleles. The physiological mechanism mediating the association between *VDR* gene variants and bone mass and bone loss are unclear, but the mechanism is likely due to the established actions of vitamin D on calcium homeostasis [11,12]. It is known that 1, 25 $(OH)_2$ D and its receptor mediate active calcium absorption, and calcium absorption has been shown to be reduced in subjects with the BB genotype and homozygous BAt haplotype [11,13]. These associations may be more pronounced among subjects with low dietary calcium intake. Premenopausal women with the Bat haplotype had 11% lower, and postmenopausal women, 37% lower calcium absorption compared to women with the

baT haplotype [11,13,14,22]. Thus, the effect of *VDR* gene variation on calcium absorption may also be modified by age or hormonal status. An effect of FokI alleles on calcium absorption has been demonstrated among children. Calcium absorption was 41.5% and 17% greater in children with the FF homozygote and heterozygote, respectively, than in those with the ff homozygote [14,23]. However, not all studies have confirmed this association between *VDR* genotype and calcium absorption [24,25]. Katsumata et al. [10] have reported that *VDR* (FokI) and *PTH* (DraII) gene polymorphisms were associated with lumbar spine BMD, in contrast to findings for estrogen receptor, calcium-sensing receptor, and β3-adrenergic receptor gene polymorphisms in Japanese girls. These results suggest that there may be differences in intestinal sensitivity to 1, 25 (OH)2 D due to *VDR* genotypes.

The main cause of osteomalacia is vitamin D deficiency. Resistance to 1, 25 (OH)2 D has been shown to be affected by *VDR* mutation [1]. *VDR* gene polymorphism has not been investigated in osteomalacia. Vitamin D deficiency, which can result from the above-mentioned etiologies, causes osteomalacia by affecting calcium metabolism in different time intervals. Although they live in the same area and have the same eating habits, some individuals are more prone to have features of osteomalacia. *VDR* gene polymorphism may account for this difference. The winter level of serum 25OHD, lumbar spine BMD, and intestinal strontium absorption were highest for the BB genotype in a study of Finnish premenopausal and postmenopausal women. The *VDR* genotype distribution was: 16%, BB; 34.5%, Bb; and 49.5%, bb in that study [15]. In our study, *VDR* gene allelic variants were found to be 31.6%, BB; 44.7%, Bb; and 23.7%, bb (Bb > BB > bb) in the patients and 19.4%, BB; 61.3%, Bb; and 19.4%, bb (Bb > BB = bb) in the healthy controls. Although heterozygotes were encountered more often than the BB and bb allelic forms in both groups, the BB genotype was encountered more often in the osteomalacia patients than in the controls. However, these findings, provided from a limited number of subjects, were not found to be significant. The effect of *VDR* genotypes on biochemical parameters and the relationship of genotypes to osteomalacia also were not significant.

In conclusion, *VDR* gene allelic forms, along with other well-known factors, may play a role in the occurrence of osteomalacia, especially in people who usually have a low calcium intake. Further studies, performed in larger groups who have varying calcium intakes, are required to verify that *VDR* gene allelic polymorphisms have a contributory role in osteomalacia.

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