J. Perinat. Med. 37 (2009) 491–495 • Copyright © by Walter de Gruyter • Berlin • New York. DOI 10.1515/JPM.2009.091

Vitamin D receptor genotype BB is associated with higher serum osteocalcin in first pregnancy

Jacqueline Feuz[1], Josef Wisser[1], Armin Heufelder[2], Werner Joba[2], Roland Zimmermann[1] and Ursula von Mandach[1,*]

[1] Department of Obstetrics, Zurich University Hospital, Switzerland

[2] The Eliscourt Clinical Research Center, Munchen, Germany

Abstract

Aim: Serum osteocalcin was shown in a previous study on first trimester pregnant women to correlate with bone density and to distinguish between fast and slow bone losers. The objective of the present study is to examine whether serum osteocalcin is related to vitamin D receptor (VDR) *BsmI* polymorphism in pregnant women.

Study design: We determined osteocalcin serum levels and VDR *BsmI* genotype in 97 healthy first trimester pregnant women consecutively recruited during six months.

Results: *BB* (21%), *Bb* (38%) and *bb* (41%) genotypes showed similar osteocalcin serum levels. However, in primigravidas (n = 38) the *BB* genotype was significantly associated with higher mean osteocalcin level (9.67 ng/mL) than the *Bb* (8.07 ng/mL) and the *bb* genotype (8.14 ng/mL), respectively ($P < 0.05$). The VDR genotype was the only independent parameter to correlate with serum osteocalcin ($P < 0.05$).

Conclusion: Only primigravidas show in the first trimester a relation between the bone formation parameter serum osteocalcin and the VDR genotype BB which indicates a higher risk of fractures. For further clinical applications serum osteocalcin and VDR genotype should be tested on a cohort of primigravidas including measurements of bone density.

Keywords: Bone metabolism; osteocalcin; osteoporosis; VDR polymorphism.

*Corresponding author:
Prof. Dr. Ursula von Mandach
Department of Obstetrics
Zurich University Hospital
Frauenklinikstrasse 10
CH-8091 Zurich
Switzerland
Tel.: +41/44/255 51 36
Fax: +41/44/255 44 30
E-mail: ursula.vonmandach@usz.ch

Introduction

Osteoporosis is a multifactorial disease that involves a reduction in bone mineral density (BMD). Since osteoporosis is a late-onset disease, the development of early diagnostic modalities and preventive measures are urgently required. Environmental and genetic factors are interacting in establishing the individual osteoporotic risk. Among multiple candidate genes in relation to BMD, such as estrogen receptor and collagen I gene polymorphisms, in addition to the TGFβ1-regulating genes [4], the vitamin D receptor (VDR) gene at the 3' end allele was the first described [15]. *BsmI* polymorphism is one of the four VDR gene polymorphisms known to date, which are differently related to BMD. Age, gender, race and diet play key roles. Therefore, crucial relations of VDR polymorphism and BMD exist from studies with different populations.

Post-menopausal women are the major at-risk subgroup for osteoporosis [8]. Moreover, pregnancy has different impact on bone mineral metabolism and density [6, 9]. In a meta-analysis, mean loss of BMD was considered as up to 5% [9]. However, it could be shown that women with many children had similar or higher BMD than their nulliparous controls. Parity obviously does not increase the risk of BMD loss or other factors may be determinants such as bone metabolism in pregnancy. We recently demonstrated that trabecular bone density in pregnancy varied between slow and fast bone losers during the first trimester [19]. Thereby, the only independent biochemical parameter significantly associated with the rate of bone loss was serum osteocalcin which could distinguish slow from fast losers. *BsmI* polymorphism occurs in 18% in Caucasian population with the *BB* genotype [4, 8]. The first study with sufficient statistical power included white nurses in a health cohort and demonstrated a two-fold increase in the risk of hip fractures among *BB* genotypes in association with older age and lower calcium intake [5].

Osteocalcin gene expression is regulated by the VDR gene [14]. It was the goal of the present study to prospectively test if higher osteocalcin levels indicating fast bone losers are also associated with the *BB* genotype. Bone chemistry in our previous study was not consistent between first and third trimester demonstrating pregnancy associated adaptations. From these results we suggested that bone metabolism may change within pregnancy and we hypothesized for our present study that gravidity i.e., the first pregnancy, could also play an

important role. Our second endpoint was, therefore, to compare the relationship between osteocalcin and VDR genotype in primi- and multigravidas.

Material and methods

Subjects

Following approval of the local ethical committee and with their informed consent, we consecutively recruited healthy first trimester pregnant primigravidas and multigravidas during routine control in the Obstetric Clinic of Zurich University Hospital, Switzerland over a 6-month period. Inclusion criteria to this prospective study were uncomplicated singleton pregnancies between 10.0 and 16.0 weeks of gestation by ultrasound. Exclusion criteria were multiple gestation, age <18 and >40.0 years, BMI >30.0 at inclusion, preexisting maternal diseases such as diabetes and hypothyroidism, and drug abuse (incl. tobacco). Patient data were obtained from the patient notes.

Venous blood (3 mL) was collected between 8:00 and 10:00 am into EDTA containing tubes which were stored at –80°C until analysis of VDR; 10 mL were collected into native tubes kept immediately at 4°C and centrifuged 10 min after venepuncture at 4°C, the serum was immediately transferred into 800 μL aliquots for storage at –20°C until analysis of osteocalcin.

Laboratory methods

Vitamin D receptor (VDR) genotype Peripheral blood collected in EDTA vacutainers was frozen (whole blood) and stored at –80°C until extraction. Genomic DNA was extracted from whole blood by MagNA pure compact instrument using the MPC nucleic acid isolation kit I according to the manufacturer's instructions (Roche Applied Science, Germany) and quantified by UV absorption at 260 nm. Genotyping of the single nucleotide polymorphism BsmI (A>G in intron 8) was performed by PCR amplification of a region of the VDR gene spanning the BsmI-site, followed by restriction endonuclease digestion. After digestion DNA fragments were separated on ethidium-bromide stained 2% agarose gels and VDR genotype was determined by UV illumination, identifying the wild type allele or the mutated allele, respectively. Homozygous presence of the wild type site (bb) resulted in two fragments of 195 base pairs (bp) and 164 bp, while homozygous absence of the restriction site (mutated site BB) resulted in a single fragment of 359 bp. A heterozygous genotype (Bb) was identified by DNA fragments of 359 bp, 195 bp and 164 bp.

Osteocalcin Osteocalcin in serum was measured by enzyme immunoassay (EIA; osteocalcin intact human) from Metra™ (www.teco-medical.ch) (intra-assay variation $<6\%$, inter-assay variation $<10\%$; detection limit 0.45 ng/mL). Samples were assayed in duplicates (the mean value was used) according to the manufacturer's protocol. In a precedent setting six different samples were analyzed by both, the EIA and the previously used IRMA method [19] which is not anymore commercially available and the values were compared by using the Bland-Altmann-Plot [2]. It could be shown that IRMA values were 2.14 lower than EIA values (mean ratio 2.14 with limits of agreement between 2.00 and 2.27).

Endpoints

The primary endpoint was the relationship between VDR genotype and serum osteocalcin in the typical population of our clinic. The second endpoint was the comparison of such a relationship between primi- and multigravidas.

Statistical analysis

Statistical analysis was performed using SPSS 10 for Windows. Data are expressed as mean ± SE. Differences between groups were examined using the Mann-Whitney *U*-test as well as the unpaired two-tailed *t*-tests of log values. Group interactions with other parameters were tested using analysis of variance (ANOVA). Correlations between variables were tested by logistic and simple regression. A $P<0.05$ was considered significant in all tests. The sample size of 20 (in each group) was adequate (power $>80\%$, $\alpha=0.05$) for detecting a difference $>20\%$ between the median of osteocalcin serum concentration in different VDR genotypes.

Results

Subjects

After the exclusion of three falsely recruited women who did not meet the inclusion criteria (one with a BMI of 31.0 kg/m^2 and two with multiple pregnancy), the population numbered n = 97 first trimester pregnant women with a mean age of 28.4 years and an actual BMI of 22.9 kg/m^2. The majority of women were Caucasians (76.3%) (Table 1). Data for the outcome of pregnancy could be recorded in n = 94; one woman had an abortion in the 18th week, and two women delivered out of the country. The outcome of these 94 women was uncomplicated in 78.7%. Four women had gestational diabetes without further problems and two had mild preeclampsia (edema; proteinuria ≤ 5 g/24 h, systolic/diastolic blood pressure $>140 \leq 160/>90 \leq 110$ mm Hg). Three women had other complications (streptococcus B infection n = 1, toxoplasmosis infection n = 1, fetal malformation n = 1). The mean gestational age at delivery was 39.3 weeks; six women delivered between 35.0 and 37.0 weeks.

Table 1 Maternal characterizing data at blood sampling (n = 97).

Age (years)	28.4 ± 0.5
Height (m)	1.64 ± 0.01
Actual weight (kg)	61.8 ± 0.9
BMI (kg/m^2)	22.86 ± 0.3
Actual gestational age (weeks)	12.4 ± 0.1
Gravidity >1 (yes; no)	59; 38
Nulliparity (yes; no)	50; 47
Caucasian (yes; no)	74; 23
Asian (yes; no)	12; 85
African (yes; no)	9; 88
Oriental (yes; no)	2; 95

Continuous values: mean ± SE.
Numeric values: n.

Vitamin D receptor (VDR) genotype and serum osteocalcin

While *bb* was the most prevalent VDR *BsmI* genotype (41.3%), *BB* was the least prevalent (20.6%) in this group of women (n = 97). In our Caucasian women (n = 74) the distribution was 23%, 37.8% and 39.2%, in Asian women (n = 12) 16.7%, 33.3% and 50%, and in African women (n = 9) 11.1%, 33.3% and 55.6% for *BB, Bb* and *bb* genotypes, respectively. The two Oriental women had both the *Bb* genotype. The *BB* homozygotes were associated with higher serum osteocalcin levels compared with the *Bb* heterozygotes or the *bb* homozygotes (Table 2). In primigravidas (n = 38) the difference was statistically significant (9.67 ng/mL in *BB* vs. 8.07 ng/mL in *Bb* and 8.14 ng/mL in *bb* genotypes; $P < 0.05$) (Figure 1A). No significant differences were found between nulliparas and multiparas (Figure 1B), nor between women with and without pregnancy complications (data not shown). There were also no differences between the subgroup of Caucasians and the whole collective (Table 2). Osteocalcin values in primigravidas ranged between 6.06 and 16.63 ng/mL suggesting a 2.14-fold higher level than in our previous study determined by the IRMA method [19]. The ROC curve showed that an osteocalcin concentration of 7.19 ng/mL in primigravidas discriminated between fast and slow losers with 53.1% sensitivity and 100% specificity (Figure 2). The VDR genotype was the only independent variable correlating with osteocalcin in fast and slow losers in a logistic regression model ($P < 0.05$) including age and BMI in primigravidas (Table 3).

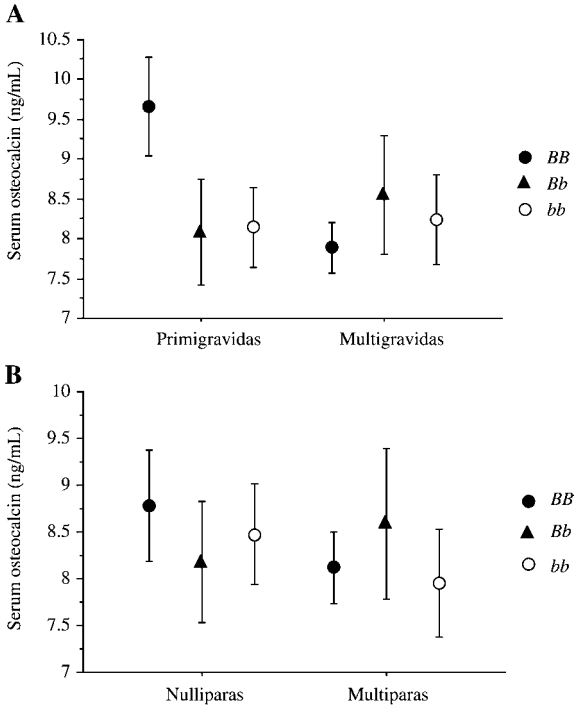

Figure 1 (A) Mean ± SE osteocalcin serum concentration (ng/mL) in VDR *BsmI* genotypes showing significantly higher levels in primigravidas with the *BB* allele (n = 6) than with the *Bb* (n = 17) or *bb* (n = 15) alleles or than in multigravidas (*BB*, n = 14; *Bb*, n = 20; *bb*, n = 25), *P < 0.05 (Mann-Whitney *U*-test and unpaired *t*-test of log values, respectively). (B) Mean ± SE osteocalcin serum concentration (ng/mL) in VDR *BsmI* genotypes showing no significantly different levels in nulliparas with the *BB* allele (n = 9) than with the *Bb* (n = 23) or *bb* (n = 19) alleles or than in multiparas (*BB*, n = 11; *Bb*, n = 14; *bb*, n = 21).

Discussion

To our knowledge, no previous study exists demonstrating higher osteocalcin levels in pregnancy and its relation to the VDR genotype *BB*.

The new finding in our study is that only primigravidas show the association between osteocalcin serum concentrations and VDR based on a number of robust findings. Blood sampling for osteocalcin was standardized (time of blood sampling, handling and storage of serum). The concentration which discriminates between fast and slow bone loser was 7.19 ng/mL corresponding to 3.36 ng/mL which is similar to that found in our previous study (3.67 ng/mL). We could also show that no other independent variables exist other than VDR genotype that correlates with osteocalcin levels. The distribution of VDR genotype at the gene locus *BsmI* in our Caucasians was similar (23%) to that in previously published studies (18–20%) [4, 8]. However, in our Asians (n = 12), the *BB*

Table 2 VDR *BsmI* genotype and corresponding serum osteocalcin (n = 97).

Genotype	Race	n (%)	Osteocalcin (ng/mL)	
			EIA raw data	To IRMA transposed data
All	All	97 (100)	8.30 ± 0.26 (4.01–16.65)	3.81 ± 0.13 (1.72–7.87)
BB	All	20 (20.6)	8.42 ± 0.34 (5.56–11.48)	3.87 ± 0.17 (2.48–5.35)
	Caucasian	17	8.57 ± 0.36 (6.54–11.48)	3.94 ± 0.18 (2.95–5.35)
Bb	All	37 (38.1)	8.33 ± 0.50 (4.19–16.65)	3.82 ± 0.24 (1.81–7.87)
	Caucasian	28	8.43 ± 0.55 (4.89–16.63)	3.87 ± 0.27 (2.14–7.86)
bb	All	40 (41.3)	8.20 ± 0.40 (4.01–14.97)	3.76 ± 0.19 (1.72–7.05)
	Caucasian	29	8.49 ± 0.48 (4.01–14.97)	3.90 ± 0.23 (1.72–7.05)

Continuous values: mean ± SE (range).
Numeric-values: n.

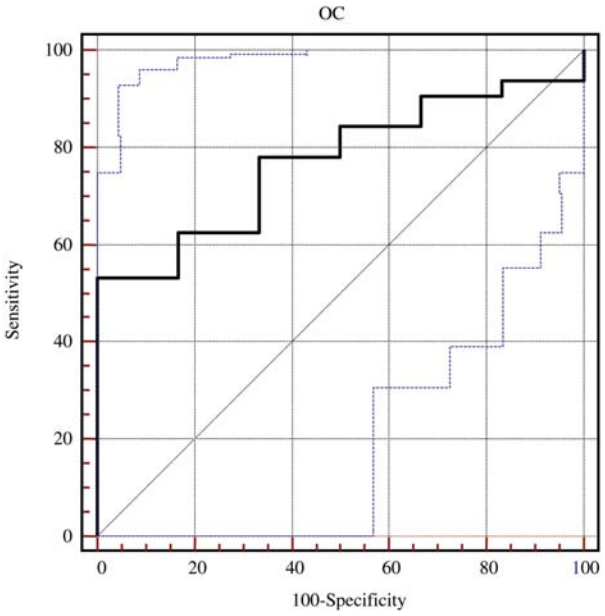

Figure 2 Serum osteocalcin (OC) ROC curve differentiating fast from slow bone losers in primigravidae (n = 38).

Table 3 Logistic regression model using age, body mass index (BMI) and vitamin D receptor (VDR) *BsmI* genotype (independent variables) in 38 primigravidas with osteocalcin $</\geq 3.36$ ng/mL (dependent variable).

	P-value	OR*	95% confidence interval
Age	0.518	0.949	0.808–1.113
BMI	0.222	0.852	0.658–1.102
VDR *BsmI* genotype**	0.038	0.066	0.005–0.860

*Adjusted. ***BB* vs. *Bb* or *bb*.

genotype occurred more frequent (16%) than in other studies with Asians (1–2%) [4, 10, 18, 20].

There are also some limitations in our study. For example, since higher osteocalcin levels correlated with the *BB* genotype, we had only a small number of primigravidas with this genotype (all, n = 38; *BB* genotype, n = 6). We also did not account the consumption of calcium because in our previous study [19] we could demonstrate that bone density was independent from calcium intake.

The fact that a relationship exists between higher osteocalcin serum levels and VDR *BB* genotype in primigravidas supports the regulatory role of VDR gene in bone metabolism as well as different bone metabolism changes in primigravidas compared to multigravidas. Simultaneous bone biochemistry in our previous study indicated that bone turnover is subject to change during pregnancy. These pregnancy associated changes have also been demonstrated by others [6]. According to our previous study, in which we could also demonstrate that serum osteocalcin reflects bone density by differentiating fast from slow bone losers, primigravidas with the *BB* genotype of the present study are also associated with higher bone mass losing. In fact, osteocalcin is related to the current bone mass and provides information about future bone loss and fracture risk, as a five-year follow-up study in premenopausal Swedish women has recently shown [12]. In addition, it could be shown that the effect of the calcitriol-stimulated osteocalcin secretion in human osteoblasts was doubled in the *bb* genotype compared with the *BB* genotype [16]. However, the association between VDR gene *BsmI* polymorphism and BMD remains controversial. Gender, age (children vs. adolescents vs. premenopausal vs. postmenopausal women), race and ethnicity are the most important influencing factors which could be accounted for the great diversity between numerous studies [1, 3, 7, 11, 13, 17].

Moreover, we failed to detect the influence of parity confirming by several studies which showed similar or higher BMD in women with many children than in nulliparous controls [9].

In conclusion, only primigravidas show in the first trimester a relation between the bone formation parameter serum osteocalcin and the VDR genotype BB which is known to indicate a higher risk of fractures. For further clinical applications, serum osteocalcin and VDR genotype should be tested on a cohort of primigravidas including measurements of bone density in pregnancy as well as before and after menopause.

Acknowledgement

Alexandra Dolder is acknowledged for her excellent laboratory work.

References

[1] Bernad M, Jaramillo MC, Aguado P, del Campo T, Coya J, Martin Mola E, et al. Polymorphisms of the gene of vitamin D receptor and bone mineral density in postmenopausal women. Med Clin (Barc). 1999;112:651–6.
[2] Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet. 1986;1(8476):307–10.
[3] Cooper GS, Umbach DM. Are vitamin D receptor polymorphisms associated with bone mineral density? A meta-analysis. J Bone Miner Res. 1996;11:1841–9.
[4] Ferrari S, Rizzoli R, Bonjour JP. Genetic aspects of osteoporosis. Curr Opin Rheumatol. 1999;11:294–300.
[5] Feskanich D, Hunter DJ, Willett WC, Hankinson SE, Hollis BW, Hough HL, et al. Vitamin D receptor genotype and the risk of bone fractures in women. Epidemiology. 1998;9: 535–9.
[6] Gallacher SJ, Fraser WD, Owens OJ, Dryburgh FJ, Logue FC, Jenkins A, et al. Changes in calciotrophic hormones and biochemical markers of bone turnover in normal human pregnancy. Eur J Endocrinol. 1994;131:369–74.

[7] Garnero P, Borel O, Sornay-Rendu E, Delmas PD. Vitamin D receptor gene polymorphisms do not predict bone turnover and bone mass in healthy premenopausal women. J Bone Miner Res. 1995;10:1283–8.
[8] Gomez C, Naves ML, Barrio Y, Diaz JB, Fernandez JL, Salido E, et al. Vitamin D receptor gene polymorphisms, bone mass, bone loss and prevalence of vertebral fracture. Differences in postmenopausal women and men. Osteoporosis Int. 1999;10:175–82.
[9] Karlsson MK, Ahlborg GH, Karlsson C. Maternity and bone mineral density. Acta Orthop. 2005;76:2–13.
[10] Kikuchi R, Uemura T, Gorai I, Ohno S, Minaguchi H. Early and late postmenopausal bone loss is associated with BsmI vitamin D receptor gene polymorphism in Japanese women. Calcif Tissue Int. 1999;64:102–6.
[11] Lim SK, Park YS, Park JM, Song YD, Lee EJ, Kim KR, et al. Lack of association between vitamin D receptor genotypes and osteoporosis in Koreans. J Clin Endocrinol Metab. 1995;80:3677–81.
[12] Lofman O, Magnusson P, Toss G, Larsson L. Common biochemical markers of bone turnover predict future bone loss: a 5-year follow-up study. Clin Chim Acta. 2005;356: 67–75.
[13] Lorentzon M, Lorentzon R, Nordstrom P. Vitamin D receptor gene polymorphism is related to bone density, circulating osteocalcin, and parathyroid hormone in healthy adolescent girls. J Bone Miner Metab. 2001;19:302–7.
[14] Morrison NA, Shine J, Fragonas JC, Verkest V, McMenemy ML, Eisman JA. 1,25-dihydroxyvitamin D-responsive element and glucocorticoid repression in the osteocalcin gene. Science. 1989;246:1158–61.
[15] Morrison NA, Qi JC, Tokita A, Kelly PJ, Crofts L, Nguyen TV, et al. Prediction of bone density from vitamin D receptor alleles. Nature. 1994;367:284–7.
[16] Naves M, Alvarez-Hernandez D, Fernandez-Martin JL, Paz-Jimenez J, Garcia-Prado P, Fernandez-Coto T, et al. Effect of VDR gene polymorphisms on osteocalcin secretion in calcitriol-stimulated human osteoblasts. Kidney Int Suppl. 2003;63:S23–7.
[17] Saadi HF, Nagelkerke N, Benedict S, Qazaq HS, Zilahi E, Mohamadiyeh MK, et al. Predictors and relationship of serum 25 hydroxyvitamin D concentration with bone turnover markers, bone mineral density, and vitamin D receptor genotype in Emirati women. Bone. 2006;39:1136–43.
[18] Tokita A, Matsumoto H, Morrison NA, Tawa T, Miura Y, Fukamauchi K, et al. Vitamin D receptor alleles, bone mineral density and turnover in premenopausal Japanese women. J Bone Miner Res. 1996;11:1003–9.
[19] Wisser J, Florio I, Neff M, Konig V, Huch R, Huch A, et al. Changes in bone density and metabolism in pregnancy. Acta Obstet Gynecol Scand. 2005;84:349–54.
[20] Zhao J, Zhou X, Meng X, Liu G, Xing X, Liu H, et al. Polymorphisms of vitamin D receptor gene and its association with bone mineral density and osteocalcin in Chinese women. Chin Med J. 1997;110:366–71.

The authors stated that there are no conflicts of interest regarding the publication of this article.

Received November 6, 2008. Revised February 25, 2009. Accepted March 30, 2009. Previously published online June 3, 2009.