The Influence of VDR Genotype and Exercise on Ultrasound Parameters in Young Adult Japanese Women

Fumihiro Omasu¹*, Jun Kitagawa¹, Katsuhiro Koyama², Kazumi Asakawa³, Juri Yokouchi², Daisuke Ando² and Yoshibumi Nakahara¹

¹) Department of Human System Science, Graduate School of Decision Science and Technology, Tokyo Institute of Technology
²) Faculty of Education and Human Sciences, University of Yamanashi
³) Yamanashi Junior College of Nursing
* Current address: PRESTO, Japan Science and Technology Corporation

Abstract  We investigated the relation between the vitamin D receptor (VDR) genotype and bone mass including the effect of exercise history as a measure of physical activity. BUA (broadband ultrasound attenuation), SOS (speed of sound) and Stiffness index of the calcaneus were measured using an ultrasound bone densitometer in 105 Japanese young adult women (age: mean ± SD 20.4 ± 4.1 years, ranged 18–37) by the calcaneal ultra sound measurement to assess bone mass. Physical activity was measured using a questionnaire about exercise and was calculated as exercise hours per week during prepuberty (elementary school), puberty (junior and senior high school) and a current period (from >18 years old). VDR genotype was determined by the BsmI restriction site of the VDR gene. Significant differences were observed in age-adjusted and menarche age-adjusted SOS and Stiffness between BsmI VDR genotypes. We also examined the interaction between VDR genotype and the amount of exercise. The association between ultrasound parameters and exercise hours per week was evaluated with simple regression analysis according to VDR genotype. There was a significant difference in the slope between VDR genotypes in regression analysis of exercise hours per week during senior high school for SOS (P < 0.05). Furthermore, we conducted multiple regression analysis to examine the contribution of each factor to ultrasound parameters. VDR genotype was a significant independent variable for SOS (P < 0.05). Exercise hours each week during senior high school was a significant independent variable for all ultrasound parameters (all: P < 0.001). In conclusion, there was a partial significant relation between VDR genotype and ultrasound parameters, but the exercise hours each week during senior high school was the strongest independent factor for bone mass in young adult Japanese women. J Physiol Anthropol Appl Human Sci 23 (2): 49–55, 2004 http://www.jstage.jst.go.jp/browse/jpa

Keywords: BUA, SOS, Stiffness, Calcaneus, BsmI

Introduction

It was shown previously that lifestyle factors such as calcium intake and physical activity are important correlates of bone mass (Eisman JA et al., 1991; Suleiman S et al., 1997). Studies of twins and families have shown that genetic factors also play an important role in bone mineral density (BMD) (Pocock NA et al., 1987; Krall EA et al., 1993). Morrison et al. (1994) reported that the bb genotype of the vitamin D receptor (VDR) locus determined by BsmI restriction enzyme had 15% higher BMD than the BB genotype while adjusting for other factors. A similar result that the BB genotype in BsmI VDR genotypes predicts lower BMD was reported for black and white women (Fleet JC et al., 1995). However, those results were not seen in all previous studies. For example, among healthy American premenopausal women (Salamone LM et al., 1996) the bb genotype was significantly related to the low BMD. Elsewhere, Spotila et al. (1996) found that BsmI VDR genotypes had no significant relation to BMD. The area of dispute appears to be the exact relationship between the VDR genotype and bone mass.

Salamone et al. (1996) reported VDR genotype, weight and physical activity to jointly determine bone mineral density in postmenopausal women. Lifestyle factors apparently interact with genetic factors to affect bone mass. Graafmans et al. (1997) found that the effect of vitamin D supplementation on bone mineral density was correlated with VDR genotype. However, Jarvien et al. (1998) suggested that there was no significant relation between the influence of physical activity on bone mass and VDR genotype. Interactions between genetics and lifestyle factors are considered important for bone mass (Suleiman S et al., 1997; Jarvien TLN et al., 1998; Fujita
Y et al., 1999). However, those studies were carried out in elderly persons among whom peak bone mass is inevitably somewhat decreased. Peak bone mass, which is achieved by the end of the second decade (Gordon CL et al., 1991; Teegarden D et al., 1995), may be linked with the future quality of life since the inevitable age-related decrease of bone mass in the elderly often results in osteoporosis and an increasing risk of fracture. A general consensus is that peak bone mass is determined by both lifestyle and genetic factors (Fujita Y et al., 1999), as well as decreasing among the elderly as stated above.

A close correlation between quantitative ultrasound and BMD measurements assessed by dual energy X-ray absorptiometry (DXA) has been shown in several studies (Diez-Perez A et al., 2003; Lopez-Rodriguez F et al., 2003). Lochmuller et al. (1998) suggested that femoral neck BMD and calcaneal ultrasound are equivalent in predicting the risk of fractures. Tromp et al. (1999) also found a significant correlation of calcaneal ultrasound with sites of BMD measurements. Very few attempts have been made to examine the relationship between VDR genotype and calcaneal ultrasound estimates of BMD in young women.

This study is designed to clarify the relationship between the VDR genotype defined by the BsmI restriction enzyme and bone mass measured using the calcaneal ultrasound method. Additionally, the influence of exercise, a lifestyle factor, on the association of VDR genotype with BMD in young adult Japanese women is examined.

Materials and Methods

Subjects

105 premenopausal young adult Japanese women aged 18-37 years old participated in this study to determine associations between the VDR genotype and bone mass. We obtained written informed consent to take blood samples and analyze VDR genotype for this study. Women who had a history of treatment and therapy that were considered to influence bone mass were excluded. VDR genotype was determined for all women. Each also was measured for height and weight, BMI (Body Mass Index) was calculated according to the formula-weight (kg)/height (m)², and ultrasound parameters and reported their exercise history age, and menarche age using a questionnaire. This study was approved by the Ethical Committee of Epidemiological Studies in the Graduate School of Decision Science and Technology, Tokyo Institute of Technology.

Bone mass measurement

The bone mass was measured at the right calcaneus using the ultrasound. In this study, BUA (broadband ultrasound attenuation), SOS (speed of sound) and Stiffness Index, which was estimated using BUA and SOS values, were used as estimates of the bone mass by means of the ultrasound bone densitometry (Lunar A-1000 Achilles). Estimates are deemed reliable because they were obtained using an expert operating the device and the coefficient of variation was within 1.5% for the calcaneus in vivo measurements.

Questionnaire of exercise

Exercise and its frequency in the past and at present were determined using a self-administered questionnaire (Paffenbarger RS et al., 1978; Raitakari OT et al., 1996; Pereira MA et al., 1997). Each subject filled in the item of exercise as extracurricular activities at under 12 years old (elementary school), 12–18 years old (junior and senior high school) and from >18 to the current period with the exercise frequency. The estimated exercise hours each week during each period was used in this examination.

VDR genotyping

The VDR genotype defined by BsmI restriction enzyme was analyzed. DNA was extracted from total blood using GFX Genomic Blood DNA purification kit (Amersham Pharmacia Biotech). The VDR gene was amplified by polymerase chain reaction (PCR) with each oligonucleotide primer as described previously (Morrison NA et al., 1994). The PCR product was digested with BsmI at 65°C for 3 hours. The digested sample was loaded onto 2% agarose gel electrophoresis and the VDR genotype was specified from the DNA fragments that were visualized by ultraviolet illumination.

Statistical analyses

To examine the influence of age, menarche age, BMI and exercise hours each week on ultrasound parameters, we analyzed the correlation of each factor using simple regression analysis. ANOVA (Analysis of variance) was conducted to test for the differences between the ultra-sound parameters divided by the VDR genotype and we also used the age- and menarche age-adjusted ultrasound parameters. The difference in the slopes between the VDR genotype in simple regression analysis with ultrasound parameters and exercise hours per week was statistically examined to clarify the interaction between the VDR genotype and exercise on bone mass. We examined the contributions of independent variables to ultrasound parameters using multiple regression analysis. Statistical analyses were performed with the SAS Statistical Package version 6.11 (SAS Institute, Cary, NC, USA).

Results

Characteristics of the subjects

Characteristics of the 105 subjects are shown in Table 1. In this study, only one subject showed the BB genotype. Therefore only two genotypes bb and Bb, excluding the BB genotype, in BsmI the VDR genotypes were investigated. There was a significant difference in age between the VDR genotypes. There was no significant difference in any ultrasound parameter between VDR genotypes.
Univariate analysis

Correlation between ultrasound parameters and each factor was examined (Table 2). The correlation of menarche age with SOS and Stiffness was significant and negative (r = -0.199, r = -0.191, P < 0.05 respectively). There was a significant correlation of SOS with height (r = 0.217, P < 0.05), and of BUA and Stiffness with weight (r = 0.348, P < 0.001, r = 0.191, P < 0.05 respectively) and BMI (r = 0.338, P < 0.001, r = 0.264, P < 0.01, respectively). Exercise hours each week during senior high school had a positive correlation with all ultrasound parameters (r = 0.285, P < 0.01, r = 0.351, P < 0.001, r = 0.355, P < 0.001, respectively). There was a significant correlation of BUA alone with exercise each week during elementary school (r = 0.245, P < 0.05).

Age- and menarche age-adjusted ultrasound parameters and the VDR genotype

The relation between the VDR genotype and age-adjusted and menarche age-adjusted ultrasound parameters was examined. The SOS and Stiffness in the adjusted ultrasound parameters showed significant differences between the VDR genotypes (bb 1527.9 ± 25.8 Bb 1537.7 ± 25.3 P < 0.05, bb 82.6 ± 11.2 Bb 84.6 ± 9.9 P < 0.05, respectively) (Fig. 1).

Interaction between the VDR genotype and exercise for the bone mass

We analyzed the relation between the ultrasound parameters and the VDR genotype, taking into account the interaction of exercise, especially exercise during senior high school, which showed a strong correlation with ultrasound parameters. We examined whether the correlation of exercise hours per week during senior high school with ultrasound parameters by simple regression analysis varied within the VDR genotypes.

Table 1  Characteristics of subjects by VDR genotype

<table>
<thead>
<tr>
<th>Subjects (n)</th>
<th>All 105</th>
<th>bb 86 (81.9%)</th>
<th>Bb 19 (18.1%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>20.4 ± 4.1</td>
<td>20.0 ± 3.6</td>
<td>21.9 ± 5.8*</td>
</tr>
<tr>
<td>Menarche age (years)</td>
<td>12.0 ± 1.0</td>
<td>12.1 ± 1.1</td>
<td>12.4 ± 1.5</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>159.1 ± 4.9</td>
<td>159.5 ± 5.1</td>
<td>157.5 ± 4.2</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>51.7 ± 6.8</td>
<td>51.9 ± 7.1</td>
<td>50.5 ± 5.1</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>20.4 ± 2.2</td>
<td>20.4 ± 2.4</td>
<td>20.4 ± 1.7</td>
</tr>
<tr>
<td>BUA</td>
<td>111.5 ± 8.4</td>
<td>111.7 ± 8.6</td>
<td>110.7 ± 7.4</td>
</tr>
<tr>
<td>SOS</td>
<td>1,529.1 ± 26.2</td>
<td>1,527.5 ± 26.0</td>
<td>1,536.0 ± 27.0</td>
</tr>
<tr>
<td>Stiffness</td>
<td>82.7 ± 11.2</td>
<td>82.4 ± 11.3</td>
<td>83.7 ± 11.4</td>
</tr>
<tr>
<td>Exercise hours/week</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elementary school</td>
<td>1.9 ± 2.2</td>
<td>1.9 ± 2.2</td>
<td>2.1 ± 2.4</td>
</tr>
<tr>
<td>Junior high school</td>
<td>7.6 ± 6.5</td>
<td>7.2 ± 6.6</td>
<td>8.7 ± 6.0</td>
</tr>
<tr>
<td>Senior high school</td>
<td>5.3 ± 7.1</td>
<td>5.0 ± 7.2</td>
<td>5.2 ± 6.1</td>
</tr>
<tr>
<td>Current period</td>
<td>0.6 ± 1.4</td>
<td>0.6 ± 1.3</td>
<td>0.8 ± 1.8</td>
</tr>
</tbody>
</table>

Excluding BB genotype

* bb vs Bb P < 0.05 Mean ± SD

Table 2  Coefficient of correlation by simple regression analysis

<table>
<thead>
<tr>
<th>Ultrasound parameter</th>
<th>BUA</th>
<th>SOS</th>
<th>Stiffness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>-0.145</td>
<td>-0.052</td>
<td>-0.048</td>
</tr>
<tr>
<td>Menarche age</td>
<td>-0.114</td>
<td>-0.199b</td>
<td>-0.191b</td>
</tr>
<tr>
<td>Height</td>
<td>0.103</td>
<td>0.217b</td>
<td>0.091</td>
</tr>
<tr>
<td>Weight</td>
<td>0.348a</td>
<td>0.053</td>
<td>0.191b</td>
</tr>
<tr>
<td>BMI</td>
<td>0.336a</td>
<td>0.177</td>
<td>0.264a</td>
</tr>
</tbody>
</table>

* p < 0.01, b: p < 0.05
The slope for Bb differed significantly from that for bb only for SOS (Fig. 2), even though the ultrasound parameters were adjusted for each factor used.

**Multivariate Analysis**

We analyzed the contribution of each factor to the ultrasound parameter with multiple regression analysis (Table 3). Independent variables included in this multivariate analysis were age, menarche age, BMI, VDR genotype and exercise hours per week variables. The VDR genotype was a significant independent variable only for SOS (p<0.05). The factor of exercise hours each week during senior high school was a strong predictor of all ultrasound parameters (p<0.001), but...
Table 3 The result of stepwise multiple regression analysis

<table>
<thead>
<tr>
<th>Ultrasound Parameter</th>
<th>BUA</th>
<th>SOS</th>
<th>Stiffness</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI</td>
<td>0.205b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exercise hours per week during senior high school</td>
<td>0.287c</td>
<td>0.318c</td>
<td>0.342c</td>
</tr>
<tr>
<td>VDR genotype</td>
<td>0.189b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Multiple R</td>
<td>0.490</td>
<td>0.405</td>
<td>0.454</td>
</tr>
<tr>
<td>R square</td>
<td>0.240</td>
<td>0.164</td>
<td>0.206</td>
</tr>
<tr>
<td>P value in model</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*: P<0.01, : P<0.05

The standardized partial regression coefficient of BMI, exercise hours per week during senior high school and VDR genotype to ultrasound parameters.

BMI was the only significant predictor for the BUA and Stiffness (p<0.01).

Discussion

Some reasons for disagreements about the relation between the VDR genotypes and bone mass in previous studies are offered. According to the study by Ferrari et al. (1998), the significant relation between the VDR genotype and BMD was observed only in prepubertal females. Kikuchi et al. (1999) reported that the postmenopausal women were strongly influenced by a number of factors other than genetics factor. Although the peak bone mass is mostly determined by genetic factors, the bone mass shows many subsequent changes influenced by environmental and lifestyle factors. There appears to be difficulties when examining the relation between the VDR genotype and bone mass in postmenopausal females because the genetic factors are confounded with environmental and lifestyle factors throughout life. The period of exposure to the lifestyle factors is shorter in the young and middle-aged than in the elderly. Thus, in the present study, the significant relation between the VDR genotype and bone mass may have been partially observed in adult women.

Others reported the possibility of an interaction between the genetic and environmental factors, according to differences in physical activity, on bone mass and between the VDR genotype in postmenopausal women (Omasu F et al., 2000). We partially showed the interaction between the VDR genotype and exercise on bone mass in young women. Blanchet et al. (2002) reported that the BB genotype had a better response to leisure physical activity than bb. Our results are in agreement with their results. Furthermore, in the present study, it was found that the Bb genotype had higher SOS than bb did. However, since exercise hours each week during senior high school showed a strong correlation with all ultrasound parameters, the influence or interaction of the VDR genotype might be surpassed as suggested by multiple regression analysis. In other words, it is quite likely that the peak bone mass is mostly determined by sufficient exercise during puberty beyond the influence of VDR genotype.

There was a significant difference in age between VDR genotypes. Tamai et al. (1997) found a significant relation between the BMD and the VDR genotype adjusting for age. A significant correlation of menarche age with SOS and Stiffness was found in this sample also. The menarche and menopause are important physiological correlates for the bone density over the life of a woman. Krall et al. (1995) found a significant relation between the VDR genotype and bone mass using years since menopause in postmenopausal women. It is likely important to consider menarche age when examining BMD of adult women who have achieved the peak bone mass.

We measured the calcaneal bone mass, which responds to mechanical stresses of physical activity and weight bearing because it is mostly composed of cancellous bone (Suominen H et al., 1991). The mechanisms whereby exercise, height, weight or BMI influence bone mass are not clear, but it is well known that mechanical stress such as weight-bearing of bone influences the bone mass (Vogel JM et al., 1998).

Each ultrasound parameter in the present study differed in relation to determinants, and only SOS reflected the VDR genotype. Race has been reported as an important predictor of SOS (Gregg EW et al., 1999). However our sample included only Japanese women, thus race does not influence our genetic association. It was also reported that the weight and exercise have strong influences on BUA (Han S et al., 1997). The significant relation between the VDR genotype and SOS in the present study might be due to the low impact of the BMI and exercise on SOS, compared with the BUA and Stiffness. Moreover, it is said that the BUA reflects the structure of bone and the SOS is related to the density of bone (Langton CM et al., 1984; Hans D et al., 1999), so it is possible that the BMD using DXA which reflects the density of bone reflects the relation of the VDR genotype with the bone density.

In the present study, there are several limitations. The frequency of BsmI VDR alleles in Japanese differs from that of Caucasians according to previous studies (Morrison NA et al., 1994; Yamagata Z et al., 1994). The frequency of BsmI VDR alleles in the present study was quite similar to that of previous studies for Japanese (Yamagata Z et al., 1994) in that there was only one subject showing the BB genotype. An investigation including the BB genotype will be needed to clarify further the association of the bone mass and VDR genotype. The difference in the results regarding the relation between the VDR genotype and bone mass in previous studies may be due to the variety of measurements for the bone mass and bone sites. Viitanen et al. (1996) suggested that the lumbar spine and femoral neck differed in the relation between the VDR genotype and BMD. Tamai et al. (1997) found a significant relation between the VDR genotype and SOS (Gregg EW et al., 1999). However our sample included only Japanese women, thus race does not influence our genetic association. It was also reported that the weight and exercise have strong influences on BUA (Han S et al., 1997). The significant relation between the VDR genotype and SOS in the present study might be due to the low impact of the BMI and exercise on SOS, compared with the BUA and Stiffness. Moreover, it is said that the BUA reflects the structure of bone and the SOS is related to the density of bone (Langton CM et al., 1984; Hans D et al., 1999), so it is possible that the BMD using DXA which reflects the density of bone reflects the relation of the VDR genotype with the bone density.

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In summary, we found a partially significant association
between the bone mass using the calcaneal ultrasound measurement and BsmI the VDR genotype in Japanese young adult women. Exercise during puberty strongly influenced the bone mass in adult, and that influence was much stronger than that of the VDR genotype. Clearly, a genetic influence on the bone mass is suggested and this needs further exploration. The possibility of interactions between the genetics and lifestyle factors also needs to be further examined.

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Correspondence to: Jun Kitagawa, Nakahara Laboratory, Department of Human System Science, Graduate School of Decision Science and Technology, Tokyo Institute of Technology, 2–12–1, O-okayama, Meguro-ku, Tokyo 152–8552, Japan Phone: +81–3–5734–2682
Fax: +81–3–5734–2682
e-mail: kitagawa@hum.titech.ac