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Short Communication

Association of vitamin D receptor polymorphism with calcaneal broadband ultrasound attenuation in Korean postmenopausal women with low calcium intake

Hee-Seon Kim[1], Ji-Sun Kim[2], Nam Soo Kim[3], Jin Ho Kim[3] and Byung-Kook Lee[3]*

[1]*Department of Food Science and Nutrition, Soonchunhyang University, Asan, 336-745, Republic of Korea*
[2]*Korea Food Research Institute, Seongnam, 463-746, Republic of Korea*
[3]*Institute of Environmental and Occupational Medicine, Soonchunhyang University, 646 Eupnae-ri, Shinchang-myun, Asan-si, Choongnam 336-745, Republic of Korea*

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This study investigated the associations among vitamin D receptor (VDR) *Bsm*I polymorphism, calcium intake and bone strength as indicated by the broadband ultrasound attenuation (BUA) measured by calcaneal quantitative ultrasound at the left calcaneus in community-dwelling subjects with a low calcium intake. The VDR *Bsm*I polymorphism was analysed in 335 women older than 65 years residing in rural Asan, Korea. Calcium intake was assessed with a 2 d, 24 h recall method. The distribution of genotypes was similar to that reported in other Asian populations (92 % bb, 7 % Bb and 1 % BB). The calcaneal BUA was significantly higher ($P=0{\cdot}013$) in the bb genotype than in the Bb or BB genotype (Bb and BB genotypes were combined due to the small number of BB subjects) in a multiple regression model after adjusting for age, body weight, height, physical activity and nutritional factors. BUA was not significantly affected by the calcium intake regardless of the genotype, cross-sectionally. The energy-adjusted average calcium intake of this population was 439·6 mg/d (432·5 mg/d for bb and 522·3 mg/d for Bb or BB), and 96 % of the subjects had dietary intakes that were less than the recommended Dietary Reference Intake for Koreans (which for calcium is 800 mg/d for women older than 65 years). In summary, the BUA in older Korean women with a low calcium intake was significantly influenced by the VDR genotype but not by the calcium intake, cross-sectionally.

Broadband ultrasound attenuation: Vitamin D receptor genotype: Calcium intake: Korean: Postmenopausal women

The last decade has yielded a wealth of information on bone health. The increased interest and advanced technology for bone mass measurements have enhanced our understanding of osteoporosis risk factors, its causes, and routes of prevention and management[1]. Nevertheless, osteoporosis remains a complex, multifactorial condition that leads to an increased risk of fractures, with inadequate calcium intake influencing bone loss and playing an important role in its pathogenesis[2]. The intakes of some nutrients by older women in rural Korea are lower than recommended levels[3], with calcium being especially significant since Korean national nutrition surveys began in the 1960s[4]. Interventions for increasing calcium intake are an important public health issue given the ageing population in Korea. However, the health benefits to be expected from calcium depend not only on how much calcium is consumed but also on how much is absorbed[5].

It has been suggested that vitamin D receptor (VDR) gene polymorphisms influence intestinal calcium absorption, with women with B variants exhibiting reduced calcium absorption efficiency when their calcium intake is low and showing lower bone mineral density (BMD) than women with bb variants[6]. The impact of this genetic difference reduces with higher calcium intakes or increasing age[7]. Therefore, many factors need to be considered when attempting to improve the calcium intake of older women significantly. The present study investigated the association among VDR genotype, calcium intake and bone strength as indicated by the broadband ultrasound attenuation (BUA) in community-dwelling older women with low calcium intake in rural Korea. Quantitative ultrasound was used since this non-invasive method is easy to apply and has been proposed as an alternative to dual-energy X-ray absorptiometry, which is the currently accepted indicator for assessing bone strength[8].

Abbreviations: BMD, bone mineral density; BUA, broadband ultrasound attenuation; VDR, vitamin D receptor.

* **Corresponding author:** Dr Byung-Kook Lee, fax +82 41 530 1778, email bklee@sch.ac.kr

Materials and methods

Subjects

In total, 350 women aged over 65 years were recruited from residents of Asan, Korea, of whom 335 (96 %) completed a 24 h recall interview, survey form, BUA measurement and blood collection. The subjects were aged from 65 to 94 years, with a mean age of 72·4 years. The study was approved by the Institute of Research Board of Soonchunhyang University and all participants provided their written, informed consent.

Data collection and analysis

Information on demographic characteristics (including physical activity on five scales) was obtained by survey and the dietary calcium intake was assessed using a 2 d, 24 h recall method. Food records were converted to nutrient intake using a computerized nutrient analysis program (CAN-pro; The Korean Society of Nutrition, Seoul, Korea). Approximately 10 ml of venous blood were collected and divided into two tubes: 4 ml were used for VDR genotype analysis, and 6 ml were immediately separated for analyses of serum total alkaline phosphatase, osteocalcin, intact parathyroid hormone and 25-hydroxyvitamin D_3. Alkaline phosphatase was analysed spectrophotometrically (TBA-40FR biochemical analyser; Hitachi, Tokyo, Japan), and osteocalcin and intact parathyroid hormone were analysed by an immunoradiometric assay at Samkwang Reference Laboratories (College of American Pathologists Accredited Laboratory 69 944-01, Seoul, Korea). 25-Hydroxyvitamin D_3 was analysed using HPLC as described previously[9]. BUA was measured with quantitative ultrasound at the left calcaneus (QUS-2; Metra Biosystems Inc., Mountain View, CA, USA). Intra- and inter-assay CV were all less than 10 %.

DNA analysis

DNA was extracted from leucocytes and amplified using the PCR. The enzyme *Bsm*I endonuclease was used to define the VDR gene allelic polymorphisms, with BB and bb representing the absence and presence of the restriction site on both alleles, respectively[10].

Statistics

All statistical analyses were performed using SPSS version 11.0 (SPSS Inc., Chicago, IL, USA). Statistical significance was defined at the $P<0{\cdot}05$ level based on Student's *t* test. To minimize extraneous errors in estimating dietary calcium intake due to individual differences in total food intake, calcium intake was adjusted for total energy intakes[11]. Multiple linear regression analysis was used to examine the relation between BUA and VDR after adjusting for age, weight, height, physical activity, calcium intake and 25-hydroxyvitamin D_3. The Bb and BB genotypes were combined in a regression model due to a small number of samples of BB type (*n* 3).

Results

The data on demographic indexes and nutritional, hormonal and bone factors for the different VDR genotypes are listed in Table 1. A total of 309 (92 %) participants were genotype bb, 23 (7 %) were Bb and 3 (1 %) were BB. The mean calcium intake of the total population was 439·6 (SD 279·6) mg/d, which is only 55 % of the recommended intake level of the Dietary Reference Intake for Koreans (which for calcium is 800 mg/d for women older than 65 years). The calcium intake did not differ between VDR genotypes, but 96 % of the participants had a calcium intake lower than the recommended Dietary Reference Intake for Koreans. The BUA was significantly higher in the bb genotype than in the

Table 1. Calcium intake, calcaneal broadband ultrasound attenuation (BUA) and bone markers for different vitamin D receptor (VDR) genotypes, and the impact of VDR on BUA as assessed using multiple linear regression analysis

	bb genotype (*n* 309, 92 %)		Bb and BB genotypes (*n* 29, 8 %)	
	Mean	SD	Mean	SD
Age (years)	72·4	5·7	72·0	5·5
Years since menopause	24·4	7·7	25·7	7·5
Weight (kg)	55·1	9·1	54·9	9·0
Height (cm)	148·7	5·8	150·4	5·7
Energy-adjusted dietary calcium intake (mg/d)	432·5	271·9	522·3	353·2
25-Hydroxyvitamin D_3 (ng/ml)	36·0	22·5	30·1	26·2
Intact parathyroid hormone (pg/ml)	17·8	4·5	19·3	5·9
BUA (dB/MHz)	62·5	15·6	56·1*	17·6
Osteocalcin (ng/ml)	7·75	6·62	8·61	6·16
Total alkaline phosphatase (U/l)	21·8	5·4	24·0	7·0
Multiple linear regression analysis†				
Variable	β	SE	*t*	*P*
Energy-adjusted dietary calcium intake	0·005	0·004	1·171	0·243
Serum 25-hydroxyvitamin D_3	0·076	0·053	1·420	0·158
VDR‡	−12·634	5·001	−2·527	0·013

Mean value was significantly different from that of the bb genotype group: *$P<0{\cdot}05$.
† Dependent variable was BUA, and age, weight, height and physical activity were controlled.
‡ VDR genotype was coded as bb = 0 and Bb/BB = 1.

Bb/BB genotype ($P<0{\cdot}05$, Student's *t*-test), while 25-hydroxyvitamin D_3, parathyroid hormone, osteocalcin and total alkaline phosphatase levels did not differ with the genotype. The multiple linear regression analysis also revealed that the VDR genotype was significantly associated with BUA after adjusting for age, weight, height, physical activity, calcium intake and 25-hydroxyvitamin D_3 ($P=0{\cdot}013$).

Discussion

We found a significant association between the VDR *Bsm*I genotype and calcaneal quantitative ultrasound results in Korean postmenopausal older women living in rural communities. Other studies involving Korean subjects have shown both the absence[12,13]and presence[14] of an association between VDR genotype and BMD or osteoporosis, and those involving various ethnic groups have produced contradictory results for the association between BMD and the b allele[15,16]. One distinguishing feature of the present study is that the calcium intake was lower than the recommended Dietary Reference Intake for Koreans (800 mg/d) in 96 % of the participants. Moreover, more than half (67 %) of them showed an intake of less than 500 mg/d, at which level active calcium transport increases (mediated by 1,25-dihydroxyl vitamin D), whereas passive diffusion accounts for an increasing proportion of the calcium absorbed as the calcium intake increases above 500 mg/d[17]. Dawson-Hughes *et al.*[6] reported that the calcium absorption efficiency was lower in women with the BB genotype with a functional defect in the intestinal VDR than in those with the bb genotype, when the calcium intake is low. Other studies[5,7] support the calcium absorption being higher in the b allele than in the B allele, although calcium intakes were not specified. The calcium intake of our population being the level at which it might be influenced by VDR may have resulted in the effect of VDR *Bsm*I genotype on bone strength being greater than that in previous studies conducted in Korea[12,13] and other countries[16]. Unfortunately, these previous studies did not examine calcium intakes. Therefore, the conflicting results of VDR *Bsm*I genotype effects on BMD may be attributable to the calcium intakes differing between the study populations.

The results of the current cross-sectional study are subject to the following limitations. Firstly, the use of a 24 h recall method may result in underreporting of dietary food intakes[18] and average 2 d, 24 h recall values may not reflect usual calcium intakes. However, the probability of technical errors or information bias is expected to be low in the current study since using energy-adjusted nutrient intake values can effectively reduce a between-person variance[11,19]. Moreover, our study population showed a relatively small within-person variance of 34 % CV for actual calcium intake, compared with a value of 49 % CV for American adult women[19]. The energy-adjusted calcium intake values rather than crude intake values further reduced the within-person variance to 28 % CV. Willett[19] and Beaton *et al.*[20] pointed out that within-person variances in nutrient intakes are largely affected by cultural factors, and the Korean elderly population (especially rural residents) – as analysed in the current study – exhibits a relative small day-to-day dietary intake variance[4]. Whilst these measures may not have avoided all of the problems associated with the 24 h recall method, it is unlikely that the non-significant association of calcium intake and BUA observed in this population is mainly attributable to measurement error. Secondly, BUA as measured by quantitative ultrasound differs from BMD and cannot be compared directly to the BMD as measured by dual-energy X-ray absorptiometry. Although dual-energy X-ray absorptiometry is considered to be the most accurate clinical method for identifying low BMD[21], in a community field situation such as in the current study, access to axial dual-energy X-ray absorptiometry is limited and screening for low BMD with dual-energy X-ray absorptiometry is not cost-effective[22]. We therefore used quantitative ultrasound to assess the calcaneal bone status since it has been used for the same purpose for almost two decades and has been shown to be clinically useful[8,23].

In conclusion, the results of the present study suggest that the BUA in populations with a low calcium intake is significantly influenced by the VDR genotype but not by the calcium intake, cross-sectionally.

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