Relationship Between Vitamin D Receptor Gene polymorphisms (BsmI, TaqI, Apal, and FokI) and Calcium Intake on Bone Mass in Young Japanese Women

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Title page

Relationship between vitamin D receptor gene polymorphisms (*BsmI*, *TaqI*, *ApaI*, and *FokI*) and calcium intake on bone mass in young Japanese women

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Abstract

Background: The increasing prevalence of low bone mass in young women in Japan has emerged as a serious health issue in recent years. Therefore, the aim of the present study was to reevaluate the relationship between genetic and dietary factors, as well as its influence on bone mass in young Japanese women, with particular emphasis on vitamin D receptor (VDR) gene polymorphisms and calcium intake.

Methods: A total of 499 Japanese women aged 20–24 years were enrolled in the study. The bone mass of the calcaneus was assessed using the quantitative ultrasound method and expressed as the osteo sono-assessment index (OSI). VDR gene polymorphisms (*BsmI*, *TaqI*, *ApaI*, and *FokI*) were analyzed using DNA extracted from saliva. Calcium intake was assessed using the Food Frequency Questionnaire based on food groups (FFQg) and adjusted with respect to the energy intake. Participants were divided into two groups based on the median calcium intake (250 mg/1000 kcal).

Results: Consequently, bone mass was significantly different among the *BsmI* genotypes and *TaqI* genotypes after adjusting the body mass index (BMI) ($p = 0.030$ and 0.019 , respectively). In addition, the *BsmI* AA and *ApaI* GT genotypes showed significant differences in bone mass between the calcium-intake groups, low OSI in the low-calcium intake group and high OSI in the high-calcium intake group, respectively, even after adjusting for covariates ($p = 0.020$ and 0.038 , respectively).

Conclusions: These findings would prove instrumental in developing a logical approach towards preventing bone loss in young Japanese women.

Keywords

vitamin D receptor gene polymorphisms; bone mass; premenopausal women; calcium intake; genotyping; osteoporosis

Background

In Japan, as well as in several developed countries, an increasing prevalence of low body weight among the younger generation poses a critical health issue. The body mass index (BMI) of Japanese men and women is the lowest among the BMI values calculated for people of other developed countries (1). Furthermore, according to a study on high school students from Japan, the United States, China, and Korea conducted by the National Institution For Youth Education in 2018, the lowest BMI was computed for the Japanese high school students among the youth of all the four countries (2). Approximately 20% of Japanese women in their 20s are underweight (BMI < 18.5), a rate that has been almost static over the past decade (3).

In women, menopause results in the rapid decline of bone mass (or density) owing to reduced estrogen production (4, 5). Furthermore, BMI demonstrates a strong positive correlation with bone mineral density (BMD) (6-8). It is known that excessive weight loss at a young age causes a deterioration in bone mass and accelerates postmenopausal osteoporosis. In a prior study, 17% of the 161 young Japanese women participants (Age 19.8 ± 1.1) showed low BMD as compared to that of women aged 50–60 years (9). To impede the progression of osteoporosis, it is crucial to prevent postmenopausal bone loss and also to boost bone mass at a younger age (10). Attaining a higher bone mass by the end of the growth period can potentially prevent fractures and conserve bone mass (10). Orito et al. reported that the BMD of 1322 young Japanese women peaked at 18–29 years (11). Thus, augmenting BMD in women in this age range is important in maintaining bone health and preventing osteoporosis throughout their lifetime.

Bone mass is also influenced by genetic factors. Genetic factors cannot be altered and determine approximately 80% of bone mass (12). Previous studies on the bone density of twins reported that monozygotic twins demonstrated a greater degree of similarity in bone density as compared to that among dizygotic twins (13-15). One such genetic factor is a single-nucleotide polymorphism (SNP). Vitamin D and its receptors are involved in calcium metabolism, and bone density is reportedly influenced by the associated genotypes, e.g., *BsmI* (rs1544410), an SNP in the vitamin D receptor (VDR) genes (14). In their meta-analysis, Gong et al. reported that VDR genotypes and bone density were related; the obtained results differed depending on factors such as gender, age, postmenopausal age, and the presence/absence of osteoporosis (16). In addition, lifestyle-related factors, including dietary calcium intake, exercise, smoking, and alcohol consumption, can also affect bone mass during growth (12, 17, 18). Thus, it is important to understand the relationship between bone mass and the VDR gene polymorphisms in the participants, considering these factors.

Several prior studies, also conducted in Japan, have investigated the effect of genetic and environmental factors, focusing on bone loss related to menopause. In contrast, there are relatively fewer studies that

assessed the relationship among bone mass, VDR gene polymorphisms, and environmental factors in premenopausal Japanese women. In these studies, the number of participants was low, and their age range was relatively wide (18–44 years) (19-21). Estrogen levels and lifestyle factors among participants can vary if a wide age range is considered in a study. Therefore, the present study aimed to investigate how VDR gene polymorphisms and calcium intake affect bone mass in young Japanese women, in a narrow-age cohort, specifically in the early twenties.

Materials and Methods

Study participants

Eligible participants comprised women aged 20–24 years, enrolled at Tokyo Kasei University. The study was conducted between April 2015 and March 2019. Written and verbal consent was obtained from 555 people who were given detailed explanations regarding bone mass measurements, questionnaires, and saliva collection. Participants who received obstetric treatment ($n = 42$), with missing data ($n = 10$), and suspected underestimation of energy ($n = 4$) were excluded; thus, the final analysis involved the processing, and further evaluation of the data was obtained from 499 participants. The study was approved by the ethics committee of Tokyo Kasei University (Approval number: ITAH26-04).

Calcaneus bone mass measurement

Bone mass at the right calcaneus was measured by quantitative ultrasound (QUS) using an AOS-100SA system (Hitachi Ltd., Tokyo, Japan); it is an indirect measure of bone mass. The correlation between bone mass (measured using QUS) and bone mineral density (measured by dual-energy X-ray absorptiometry) was strong ($r = 0.804$, $p < 0.001$) (22). Furthermore, QUS is a non-invasive, painless method and does not involve exposure to radiation (22). It is commonly carried out in Japan to screen patients for osteoporosis. Speed of sound (SOS) and transmission index (TI) were measured, and the osteo sono-assessment index (OSI; $OSI = TI \times SOS^2$) was used to calculate the corresponding bone mass. All OSI values were divided by 10^6 and used in the study.

DNA extraction and genotyping

Saliva samples were collected from each participant using Oragene® DNA OG-500 (DNA Genotek Inc., Ottawa, ON, Canada) and stored at 20–25 °C until further processing for DNA extraction. Genomic DNA was isolated from saliva in accordance with the manufacturer's protocol. The extracted DNA was diluted with sterile water to a concentration lower than 100 ng/μL and stored at –20 °C. Genotyping was performed by real-time PCR using the Thermal Cycler Dice® Real Time System (Takara Bio Inc., Shiga, Japan). Genotyping of VDR gene polymorphisms *BsmI* (rs1544410) and *TaqI* (rs731236) was detected using the Cycleave PCR Reaction Mix (Takara Bio Inc., Shiga, Japan). The genotyping of *ApaI* (rs7975232) and *FokI* (rs2228570) was conducted using the *TaqMan*™ GTXpress™ Master Mix (Applied Biosystems Inc., Foster

City, CA, USA).

Measurement of dietary calcium intake and BMI

Daily energy and calcium intake were assessed using the Food Frequency Questionnaire based on food groups (FFQg) Ver.3.0. To analyze the data, Excel add-in software (Excel Eiyoun-kun, Kenpakusha Co. Ltd., Tokyo, Japan) was used. Daily energy and calcium intake calculated based on FFQg correlated significantly with that calculated using 7-day weighed dietary records; the evaluation was performed on 66 participants aged 19–60 years ($r = 0.465, 0.410$, respectively) (23, 24). Calcium intake was adjusted according to the energy intake, and the participants were divided into two groups based on median calcium intake. In addition, body mass index (BMI, kg/m^2) was calculated from height and weight data with a self-administered questionnaire.

Statistical Analysis

All data were statistically evaluated using SPSS version 24 software (SPSS Inc., Chicago, IL, USA). The χ^2 test was used to compare the frequency of the observed genotypes with the Hardy–Weinberg equilibrium. The characteristics of the genotype groups were comparatively analyzed using analysis of variance (ANOVA). Analysis of covariance (ANCOVA) was used to evaluate the effect of calcium intakes on OSI based on VDR genotypes. The participants were divided into two groups, based on high and low calcium intake. The association between VDR genotypes and OSI was evaluated in each group by ANOVA and ANCOVA. A value of $p < 0.05$ was regarded as statistically significant.

Results

Characteristics of the participants

The OSI, an indicator of bone mass, was 2.72 ± 0.26 (Table 1). The minimum and maximum OSI values were 2.22 and 3.77, respectively. The OSI measured by the Japan Osteoporosis Society, using the same device, was equivalent in women of the same age group. The genotype frequencies of the four VDR gene polymorphisms (*BsmI*, *TaqI*, *ApaI*, and *FokI*) were similar to those reportedly previously in Japanese participants (25–27). No significant difference was observed in Hardy–Weinberg equilibrium ($p = 0.606, 0.907, 0.948$, and 0.382 , respectively). A significant difference in OSI was observed among the *BsmI* and *TaqI* genotypes after adjusting for covariates (BMI) ($p = 0.030$ and 0.019 , respectively) (Table 2); however, no significant difference was observed between the other two genotypes (*ApaI* and *FokI*) (Table 2).

Calcium intake

Median calcium intake (250 mg/1000 kcal) was used to divide the participants into two groups (low-intake group, ≤ 250 mg/1000 kcal, and high-intake group, ≥ 251 mg/1000 kcal). No significant difference was observed in participant characteristics, including the OSI, between the groups (OSI, low-intake group, 2.70

± 0.27, and high-intake group, 2.73 ± 0.25 , $p = 0.208$).

VDR genotypes, calcium intake, and OSI

OSI was analyzed between VDR genotypes and the calcium-intake groups. A significant difference in the OSI of the calcium-intake groups was observed for the *BsmI* AA genotype, even after adjusting for BMI ($p = 0.011$ and 0.020 , respectively) (Table 3). A significant difference in OSI was also observed for the *TaqI* CC genotype; however, the significance disappeared after adjustment for BMI ($p = 0.026$ and 0.057 , respectively) (Table 4). Furthermore, a significant difference in the OSI was observed between the calcium-intake groups of the *ApaI* GT genotype, even after adjusting for covariates ($p = 0.026$ and 0.038 , respectively) (Table 5). OSI values of the *FokI* genotypes did not change between the calcium-intake groups (data not shown).

Table 1. Characteristics of the participants (n = 499).

Variable	Value
Age (years)	20.0 ± 0.3
Height (cm)	158.7 ± 5.3
Weight (kg)	51.4 ± 6.0
BMI (kg/m^2)	20.4 ± 2.0
OSI	2.72 ± 0.26
Energy (kcal/day)	1724 ± 388
Calcium (mg/day)	457 ± 170
Calcium (mg/1000 kcal)	263 ± 69

Values are means ± standard deviation (SD) or percentage.

Table 2. Genotype frequencies and characteristics according to VDR genotypes (*BsmI*, *TaqI*, *ApaI* and *FokI*).

<i>BsmI</i> (rs1544410)				
	GG	GA	AA	<i>p</i>
Number (%)	399 (80.0)	92 (18.4)	8 (1.6)	-
Age (years)	20.1 ± 0.0	20.0 ± 0.0	20.0 ± 0.1	0.901
BMI (kg/m ²)	20.5 ± 0.1	20.1 ± 0.2	19.4 ± 0.6	0.100
Calcium (mg/1000 kcal)	264 ± 3	262 ± 7	247 ± 11	0.780
OSI Not adjusted	2.73 ± 0.01	2.69 ± 0.02	2.45 ± 0.07	0.009**
OSI Adjusted for BMI	2.72 ± 0.01	2.70 ± 0.03	2.49 ± 0.09	0.030*
<i>TaqI</i> (rs731236)				
	TT	TC	CC	<i>p</i>
Number (%)	389 (78.0)	102 (20.4)	8 (1.6)	-
Age (years)	20.1 ± 0.0	20.0 ± 0.0	20.0 ± 0.0	0.545
BMI (kg/m ²)	20.4 ± 0.1	20.5 ± 0.2	21.0 ± 0.9	0.622
Calcium (mg/1000 kcal)	266 ± 7	254 ± 12	255 ± 3	0.291
OSI Not adjusted	2.73 ± 0.01	2.69 ± 0.03	2.52 ± 0.07	0.058
OSI Adjusted for BMI	2.73 ± 0.01	2.69 ± 0.02	2.49 ± 0.09	0.019*
<i>ApaI</i> (rs7975232)				
	GG	GT	TT	<i>p</i>
Number (%)	226 (45.3)	222 (44.5)	51 (10.2)	-
Age (years)	20.1 ± 0.0	20.0 ± 0.0	20.0 ± 0.0	0.357
BMI (kg/m ²)	20.3 ± 0.1	20.6 ± 0.1	20.4 ± 0.3	0.382
Calcium (mg/1000 kcal)	266 ± 4	265 ± 5	246 ± 6	0.156
OSI Not adjusted	2.71 ± 0.02	2.73 ± 0.02	2.68 ± 0.03	0.458
OSI Adjusted for BMI	2.72 ± 0.02	2.72 ± 0.02	2.68 ± 0.03	0.518
<i>FokI</i> (rs2228570)				
	CC	CT	TT	<i>p</i>
Number (%)	195 (39.1)	222 (44.5)	82 (16.4)	-
Age (years)	20.0 ± 0.0	20.1 ± 0.0	20.0 ± 0.0	0.275
BMI (kg/m ²)	20.6 ± 0.2	20.2 ± 0.1	20.4 ± 0.2	0.140
Calcium (mg/1000 kcal)	256 ± 5	271 ± 5	259 ± 7	0.084
OSI Not adjusted	2.70 ± 0.02	2.72 ± 0.02	2.74 ± 0.03	0.371
OSI Adjusted for BMI	2.69 ± 0.02	2.73 ± 0.02	2.74 ± 0.03	0.133

Values are means ± standard error (SE) or percentage. The *p* values indicate the significance of difference based on genotype. Continuous variables were assessed by ANOVA. *, *p* < 0.05; **, *p* < 0.01

Table 3. Effect of *BsmI* genotype and calcium intake on OSI.

<i>BsmI</i> genotype	Calcium intake	n	Not adjusted		Adjusted [†]	
			OSI	<i>p</i>	OSI	<i>p</i>
GG	Low	192	2.72 ± 0.02	0.772	2.72 ± 0.02	0.729
	High	207	2.73 ± 0.02		2.73 ± 0.02	
GA	Low	50	2.66 ± 0.03	0.098	2.66 ± 0.03	0.194
	High	42	2.74 ± 0.04		2.73 ± 0.04	
AA	Low	5	2.33 ± 0.06	0.011*	2.33 ± 0.06	0.020*
	High	3	2.65 ± 0.07		2.65 ± 0.08	

Values are means ± SE. The *p* values indicate the significance of difference in OSI between calcium-intake groups. Differences were assessed using ANCOVA. †, adjusted for BMI. *, *p* < 0.05

Table 4. Effect of *TaqI* genotype and calcium intake on OSI.

<i>TaqI</i> genotype	Calcium intake	n	Not adjusted		Adjusted [†]	
			OSI	<i>p</i>	OSI	<i>p</i>
TT	Low	184	2.72 ± 0.02	0.586	2.72 ± 0.02	0.613
	High	205	2.73 ± 0.02		2.73 ± 0.02	
TC	Low	60	2.67 ± 0.03	0.221	2.67 ± 0.03	0.333
	High	42	2.73 ± 0.04		2.72 ± 0.04	
CC	Low	3	2.33 ± 0.08	0.026*	2.35 ± 0.09	0.057
	High	5	2.63 ± 0.06		2.62 ± 0.07	

Values are means ± SE. The *p* values indicate the significance of difference in OSI between calcium-intake groups. Differences were assessed using ANCOVA. †, adjusted for BMI. *, *p* < 0.05

Table 5. Effect of *ApaI* genotype and calcium intake on OSI.

<i>ApaI</i> genotype	Calcium intake	n	Not adjusted		Adjusted [†]	
			OSI	<i>p</i>	OSI	<i>p</i>
GG	Low	105	2.72 ± 0.03	0.768	2.72 ± 0.02	0.743
	High	121	2.71 ± 0.02		2.71 ± 0.02	
GT	Low	110	2.69 ± 0.03	0.026*	2.69 ± 0.02	0.038*
	High	112	2.77 ± 0.03		2.76 ± 0.02	
TT	Low	32	2.69 ± 0.04	0.571	2.69 ± 0.04	0.509
	High	19	2.65 ± 0.06		2.65 ± 0.05	

Values are means ± SE. The *p* values indicate the significance of difference in OSI between calcium-intake groups. Differences were assessed using ANCOVA. †, adjusted for BMI. *, *p* < 0.05

Discussion

The present study focused on the VDR genotypes, bone mass, and calcium intake in young Japanese women. As the relationship between bone mass and Cdx2 (rs11568820), a representative SNP of VDR genes (28), had been elucidated by us previously, the focus was on four representative VDR gene polymorphisms other

than Cdx2 in the present study; these included *BsmI* (rs1544410), *TaqI* (rs731236), *ApaI* (rs7975232), and *FokI* (rs2228570).

Several prior studies conducted worldwide have reported the relationship between the VDR genotypes and bone mass. Among these, a meta-analysis reported that the bone mass of the spine was significantly lower in the *BsmI* AA genotype than in the *BsmI* GG and GA genotypes in postmenopausal women (51–75 years) (29). However, the effects of the *BsmI* AA and *TaqI* CC genotypes on bone mass in Japanese women have rarely been examined because these genotypes are present at a relatively lower proportion than that seen in European countries; e.g., the respective allelic frequencies for the *BsmI* genotype G and A in the European population were 59% and 41%, respectively, compared to 88% and 12% in the Japanese population, (25, 30), indicating a clear racial difference. In previous studies, less than 200 Japanese women participants were observed, a few of whom expressed the *BsmI* AA and *TaqI* CC genotypes ($n = 0\text{--}2$); hence, the relevant data could not be included in the analyses (20, 21, 31–33). However, in the present study involving 499 participants, these genotypes were recognized ($n = 8$ for both) and included in the analysis. Consistent with the findings obtained in previous studies, the *BsmI* genotype showed a significant difference in BMD also in young Japanese women; the OSI of the *BsmI* AA genotype was lower than that for other genotypes.

The OSI values of the *TaqI* CC genotype differed significantly from those of other genotypes only after adjusting for BMI ($p = 0.019$). Previous studies on the *TaqI* genotype and bone mass in premenopausal Japanese women did not report consistent results, and reported no significant difference (19, 34–37); or, participants with the *TaqI* TT genotype displayed higher bone mass than those with the TC genotype (21, 31). These studies, however, did not include the participants with the *TaqI* CC genotype because of a limited number of participants harboring this genotype. In a previous large-scale cohort study ($n = 778$) on representative samples obtained from Japanese participants, the presence of the *TaqI* genotype, including the CC genotype ($n = 11$, 1.4%), did not significantly influence BMD in premenopausal women; however, this study was not adjusted for BMI (37). Furthermore, a study with a sufficient number of participants would be needed to evaluate the effect of the *TaqI* CC genotype on BMD in Japanese women. The OSI values of the *ApaI* and *FokI* genotypes were not significantly different in the present study; this observation was consistent with the results of previous studies on these specific genotypes in Japanese women, which did not report significant differences in genotype and bone mass (19, 31, 35, 37).

Several environmental factors, especially dietary calcium intake, have been thought to affect the relationship between bone mass and VDR genotypes.

From our previous study, it was reported that the relationship between the VDR Cdx2 genotype and bone mass differs depending on dietary calcium intake (28). Furthermore, certain studies have implied that VDR genotypes modulate the calcium absorption rate (38–40). Gennari et al. reported that postmenopausal participants with the *BsmI* AA and *TaqI* CC genotypes demonstrated reduced intestinal calcium absorption (39). Consequently, our study showed a significant difference in the OSI values between the calcium-intake groups of the *BsmI* AA genotype, even after adjusting for BMI ($p = 0.020$). Therefore, this genotype may

interact with calcium intake and affect bone mass in young Japanese women. In contrast, no effect of the interaction between the *TaqI* CC genotype and calcium intake on bone mass was observed. Intriguingly, there was a significant difference between the calcium-intake groups of the *ApaI* GT genotype; OSI was higher in the high-calcium intake group. Similar to our study, a previous study also showed that bone mass in individuals with the *ApaI* GT genotype was elevated concomitantly with high calcium intake; this estimation was made based on the frequency of consumption of seven food groups considered to be rich in calcium: milk, milk products, bean and bean products, meat, fish, dried fish with bone, and green vegetables (35).

The present study has a few limitations. Primarily, the phenotypic ratio of VDR gene polymorphisms varies among genotypes; hence, the statistical power of OSI could differ. Therefore, uniformity in the number of participants is essential. In particular, there were only a limited number of participants for the *BsmI* AA and *TaqI* CC genotypes because of the low proportion of these genotypes in the Japanese population; however, it is our expectation that the data from the present study would prove to be valuable. Second, serum vitamin D levels related to bone formation were not considered. However, there is reportedly no significant difference in serum vitamin D levels among genotypes for these four VDR gene polymorphisms (*BsmI*, *TaqI*, *ApaI*, and *FokI*) (41, 42). Third, there are several environmental factors that may affect bone mass, such as smoking and alcohol consumption; however, these factors were not considered here. Smoking and alcohol consumption by the age of 20 are legally prohibited in Japan; hence, the influence of these factors would be minimal among the participants enrolled in the present study. Furthermore, a previous study showed that smoking and alcohol consumption did not affect BMD at younger ages (9).

Conclusion

The present study shows that the VDR *BsmI* and *TaqI* genotypes are potentially associated with bone mass in young Japanese women. Among them, the *BsmI* AA genotype may interact with the calcium intake and be related to a lower bone mass under conditions of low calcium intake. In addition, the VDR *ApaI* GT genotype is associated with increased bone mass concomitant with higher calcium intake. Finally, our results would be immensely valuable in the development of a preventive strategy to tackle bone loss and future osteoporosis in young women.

Abbreviations

ANCOVA: Analysis of covariance

ANOVA: analysis of variance

BMD: bone mineral density

BMI: body mass index

FFQg: Food Frequency Questionnaire based on food groups

OSI: osteo sono-assessment index

SNP: single-nucleotide polymorphism

VDR: vitamin D receptor

Declarations

Ethics approval and consent to participate

All participants signed an informed consent form. They provided samples by bone mass measurements and saliva collection, in addition to answering questionnaires. The study was approved by the ethics committee of Tokyo Kasei University (Approval number: ITAH26-04).

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analysed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests

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Authors' Contributions

YS and YT planned the overall objective. YS and FO planned and conducted the study. KI and PW provided statistical advice on the analyses. YS wrote the first draft. YS and YT are responsible for the overall content as the guarantor of the paper. All authors revised the paper critically for intellectual content and approved the final version. All authors agree to be accountable for the study and to ensure that any questions relating to the accuracy and integrity of the paper are investigated and properly resolved.

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Authors' information (optional)

Not applicable.

References

1. World Health Organization. Global Health Observatory data repository. 2016, <https://apps.who.int/gho/data/node.main.BMIMEANC?lang=en>. Accessed 6 Aug 2020.
2. The National Institute for Youth Education. Survey of High School Students' Views on Mental and Physical Health. 2018. http://www.niye.go.jp/kenkyu_houkoku/contents/detail/i/126/. Accessed 6 Aug 2020.
3. Ministry of Health, Labor and Welfare. National Health and Nutrition Survey. 2018. https://www.mhlw.go.jp/bunya/kenkou/kenkou_eiyou_chousa.html. Accessed 6 Aug 2020.
4. Zhang HC, Kushida K, Atsumi K, Kin K, Nagano A. Effects of age and menopause on spinal bone mineral density in Japanese women: a ten-year prospective study. *Calcif Tissue Int*. 2002;70(3):153-7.
5. Pérez-López FR, Chedraui P, Cuadros-López JL. Bone mass gain during puberty and adolescence: deconstructing gender characteristics. *Curr Med Chem*. 2010;17(5):453-66.
6. Al-Shoumer KA, Nair V. Prevalence of low bone mass in postmenopausal Kuwaiti women residents in the largest province of Kuwait. *Arch Osteoporos*. 2012;7:147-53.
7. Méndez JP, Rojano-Mejía D, Pedraza J, Coral-Vázquez RM, Soriano R, García-García E, et al. Bone mineral density in postmenopausal Mexican-Mestizo women with normal body mass index, overweight, or obesity. *Menopause*. 2013;20(5):568-72.
8. Gerber LM, Bener A, Al-Ali HM, Hammoudeh M, Liu LQ, Verjee M. Bone mineral density in midlife women: the Study of Women's Health in Qatar. *Climacteric*. 2015;18(2):316-22.
9. Hirota T, Nara M, Ohguri M, Manago E, Hirota K. Effect of diet and lifestyle on bone mass in Asian young women. *Am J Clin Nutr*. 1992;55(6):1168-73.
10. Bonjour JP, Chevalley T, Ferrari S, Rizzoli R. The importance and relevance of peak bone mass in the prevalence of osteoporosis. *Salud Publica Mex*. 2009;51 Suppl 1:S5-17.
11. Orito S, Kuroda T, Onoe Y, Sato Y, Ohta H. Age-related distribution of bone and skeletal parameters in 1,322 Japanese young women. *J Bone Miner Metab*. 2009;27(6):698-704.
12. Davies JH, Evans BA, Gregory JW. Bone mass acquisition in healthy children. *Arch Dis Child*. 2005;90(4):373-8.
13. Pocock NA, Eisman JA, Hopper JL, Yeates MG, Sambrook PN, Eberl S. Genetic determinants of bone mass in adults. A twin study. *J Clin Invest*. 1987;80(3):706-10.
14. 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(6460):284-7.
15. Takeshita T, Yamagata Z, Iijima S, Nakamura T, Ouchi Y, Orimo H, et al. Genetic and environmental factors of bone mineral density indicated in Japanese twins. *Gerontology*. 1992;38 Suppl 1:43-9.

16. Gong G, Stern HS, Cheng SC, Fong N, Mordeson J, Deng HW, et al. The association of bone mineral density with vitamin D receptor gene polymorphisms. *Osteoporos Int.* 1999;9(1):55-64.
17. Miyabara Y, Onoe Y, Harada A, Kuroda T, Sasaki S, Ohta H. Effect of physical activity and nutrition on bone mineral density in young Japanese women. *J Bone Miner Metab.* 2007;25(6):414-8.
18. Weaver CM, Gordon CM, Janz KF, Kalkwarf HJ, Lappe JM, Lewis R, et al. The National Osteoporosis Foundation's position statement on peak bone mass development and lifestyle factors: a systematic review and implementation recommendations. *Osteoporos Int.* 2016;27(4):1281-386.
19. Kurabayashi T, Matsushita H, Kato N, Nagata H, Kikuchi M, Tomita M, et al. Effect of vitamin D receptor and estrogen receptor gene polymorphism on the relationship between dietary calcium and bone mineral density in Japanese women. *J Bone Miner Metab.* 2004;22(2):139-47.
20. Omasu F, Kitagawa J, Koyama K, Asakawa K, Yokouchi J, Ando D, et al. The influence of VDR genotype and exercise on ultrasound parameters in young adult Japanese women. *J Physiol Anthropol Appl Human Sci.* 2004;23(2):49-55.
21. Funakoshi Y, Omori H, Katoh T. Relation of bone mineral density to vitamin D receptor gene polymorphism and lifestyle factors in Japanese female workers aged 22-44 years: a cross-sectional study. *J Nutr Sci Vitaminol (Tokyo).* 2010;56(1):27-33.
22. Greenspan SL, Bouxsein ML, Melton ME, Kolodny AH, Clair JH, Delucca PT, et al. Precision and discriminatory ability of calcaneal bone assessment technologies. *J Bone Miner Res.* 1997;12(8):1303-13.
23. Takahashi K, Yoshimura Y, Kaimoto T, Kunii D, Komatsu T, Yamamoto, S. Validation of a Food Frequency Questionnaire Based on Food Groups for Estimating Individual Nutrient Intake. *Jpn J. Nutr. Diet;* 2001; 59:221-232. (In Japanese)
24. Takahashi K. Food Frequency Questionnaire Based on Food Groups for Estimating Individual Nutrient Intake: *Jpn J. Nutr. Diet;* 2003; 61:161-69. (In Japanese)
25. Lurie G, Wilkens LR, Thompson PJ, McDuffie KE, Carney ME, Terada KY, et al. Vitamin D receptor gene polymorphisms and epithelial ovarian cancer risk. *Cancer Epidemiol Biomarkers Prev.* 2007;16(12):2566-71.
26. Tanaka K, Miyake Y, Hanioka T, Arakawa M. VDR gene polymorphisms, interaction with smoking and risk of periodontal disease in Japanese women: the Kyushu Okinawa maternal and child health study. *Scand J Immunol.* 2013;78(4):371-7.
27. Inoue N, Watanabe M, Ishido N, Katsumata Y, Kagawa T, Hidaka Y, et al. The functional polymorphisms of VDR, GC and CYP2R1 are involved in the pathogenesis of autoimmune thyroid diseases. *Clin Exp Immunol.* 2014;178(2):262-9.
28. Oono F, Sakamoto Y, Tachi Y, Mabashi-Asazuma H, Iida K. Effect of Cdx2 Polymorphism on the Relationship between Dietary Calcium Intake and Peak Bone Mass in Young Japanese Women. *Nutrients.* 2020;12(1).

29. Thakkestian A, D'Este C, Eisman J, Nguyen T, Attia J. Meta-analysis of molecular association studies: vitamin D receptor gene polymorphisms and BMD as a case study. *J Bone Miner Res.* 2004;19(3):419-28.
30. 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(7):1003-9.
31. Fujita Y, Katsumata K, Unno A, Tawa T, Tokita A. Factors affecting peak bone density in Japanese women. *Calcif Tissue Int.* 1999;64(2):107-11.
32. Kubota M, Yoshida S, Ikeda M, Okada Y, Arai H, Miyamoto K, et al. Association between two types of vitamin d receptor gene polymorphism and bone status in premenopausal Japanese women. *Calcif Tissue Int.* 2001;68(1):16-22.
33. Katsuyama H, Ideguchi S, Fukunaga M, Saijoh K, Sunami S. Usual dietary intake of fermented soybeans (Natto) is associated with bone mineral density in premenopausal women. *J Nutr Sci Vitaminol (Tokyo).* 2002;48(3):207-15.
34. Kitagawa I, Kitagawa Y, Kawase Y, Nagaya T, Tokudome S. Advanced onset of menarche and higher bone mineral density depending on vitamin D receptor gene polymorphism. *Eur J Endocrinol.* 1998;139(5):522-7.
35. Kitagawa I, Kitagawa Y, Nagaya T, Tokudome S. Interplay of physical activity and vitamin D receptor gene polymorphism on bone mineral density. *J Epidemiol.* 2001;11(5):229-32.
36. Katsumata K, Nishizawa K, Unno A, Fujita Y, Tokita A. Association of gene polymorphisms and bone density in Japanese girls. *J Bone Miner Metab.* 2002;20(3):164-9.
37. Morita A, Iki M, Dohi Y, Ikeda Y, Kagamimori S, Kagawa Y, et al. Prediction of bone mineral density from vitamin D receptor polymorphisms is uncertain in representative samples of Japanese Women. The Japanese Population-based Osteoporosis (JPOS) Study. *Int J Epidemiol.* 2004;33(5):979-88.
38. Dawson-Hughes B, Harris SS, Finneran S. Calcium absorption on high and low calcium intakes in relation to vitamin D receptor genotype. *J Clin Endocrinol Metab.* 1995;80(12):3657-61.
39. Gennari L, Becherini L, Masi L, Gonnelli S, Cepollaro C, Martini S, et al. Vitamin D receptor genotypes and intestinal calcium absorption in postmenopausal women. *Calcif Tissue Int.* 1997;61(6):460-3.
40. Ames SK, Ellis KJ, Gunn SK, Copeland KC, Abrams SA. Vitamin D receptor gene FokI polymorphism predicts calcium absorption and bone mineral density in children. *J Bone Miner Res.* 1999;14(5):740-6.
41. Sanwalka N, Khadilkar A, Chiplonkar S, Khatod K, Phadke N, Khadilkar V. Vitamin D receptor gene polymorphisms and bone mass indices in post-menarchal Indian adolescent girls. *J Bone Miner Metab.* 2013;31(1):108-15.
42. Oliveira Junior LR, Carvalho TB, Santos RMD, Costa É, Pereira PCM, Kurokawa CS.

- 398 Association of vitamin D3, VDR gene polymorphisms, and LL-37 with a clinical form of Chagas Disease.
399 Rev Soc Bras Med Trop. 2019;52:e20190133.