Original Article

Relationship between changes of bone mineral density over seven years and A1330V polymorphism of the low-density lipoprotein receptor-related protein 5 gene or lifestyle factors in Japanese female workers

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A longitudinal study was conducted to investigate the relation between the changes of bone mineral density (BMD) over a seven-year period and A1330V polymorphism of the *low-density lipoprotein receptor-related protein 5 (LRP5)* gene or lifestyle factors. The subjects were 113 premenopausal female employees from a large-scale integrated manufacturing facility in Japan aged 25.6±4.2 years (mean ± standard deviation) at baseline. BMD was measured at the radius by dual energy X-ray absorptiometry. Lifestyle information was obtained by a questionnaire. The genotype frequencies of *LRP5* gene polymorphism were 52%, 39%, and 9% for *AA*, *AV*, and *VV*, respectively. After seven years, BMD showed a significant decrease (from 0.463±0.045 to 0.456±0.046 g/m^2) in subjects with the *AV* or *VV* genotypes, but not in subjects with the *AA* genotype. Analysis of covariance with adjustment for age and body mass index showed that subjects who drank alcohol displayed a significantly greater change of BMD if they had the *AV* or *VV* genotype than if they had the *AA* genotype (F=4.547, p=0.036). Investigation of *LRP5* A1330V polymorphism may be useful for identifying individuals who are susceptible to osteoporosis, allowing early preventive measures to be provided.

Key Words: bone mineral density, genetic polymorphism, Japan, lifestyle, low-density lipoprotein receptor-related protein 5 gene

INTRODUCTION

Osteoporosis is defined as a skeletal disorder characterized by loss of bone strength that predisposes to an increased risk of fracture.[1] The incidence of hip fractures was reported in national osteoporosis surveys which were conducted every five years: 1987, 92, 97, 2002, and 2007.[2] They clearly demonstrate that the number of new cases has been rising with each survey. Women were especially affected, the number of new cases of hip fracture having nearly tripled from 39,700 in 1987 to 116,800 in 2007.[2] Bone strength primarily depends on the density and quality of bone.[1] Bone mineral density (BMD) is frequently used as a measure of strength since it accounts for approximately 70% of bone strength.[1] Thus, a diagnosis of osteoporosis is made by measurement of BMD,[3] and a low BMD has been established as an important predictor of future fracture risk.[4] During the first 5 years after menopause, women experience a rapid decrease in bone mass due to an increase of bone turnover.[5] According to a previous study,[6] which reported on the natural history of BMD in the distal radius in healthy Japanese women, suggested that BMD reached a peak in the early part of the fourth decade, and then declined. The decrease began in the sixth decade, accelerated rapidly in the seventh, and reached a plateau around the ninth decade. Because a decrease of BMD is inevitable with aging, particularly in women after menopause, it is necessary to identify and manage any premenopausal decrease of BMD. In contrast to elderly women, however, the risk factors for low BMD in premenopausal women have not been thoroughly investigated.

BMD is influenced by both genetic factors and lifestyle factors such as exercise, smoking, alcohol and diet,[7,8] but it remains unclear whether the lifestyle of Japanese females lead to changes of BMD risk factors over the long-term. Results of twin and family studies have provided further evidence that gene polymorphism plays a major role in the determination of BMD.[9,10] A recent large-scale study that involved a analysis of individual-level data from the full Genetic Markers for Osteoporosis (GENOMOS) consortium, including data on 37,534 individuals from 18 participating teams, found that rs3736228 (A1330V) of the *low-density lipoprotein receptor-related protein 5 (LRP5)* gene on chromosome 11 was strongly associated with the BMD in Europeans and North Americans.[11] Thus, *LRP5* A1330V polymorphism has become the focus of attention as a useful genetic marker for

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predicting BMD. Another genome-wide association study12 and two meta-analyses[13,14] have also shown that LRP5 A1330V polymorphism is associated with BMD. A previous study[15] investigated the relation between *LRP5* A1330V polymorphism and BMD in the general Japanese female population, but it was performed with a cross-sectional design. Not only genes, but also lifestyle factors and their interaction with genetic variation play a prominent role in the regulation of BMD,[7,8] but the interactions between *LRP5* A1330V polymorphism and lifestyle factors remain poorly understood. Improved understanding of their combined effect may allow us to identify persons with a high risk of osteoporosis and lead to more effective prevention of this condition. Accordingly, the purpose of the present longitudinal study was to investigate the relation of changes in BMD over a seven-year period with *LRP5* A1330V polymorphism and lifestyle factors in premenopausal Japanese female workers.

MATERIALS AND METHODS

Subjects

The subjects were 113 premenopausal female employees working at a large-scale integrated manufacturing facility in Japan. The menstrual history was recorded at an individual interview and premenopausal status was defined on the basis of regular menstruation. The criteria for entry into this study were no prior diagnosis of osteoporosis, no systemic diseases, and no medications known to influence bone or calcium metabolism. Height, weight, and BMD were measured during a comprehensive health check. Body mass index (BMI) was calculated as the weight in kilograms divided by the height in meters squared. Lifestyle information, such as the past history of exercise, current exercise, daily milk intake, number of meals per day, history of dieting, smoking status, and alcohol intake was obtained from a self-reporting questionnaire.[16]

Measurement of bone mineral density (BMD)

BMD was measured at the distal 1/3 site of the radius on the non-dominant side by DXA (Osteometer DTX200) according to the manufacturer's protocol (CV for precision error<1.0% in vivo). This measurement has been validated as being highly predictive of fracture risk.[17]

DNA extraction and genotyping

Genomic DNA was extracted from peripheral blood leukocytes using a DNA Extractor WB Kit (Wako Pure Chemical Industries, Osaka, Japan) according to the manufacturer's instructions.

A real-time quantitative polymerase chain reaction assay was performed using a Step One Sequence Detector (Applied Biosystems). *LRP5* A1330V (rs3736228) polymorphism was genotyped with a Custom TaqMan Genotyping Assay (Applied Biosystems) according to the manufacturer's protocol.

Statistical analysis

Statistical analysis was performed with IBM SPSS Statistics 18 software. Results are presented as the mean ± SD. The chi-square test was employed to verify the Hardy-Weinberg equilibrium of genotype frequencies. The paired *t*-test was used to assess the significance of changes in the measureable variables from baseline. Student's *t*-test and Mann-Whitney U test were used to assess the differences between each type of polymorphism. Analysis of covariance (ANCOVA) with adjustment for age and BMI was employed to determine the influence of *LRP5* A1330V polymorphism and lifestyle factors on changes of BMD. We then confirmed that these interactions were not significant. Statistical significance was accepted at $p<0.05$.

RESULTS

Table 1 shows the baseline characteristics of the subjects stratified according to *LRP5* A1330V polymorphism. Their mean baseline age was 25.6±4.2 years and mean BMD was 0.465±0.044 g/cm^2. *LRP5* A1330V polymorphism (n=59 *AA*, n=44 *AV*, and n=10 *VV*) showed a distribution that followed the Hardy-Weinberg equilibrium (p=0.189). The allele frequency of *LRP5* A1330V polymorphism (52% *AA*, 39% *AV*, and 9% *VV)* was similar to that reported previously in a Japanese population (46% *AA*, 45% *AV*, and 9% *VV*).[15] It was necessary to combine the heterozygous (*AV*) and homozygous *(VV*) genotypes when comparing characteristics due to the low frequency of the *VV* genotype in the Japanese population. No significant differences of *LRP5* A1330V polymorphism were seen in relation to age, height, weight, BMI, and BMD. There were also no differences in the past history of exercise, current exercise, daily milk intake, number of meals per day, history of dieting, smoking status, and alcohol intake between *LRP5* A1330V genotypes.

Table 2 shows the changes in various parameters over seven years. There was a significant increase of height, weight, and BMI according to the paired *t*-test, but BMD significantly decreased after seven years in all subjects. BMD significantly decreased in subjects with the *AV* or *VV* genotype (according to the paired Student's *t*-test), but not in subjects with the *AA* genotype. In addition, the weight and BMI of subjects with the *AA* genotype and the height of subjects of both genotypes (*AA* and *AV*+*VV*) had increased significantly from the baseline values.

Table 3 shows the combined effects of lifestyle factors and *LRP5* A1330V polymorphism on the changes of BMD. After adjustment for age and BMI by ANCOVA, subjects who drank alcohol showed a significantly greater change of BMD if they had the *AV* or *VV* genotype than if they had the *AA* genotype (F=4.547, p=0.036), and there was no significant interaction among these variables (F=2.202, p=0.118). On the other hand, there was no significant association between BMD and the other lifestyle factors investigated (past history of exercise, current exercise, daily milk intake, number of meals per day, history of dieting, and smoking status).

DISCUSSION

In this study, we found that Japanese female workers with the *AV* or *VV* genotype showed a significant decrease of BMD after follow-up for seven years. In addition, we found that subjects who drank alcohol showed a significantly greater change of BMD if they had the *AV* or *VV* genotype than if they had the *AA* genotype. *LRP5* A1330V polymorphism has already been reported to influence BMD in Japanese women,[15] but previous studies have not assessed the long-term effects. To our knowl-

Table 1. Baseline characteristics of the subjects stratified according to *low-density lipoprotein receptor related protein 5 (LRP5)* A1330V polymorphism

Valuable	All (*n*=113)	*AA* (*n*=59)	*AV* + *VV* (*n*=54)
Age (years)	25.6±4.2	25.4±4.4	25.9±4.0
Height (cm)	157.3±5.8	157.8±6.3	156.7±5.1
Weight (kg)	50.3±6.6	50.1±5.8	50.5±7.3
BMI (kg/m^2)	20.2±2.4	20.1±2.2	20.4±2.7
BMD (g/m^2)	0.465±0.044	0.467±0.044	0.463±0.045
Past history of exercise (%)			
No	43 (38.4)	25 (42.4)	18 (34.0)
Yes	69 (61.6)	34 (57.6)	35 (66.0)
Current exercise (%)			
No	87 (77.0)	46 (78.0)	41 (75.9)
Yes	26 (23.0)	13 (22.0)	13 (24.1)
Daily milk intake (%)			
No	84 (74.3)	43 (72.9)	41 (75.9)
Yes	29 (25.7)	16 (27.1)	13 (24.1)
Number of meals daily (%)			
3 meals/day	23 (20.4)	9 (15.3)	14 (25.9)
2 meals/day	90 (79.6)	50 (84.7)	40 (74.1)
History of dieting (%)			
No	68 (60.2)	34 (57.6)	34 (63.0)
Yes	45 (39.8)	25 (42.4)	20 (37.0)
Smoking status (%)			
Non-smoker	97 (85.8)	50 (84.7)	47 (87.0)
Smoker	16 (14.2)	9 (15.3)	7 (13.0)
Alcohol intake (%)			
Non-drinker	34 (30.1)	22 (37.3)	12 (22.2)
Drinker	79 (69.9)	37 (62.7)	42 (77.8)

Values are the mean ± SD.
Abbreviations: BMI=body mass index, BMD=bone mineral density.
A past history of exercise was determined from the exercise habits until the age of 20 years.
Each characteristic was not different between *AA* and *AV* + *VV* significantly by Student's *t*-test and Mann-Whitney U test.

Table 2. Changes in height, weight, BMI and bone mineral density over seven years

Valuable	All (*n*=113)	*AA* (n=59)	*AV* + *VV* (*n*=54)
Change in height (cm)	0.5±0.7*	0.4±0.6*	0.6±0.7*
Change in weight (kg)	1.5±4.1*	2.3±4.0*	0.7±4.0
Change in BMI (kg/m^2)	0.6±1.7*	0.9±1.6*	0.3±1.7
Change in BMD (g/m^2)	-0.003±0.018*	-0.001±0.019	-0.006±0.017*

Values are the mean ± SD.
Abbreviations: BMI=body mass index, BMD=bone mineral density.
Data were analyzed using paired Student's *t*-test.
*p<0.05 compared to baseline.

edge, this is the first report of an association between changes of BMD and lifestyle factors or *LRP5* A1330V polymorphism in Japanese women followed for seven years.

Chromosome region 11q12-13 contains the *LRP5* gene and has been shown to be a key quantitative trait locus (QTL) contributing to variations of BMD.[18] Previous studies have demonstrated that the LRP5 family is involved in the Wnt signaling pathway.[19,20] This pathway induces the upregulation of OPG expression and down-regulation of receptor activator of nuclear factor κB ligand (RANKL) expression in osteoblasts, resulting in the inhibition of bone resorption.[21] A previous study has provided extensive genetic and functional data indicating that the *LRP5* gene and the Wnt signaling pathway have a key influence on bone formation and the risk of osteoporosis, and that LRP5 signaling is essential for normal morphology, normal development, and bone health.[22] A recent study indicated that *LRP5* A1330V polymorphism influences BMD because it has an effect on Wnt signaling.[23] Ezura *et al*. performed a cross-sectional study that showed an association between *LRP5* A1330V polymorphism and BMD in 384 Japanese women aged 58.4 ± 8.6 (range: 32-69 years) recruited from the general population.[15] However, there

Table 3. Change in bone mineral density according to lifestyle factors and *low-dernsity lipoprotein receptor related protein 5 (LRP5)* A1330V polymorphism

Valuable	All (*n*=113)		*AA* (n=59)		*AV* + *VV* (*n*=54)	
	Case (%)	Change in BMD	Case (%)	Change in BMD	Case (%)	Change in BMD
Past history of exercise						
No	43 (38.4)	-0.005±0.018	25 (42.4)	-0.001±0.020	18 (34.0)	-0.011±0.015
Yes	69 (61.6)	-0.002±0.018	34 (57.6)	-0.001±0.018	35 (66.0)	-0.004±0.017
Current exercise						
No	87 (77.0)	-0.003±0.018	46 (78.0)	-0.001±0.018	41 (75.9)	-0.005±0.017
Yes	26 (23.0)	-0.006±0.019	13 (22.0)	-0.001±0.021	13 (24.1)	-0.011±0.016
Daily milk intake						
No	84 (74.3)	-0.003±0.018	43 (72.9)	-0.001±0.019	41 (75.9)	-0.004±0.017
Yes	29 (25.7)	-0.006±0.018	16 (27.1)	-0.001±0.019	13 (24.1)	-0.012±0.014
Number of meals per day						
3 meals/day	23 (20.4)	-0.007±0.017	9 (15.3)	0.001±0.018	14 (25.9)	-0.013±0.015
2 meals/day	90 (79.6)	-0.002±0.018	50 (84.7)	-0.001±0.019	40 (74.1)	-0.004±0.017
History of dieting						
No	68 (60.2)	-0.002±0.018	34 (57.6)	0.002±0.019	34 (63.0)	-0.005±0.017
Yes	45 (39.8)	-0.006±0.018	25 (42.4)	-0.004±0.019	20 (37.0)	-0.008±0.017
Smoking status						
Non-smoker	97 (85.8)	-0.004±0.019	50 (84.7)	-0.001±0.020	47 (87.0)	-0.007±0.017
Smoker	16 (14.2)	-0.001±0.012	9 (15.3)	-0.001±0.012	7 (13.0)	-0.001±0.014
Alcohol intake						
Non-drinker	34 (30.1)	-0.003±0.019	22 (37.3)	-0.003±0.019	12 (22.2)	-0.001±0.018
Drinker	79 (69.9)	-0.004±0.018	37 (62.7)	0.001±0.018	42 (77.8)	-0.008±0.016*

Values are the mean ± SD.
Abbreviation: BMD=bone mineral density.
Change in BMD was adjusted for age and BMI at baseline by ANCOVA (analysis of covariance).
*$p<0.05$ compared to *AA* subjects.

were no differences in terms of BMD between *LRP5* A1330V genotypes when a cross-sectional analysis was done at baseline in the present study. The most likely explanation for this difference is that our subjects were much younger than those studied by Ezura. Although the exact mechanism by which *LRP5* A1330V polymorphism influences BMD is not fully understood, it can be suggested that this polymorphism is probably a useful genetic marker for predicting age-related bone loss in premenopausal Japanese women.

In subjects who drank alcohol, the *AV* or *VV* genotype was associated with a significantly larger change of BMD than the *AA* genotype. An adverse influence of alcohol on the bone in subjects with the *AV* or *VV* genotype might be mediated through *LRP5* A1330V polymorphism. Although the mechanisms through which the *AV* or *VV* genotypes have an adverse impact on the bone in drinkers have not been studied extensively, alcohol intake may have a modest adverse effect on the preservation of bone mass in premenopausal women, mainly by suppressing bone formation.[24] We also found that drinkers tend to skip meals (3 meals/day in 15.2% of drinkers vs. 32.4% of non-drinkers and 2 meals/day in 84.8% of drinkers vs. 67.6% of non-drinkers, p=0.039, Mann-Whitney U test). Unhealthy behavior such as skipping meals may have an adverse effect on bone metabolism and the general condition. A dose-dependent effect of alcohol on bone metabolism has also been reported.[25] A recent meta-analysis revealed that persons consuming more than 0.5 to one drink per day had a lower risk of hip fracture than abstainers, and persons consuming more than two drinks per day had a higher risk.[26] However, the precise mechanism by which moderate intake of alcohol alters bone metabolism is still unknown. We did not assess the alcohol consumption of drinkers in the present study. The amount and frequency of alcohol consumption may be important factors determining the influence of alcohol on BMD. Further studies are needed to assess the association between alcohol consumption and BMD, as well as the influence of *LRP5* A1330V polymorphism.

In conclusion, *LRP5* A1330V polymorphism was associated with age-related bone loss in Japanese female workers after follow-up for seven years. This finding suggests that investigation of *LRP5* A1330V polymorphism may be useful for identifying individuals who are susceptible to osteoporosis, and such studies could lead to more effective prevention of osteoporosis and promotion of better health.

AUTHOR DISCLOSURES
None.

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Original Article

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日本女性僱員的骨密度改變與低密度脂蛋白受體相關蛋白 5 的基因型或生活型態有相關

以一個縱貫性研究來探討七年間骨密度(BMD)的改變與低密度脂蛋白受體相關蛋白 5(LRP5)基因 A1330V 多型性或生活型態因子間的關係。受試者為日本一個大型綜合製造廠的僱員，共包含 113 位停經前婦女，納入研究時平均年齡為 25.6±4.2 歲。以雙能 X 光骨密度儀(DXA)測量橈骨決定骨密度。生活型態方面資訊則以問卷取得。LRP5 基因的不同型頻率分別為 52%(AA)、39%(AV)及 9%(VV)。七年後，只有在基因型為 AV 與 VV 的受試者，其骨密度有顯著的減少(0.463±0.045 至 0.456±0.046 g/m^2)。在校正年齡與身體質量指數後，發現有飲酒的受試者，當其有 AV 與 VV 的基因型，比起基因型為 AA 的人，骨密度變化更大(F=4.547, p=0.036)。調查 LRP5 基因 A1330V 多型性可能有助於辨識個體是否易患骨質疏鬆症，以提供早期的預防方法。

關鍵字：骨質密度、基因多型性、日本、生活型態、低密度脂蛋白受體相關蛋白 5 基因