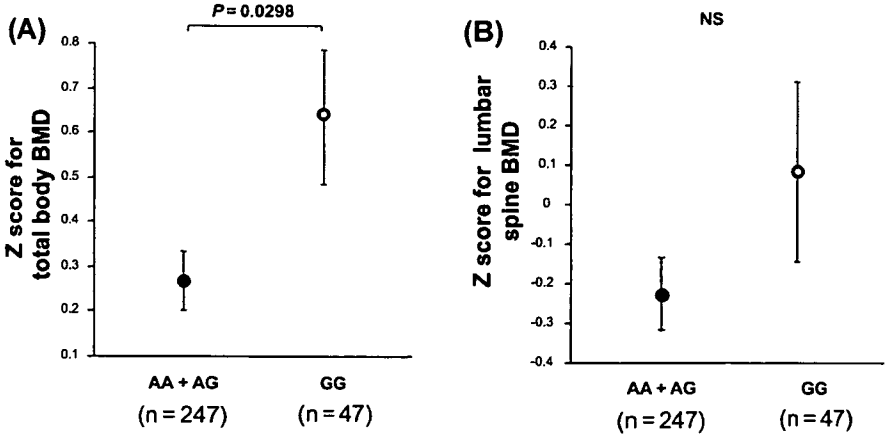

Figure 2 Z score values of total body and lumbar spine bone mineral density (BMD) in the groups of genotype AA + AG and genotype GG of the *SXR* polymorphism in intron 1 (IVS1-579). (a) Z score values for total BMD are shown for genotype AA + AG and for genotype GG. Values are expressed as mean ± SE. Number of subjects is shown in parentheses. (b) Z scores for lumbar BMD as shown in the same manner as (a).

Table 1 Comparison of background, bone mineral density and biochemical data between subjects bearing at least one A allele (AA + GA) and subjects with no A allele (GG) in the steroid and xenobiotic receptor (*SXR*) gene (IVS1-579A/G)

Items	Genotype (mean ± SD)		*P*-value
	AA + AG	GG	
No. of subjects	247	47	
Age (years)	65.2 ± 9.0	66.7 ± 8.7	NS
Height (cm)	150.8 ± 6.5	151.6 ± 5.4	NS
Body weight (kg)	50.5 ± 8.1	51.5 ± 7.7	NS
Lumber spine BMD (Z score)	−0.224 ± 1.475	0.083 ± 1.547	NS
Total body BMD (Z score)	0.268 ± 1.061	0.635 ± 1.031	0.0298
ALP (IU/L)	190.9 ± 62.7	177.3 ± 57.8	NS
I-OC (ng/mL)	8.3 ± 4.2	7.5 ± 3.1	NS
DPD (pmol/μmol Cr)	7.8 ± 4.4	6.8 ± 2.5	NS
Intact PTH (pg/mL)	34.7 ± 16.8	33.7 ± 8.6	NS
Calcitonin (pg/mL)	22.4 ± 10.4	20.7 ± 14.7	NS
1,25 $(OH)_2D_3$ (pg/mL)	35.1 ± 11.3	34.5 ± 10.3	NS
% fat	31.6 ± 8.1	32.6 ± 6.2	NS
BMI	22.1 ± 3.2	22.4 ± 3.1	NS

ALP, alkaline phosphatase; BMD, bone mineral density; BMI, body mass index; DPD, deoxypyridinoline; I-OC, intact-osteocalcin; NS, not significant; PTH, parathyroid hormone. Statistical analysis was performed according to the method described in the text.

We compared Z scores for BMD of total body and lumbar spine between the subjects bearing at least one A allele (AA + AG) and subjects without the A allele (GG). Comparison of the Z scores of the lumbar BMD between those with and without A allele showed a higher average value for GG homozygote group, but its difference was not statistically significant (Z score; 0.083 ± 1.547 vs −0.224 ± 1.475; $P = 0.195$) (Fig. 2b). On the other hand, Z score of the total body BMD in GG homozygote group was significantly higher than the other group (Z score; 0.635 ± 1.031 vs 0.268 ± 1.061; $P = 0.0298$) (Fig. 2a). The background and biochemical data were not statistically different between these two groups (Table 1).

Discussion

The nuclear receptor SXR (also known as PXR and NR1I2) plays a central role in the transcriptional regulation of xenobiotic detoxifying enzymes and transporters such as CYP3A4 and MDR1.[22–24] The SXR is activated by a diverse array of pharmaceutical agents, including Taxol, rifampicin, SR12813, clotrizole, phenobarbital, hyperforin, the herbal antidepressant Saint John's wort, and peptide mimetic HIV protease inhibitors such as ritonavir.[25–27] Recently, it was shown that vitamin K2 was a novel ligand for the SXR and could induce bone marker genes through the SXR.[16] The SXR is a member of nuclear receptor NR1I subfamily. The

vitamin D receptor (VDR, NR1I1) is a close relative of the SXR in terms of amino acid sequence similarity and belongs to the same subfamily.[22] Many reports showed that the VDR is expressed and regulated in the bone cells and *VDR* gene allelic variants could predict bone mineral density.[28] These data prompted us that the SXR may have a role in the bone homeostasis, especially in osteoporosis, as like other NR1I subfamily members.

In the present study, during the course of primary osteoblast differentiation, the increase of ALP expression, that is a marker of osteoblast differentiation[29] was followed by the increase of the SXR expression. A recent report also demonstrated that the SXR expression was detected in human osteosarcoma cell lines HOS, MG-63 and SaOS2.[16] Interestingly, the vitamin K2, one of the ligands for the SXR, upregulated the steady state mRNA levels for a panel of osteoblastic bone markers including ALP in these cells. Thus, it is possible that the SXR is involved in the differentiation of osteoblasts and the regulation of ALP gene. We have shown that the SXR expression was detected in human primary chondrocytes as well as in primary osteoblasts and increased in parallel with the increased expression of COL2A, which is a marker of chondrocyte differentiation.[20] It is also possible that the SXR is involved in the cartilage metabolism. Future studies should be required on how vitamin K and the SXR signaling could be delivered to the regulation of skeletal differentiation.

To our knowledge, the present study is the first to investigate the influence of a polymorphism of the *SXR* gene on the BMD. We demonstrated that the Japanese postmenopausal women who had two alleles of an intronic change of A-G transition showed significantly higher total-body BMD. Lumbar BMD was also higher in the subjects bearing at two G alleles, although the difference was not statistically significant. Lower BMD in postmenopausal women can be considered as a result of abnormally rapid bone loss and/or lower peak bone mass. The SNP analyzed in this study would be useful as a genetic marker for low BMD and the susceptibility to osteoporosis. Although the biological meanings of this polymorphism should be revealed by functional studies, the *SXR* IVS1-579A/G polymorphism may modulate BMD by influencing transcription and/or expression levels of the SXR.

In conclusion, our findings suggest that the *SXR* gene may be a genetic determinant of BMD in postmenopausal women as is the case with its related nuclear receptor, VDR. Examining the variation in the SXR gene will hopefully enable us to elucidate one of mechanisms of involutional osteoporosis. Furthermore, the variation may be a potential genetic susceptibility factor that need to be further evaluated with regard to the condition of other metabolisms in which the *SXR* have been clearly implicated, including lipid and drug metabolisms.[22,24]

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Q89R Polymorphism in the LDL Receptor-Related Protein 5 Gene Is Associated With Spinal Osteoarthritis in Postmenopausal Japanese Women

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Study Design. An association study investigating the genetic etiology for spinal osteoarthritis.

Objective. To determine the association of single-nucleotide polymorphism (SNP) causing an amino-acid change (Q89R) in the low-density lipoprotein receptor-related protein 5 (LRP5) coding region with spinal osteoarthritis.

Summary of Background Data. Wnt/β-catenin signaling pathway regulates bone density through a Wnt coreceptor LRP5. This pathway is also involved in cartilage development and homeostasis, suggesting that genetic variation in LRP5 gene may affect the pathogenesis of cartilage-related diseases, such as osteoarthritis.

Methods. We evaluated the presence of osteophytes, endplate sclerosis, and narrowing of disc spaces in 357 Japanese postmenopausal women. Missense coding SNP for Q89R of LRP5 gene was determined using TaqMan polymerase chain reaction (PCR) method.

Results. We found that subjects without the R allele (QQ; n = 321) had a significantly lower osteophyte formation score than did subjects bearing at least one R allele (QR + RR; n = 36) (7.80 *vs.* 10.89, $P = 0.0019$ by analysis of covariance).

Conclusions. We suggest that a genetic variation at the LRP5 gene locus is associated with spinal osteoarthritis, in line with the involvement of the LRP5 gene in the bone and cartilage metabolism.

Key words: single-nucleotide polymorphism (SNP), low-density lipoprotein receptor-related protein 5 (LRP5), spinal osteoarthritis, osteophytosis. **Spine 2007;32:25–29**

Osteoarthritis of the spine is a very common condition in the axial skeletons of aged people.[1] Vertebral osteophytes, endplate sclerosis, and intervertebral disc narrowing are recognized as characteristic features of spinal degeneration. Recent studies indicate that the appearance of these radiographic features is influenced by genetic factors, physical loading, and other environmental factors.[2,3] Association studies in using various definitions of osteoarthritis have been performed, mainly investigating genes encoding structural proteins of the extracellular matrix of cartilage (*e.g.*, collagen Type II α1, cartilage matrix protein, and aminoguanidine) or genes playing a role in the regulation of bone density and mass (*e.g.*, vitamin D receptor, insulin-like growth factor-I, and estrogen receptor α).[4,5]

The Wnt (wingless-type MMTV integration site family) represents a large group of secreted signaling proteins that are involved in cell proliferation, differentiation, and morphogenesis.[6] The name of "Wnt" is derived from wingless gene in *Drosophila melanogaster*[7] and murine int-1 oncogene identified in tumors induced by mouse mammary tumor virus.[8] It is also known that Wnt and bone morphogenetic protein (BMP) signals control apical ectodermal ridge formation and dorsal-ventral patterning during limb development.[9,10] Wnt proteins activate signal transduction through Frizzled, which act as receptors for Wnt proteins[11] and induce stabilization of cytoplasmic β-catenin protein, which also regulates target gene expression as a transcriptional coactivator. The physiologic role of Wnt in the regulation of osteoblastogenesis has been studied in experimental models, in embryonic mesenchymal progenitor cells expressing Wnt3a[12] or in mice expressing Wnt10b transgene in bone marrow.[13] It is also shown that activated β-catenin modulate osteoblast and chondrocyte differentiation.[14,15] Meanwhile, LDL receptor-related protein 5 and 6 (LRP5/6) were also found to be required for Wnt coreceptors.[16,17] Recent reports demonstrated that the Wnt/β-catenin signaling pathway regulates bone density through LRP5.[18–21] These findings indicate that Wnt-β-catenin signaling pathway plays important roles in the skeletal biology.

In addition to the regulation of limb development and bone metabolism, Wnt/β-catenin signaling may be involved in maintenance and pathophysiology of cartilage. This possibility is indirectly supported by the observation that several Wnt proteins and Frizzled receptors are expressed in synovial tissue of arthritic cartilage.[22] In

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addition, a secreted Frizzled-related protein (FrzB-2) that act as an antagonist for Frizzled receptor is strongly expressed in osteoarthritic cartilage and may regulate chondrocyte apoptosis.[23] It is also reported that chondrocytes express β-catenin at a low level and accumulation of β-catenin is sufficient to cause dedifferentiation of chondrocytes, suggesting that Wnt signaling is involved in cartilage metabolism.[24] Thus, it is assumed that LRP5 modulates Wnt/β-catenin signaling pathway in the bone and cartilage homeostasis. In the present study, we examine an association between a polymorphism in LRP5 gene and radiographic features of spinal osteoarthritis, including osteophyte formation, endplate sclerosis, and disc space narrowing number to investigate a possible contribution of LRP5 to human bone and cartilage metabolism.

■ Materials and Methods

Subjects. Genotypes were analyzed in DNA sample obtained from 357 healthy postmenopausal Japanese women (mean age ± SD; 65.22 ± 8.20 years) living in central area of Japan. Exclusion criteria included endocrine disorders such as hyperthyroidism, hyperparathyroidism, diabetes mellitus, liver disease, renal disease, use of medications known to affect bone metabolism (*e.g.*, corticosteroids, anticonvulsants, heparin sodium), or unusual gynecologic history. Patients with severe hip and knee arthritis were excluded from the present study. The eligibility of subjects was determined by taking history-physical examination. All were nonrelated volunteers and provided informed consent before this study. Ethical approval for the study was obtained from appropriate ethics committees.

Radiographic Grading of Osteoarthritis of the Spine. Conventional thoracic and lumbar spinal plain roentgenograms in lateral and anteroposterior projection were obtained from all participants. The severities of spinal degeneration, including osteophyte formation, endplate sclerosis, and disc space narrowing, were assessed semiquantitatively from T4–T5 to L4–L5 disc level or from T4 to L5 vertebrae by using the grading scale of Yu *et al.*[25] Briefly, osteophyte formation at a given disc was graded 0° to 3°, endplate sclerosis at given vertebra was graded 0° to 2°, and disc space narrowing was graded 0° to 1°. Then we defined sum of each degree from T4–T5 to L4–L5 disc level for osteophyte formation on anteroposterior radiographs as a score of osteophyte formation. We also defined sum of each degree from T4 to L4 vertebra for endplate sclerosis and that from T4–T5 to L4–L5 disc level for disc space narrowing on lateral radiographs as a score of endplate sclerosis and disc narrowing, respectively. Then we defined sum of each 13 grade for osteophyte formation on anteroposterior radiographs as a score of osteophyte formation. We also defined sum of 13 grade for endplate sclerosis and disc space narrowing on lateral radiographs as a score of endplate sclerosis and disc narrowing, respectively.

Measurement of Bone Mineral Density (BMD) and Biochemical Markers. The lumbar spine BMD and total body BMD (in g/cm^2) of each participant were measured by dual-energy radiograph absorptiometry using fast-scan mode (DPX-L; Lunar, Madison, WI). The BMD data were recorded as "Z scores," that is, deviation from the weight-adjusted average BMD for each age. Z scores were calculated using installed software (Lunar DPX-L) on the basis of data from 20,000 Japanese women.

We measured serum concentration of calcium (Ca), phosphate (P), alkaline phosphatase (ALP), intact-osteocalcin (I-OC, ELISA; Teijin, Tokyo, Japan), intact parathyroid hormone (PTH), calcitonin (CT), and 1, 25(OH)2D3. We also measured urinary ratios of urinary deoxypyridinoline (DPD, HPLC method) to creatinine.

Determination of a Single Nucleotide Polymorphism in the LRP5 Gene. DNA was extracted from peripheral leukocytes by standard techniques. Missense coding SNP for Q89R (c. 266A>G) of the LRP5 gene was determined using Assays by Design SNP Genotyping Products (Applied BioSystems) that based on the TaqMan PCR method.[26] Missense coding means that the alteration of a codon (an array of three consecutive bases in mRNA) that encodes a different amino acid. TaqMan PCR method uses two kinds of TaqMan probes that correspond to a DNA fragment including the target SNP site with different alleles and the 5′–3′ nuclease activity of Taq polymerase that is essential for PCR. TaqMan probes include fluorescence dyes at their 5′ ends and a quencher at their 3′ ends. During PCR cycles, TaqMan probes will anneal to target DNA and will be excised by the 5′–3′ nuclease activity of Taq polymerase if there is no mismatch between the probes and target sequences. Then the fluorescence dyes will be released from the probes and the intensity of fluorescence can be monitored by using ABI PRISM 7000 (Applied Biosystems) as a fluorescence detector. The allele frequencies of Q89R polymorphism were confirmed as they were not significantly deviated from Hardy-Weinberg equilibrium. Since Hardy-Weinberg equilibrium is based on the following assumptions including no genetic drift, no gene flow, no natural selection, negligible mutations, and random mating, the population under the equilibrium is not evolving and its genotype and allele frequencies are predicted to remain unchanged over successive generations. Thus, we considered that our subjects were eligible for the correlation study.

Statistical Analysis. We divided subjects into those having one or two chromosomes of the minor G-allele (QR + RR) and those with only the major A-allele (QQ) encoded at the same locus. Comparisons of Z scores of lumbar spine and biochemical markers between these two groups were subjected to statistical analysis (unpaired *t* test; StatView-J 4.5, SAS Institute Inc.). The association between these two groups and osteoarthritis parameters (number of osteophyte, endoplate sclerosis, and disc narrowing) was assessed by unpaired *t* test and by analysis of covariance (ANCOVA) with adjustment of confounding clinical variables (age, body weight, and height). A P value less than 0.05 was considered statistically significant.

■ Results

We analyzed the genotypes for the SNP of LRP5 at Q89R (c.266 A>G) in subjects, using TaqMan methods. Among 357 postmenopausal Japanese women, 321 were QQ homozygotes, 35 were QR heterozygotes, and 1 was RR homozygote. The allelic frequencies of this SNP in the present study were in Hardy-Weinberg equilibrium.

Because only 1 of these subjects carried the RR genotype of the Q89R polymorphism, we compared those who carried the R allele (QR or RR) with those who did

Table 1. Comparison of Background, Clinical Characteristics Between Subjects Bearing at Least 1 R allele (QR + RR) and Subjects With No R allele (QQ) in the *LRP5 Gene Coding Region* (Q89R)

Item	Genotype (mean ± SD) QQ	QR + RR	P
No. of subjects	321	36	
Age (yr)	65.0 ± 8.2	67.3 ± 8.0	NS
Height (cm)	150.7 ± 5.7	151.1 ± 7.1	NS
Body weight (kg)	50.5 ± 7.6	51.3 ± 7.9	NS
Lumber spine BMD (Z score)	−0.28 ± 1.40	−0.17 ± 1.89	NS
ALP (IU/L)	190.8 ± 61.3	194.8 ± 81.1	NS
I-OC (ng/mL)	8.2 ± 4.0	7.4 ± 3.0	NS
DPD (pmol/μmol of Cr)	7.6 ± 4.0	7.6 ± 2.3	NS
Intact PTH (pg/mL)	35.6 ± 16.7	34.6 ± 14.1	NS
1,25 $(OH)_2D_3$ (pg/mL)	36.1 ± 10.8	37.3 ± 14.6	NS
BMI	22.1 ± 3.0	22.8 ± 3.1	NS

BMD indicates bone mineral density; ALP, alkaline phosphatase; I-OC, intact-osteocalcin; DPD, deoxypyridinoline; PTH, parathyroid hormone; BMI, body mass index; NS, not significant. Statistical analysis was performed according to the method described in the text.

not (QQ). The lumbar BMD was not statistically different between these groups (Table 1). The background and biochemical data were not statistically different between these groups (Table 1). On ANCOVA analysis, we found significant associations between LRP5 Q89R genotype and osteophyte formation score after controlling for age, weight, and height. Women without the R allele (QQ; n = 321) had a significantly lower osteophyte formation score than did subjects bearing at least one R allele (QR + RR; n = 36) (7.80 ± 6.51 *vs.* 10.89 ± 7.6, $P = 0.0019$, Figure 1A; Table 2). We also found significant association between them on unpaired *t* test ($P = 0.0083$, Table 1). On the other hand, the occurrence of disc narrowing and endplate sclerosis did not significantly differ in those with and without at least one R allele (Figure 1B, C; Table 2).

Table 2. Association of the *LRP5* SNP Genotype

Item	Genotype (mean ± SD) QQ	QR + RR	P (unpaired *t* test)	P (ANCOVA)
No. of subjects	321	36		
Osteophyte formation score	7.80 ± 6.51	10.89 ± 7.6	0.0083	0.0019
Endoplate sclerosis score	0.368 ± 0.845	0.389 ± 0.994	NS	NS
Disc space narrowing score	2.03 ± 1.88	2.06 ± 1.84	NS	NS

ANCOVA indicates analysis of covariance; NS, not significant.

■ Discussion

The present study is the first report that shows the influence of a single-nucleotide polymorphism of LRP5 gene on spinal osteoarthritis as far as we know. Targeting the pathogenesis of low back pain, we have previously investigated associations of genetic factors with osteoporosis. LRP5 has been shown as one of the correlated genes in Japanese postmenopausal women.[27] Because spinal osteoarthritis is another major reason for low back pain, we have extended our association study of LRP5 polymorphism with spinal osteoarthritis. We demonstrated that the Japanese postmenopausal women who had one or two allele(s) of a nonsynonymous change (Q89R) in LRP5 gene showed significantly higher osteophyte formation score of spine. Our finding may also be supported by genome-wide scan for osteoarthritis-susceptibility loci that showed a linkage to chromosome 11q12-13,[28,29] which includes the LRP5 gene locus on 11q13.4. It has been recently shown that single-nucleotide polymorphisms in LRP5 gene provided no correlation with knee osteoarthritis status while haplotype analysis revealed that there was a common haplotype that provided a 1.6-fold increased risk,[30] suggesting that LRP5 might be involved in the pathogenesis of osteoarthritis also in

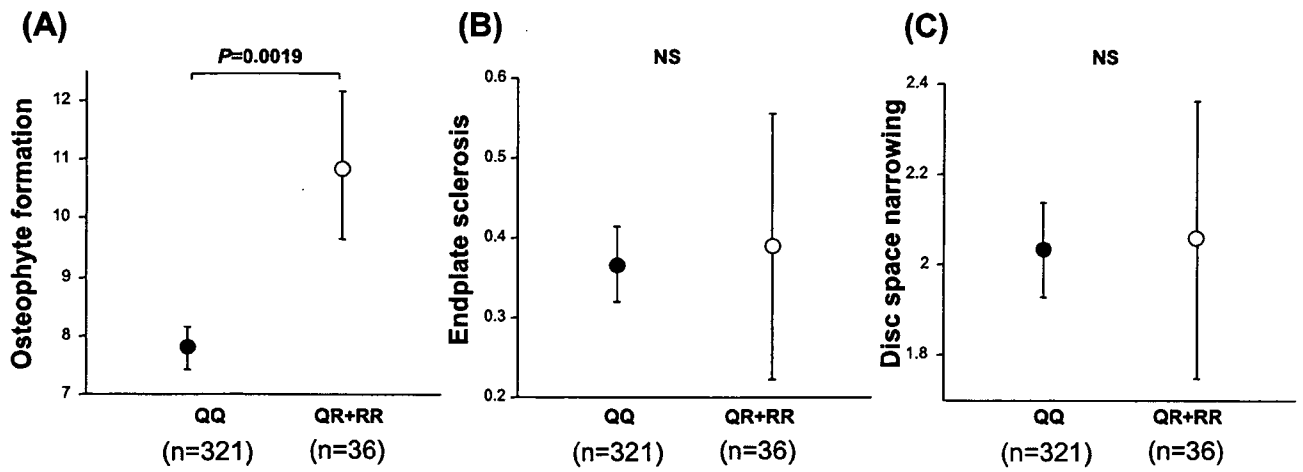

Figure 1. Scores of spinal osteoarthritis between the genotypes of polymorphism at Q89R (QQ *vs.* QR + RR). **A**, Scores of osteophyte formation are shown for genotype QQ and for genotype QR + RR. Scores are expressed as mean ± SE. Numbers of subjects are shown in parentheses. **B**, Scores of endplate sclerosis. **C**, Disc space narrowing scores. The association of the two genotype groups with osteoarthritis parameters was determined by ANCOVA, a type of multifactorial analysis, with adjustment of confounding clinical variables (age, body weight, and height).

other joints. It is also reported that there was a significant association of a functional gene variant of secreted frizzled-related protein 3 (sFRP3), which antagonizes Wnt signaling, with hip osteoarthritis in women.[31] Taken together, our results and the recent evidence suggest that the canonical Wnt signaling pathway including LRP5 is critical in the pathogenesis of skeletal abnormality, including osteoarthritis and osteoporosis.

Recently, mutations of the LRP5 gene have been described to be associated with both osteoporosis-pseudoglioma syndrome and the high bone mass phenotype.[18–21] It was found that loss-of-function of LRP5 in both human[18] and mice[19] yielded a decrease in bone formation, or an active mutation of LRP5 that cannot bind to a Wnt inhibitor Dickkopf-1 resulted in a high bone mass trait.[20,21] Moreover, our group and several other groups have reported that single-nucleotide polymorphisms in LRP5 gene predicted the bone mass.[27,32–36] These SNPs included three of different missense variations; Q89R,[33,34] V667 M,[35] and A1330V.[36] In the present study, we investigated a possible contribution of Q89R LRP5 polymorphism to spinal osteoarthritis in Japanese women. V667 M polymorphism was not detected in our Japanese population. Regarding A1330V polymorphism, we could not detect an association of the SNP with spinal osteoarthritis (data not shown).

Two groups reported consistent association of Q89R with Ward's triangle BMD but not with lumbar BMD in Korean young men[33] and Chinese premenopausal women.[34] In our Japanese population, we did not find an association of Q89R polymorphism with lumbar spine. The present data together with published data related to osteoporosis suggest that Q89R polymorphism may be involved in the pathogenesis of both osteoporosis and spinal osteoarthritis and QQ genotype in LRP5 might be preventive for both diseases. Meanwhile, there are other cases in which genetic factors contribute to the pathogenesis of osteoporosis and osteoarthritis in an opposite way. For example, it has been reported that transforming growth factor-β1 (TGF-β1) gene polymorphism T869C, which gives Leu>Pro substitution contributes differentially to osteoporosis and osteoarthritis; people with CC genotype had significantly higher BMD than those with TC or TT, whereas this CC genotype was related to significantly greater osteophytes than TT or TC.[37]

Osteoarthritis occurs as result of both mechanical and biologic events that destabilize the normal coupling of degradation and synthesis of articular cartilage chondrocytes and extracellular matrix as well as subchondral bone.[1,38] Cartilage destruction during osteoarthritis involves the loss of differentiated phenotype and apoptotic death of chondrocytes,[39] Wnt proteins were shown to regulate dedifferentiation of apoptosis of chondrocytes.[40] It is also demonstrated the interaction of β-catenin with SOX9, a transcriptional factor that is required in successive steps of chondrogenesis, controls chondrocyte differentiation.[41] These data suggest Wnt/β-catenin may participate in the pathogenesis of cartilage diseases, such as osteoarthritis. Further studies will be required to clarify the role of Q89R missense variant of the LRP5 in the pathogenesis of osteophyte formation and osteoporosis.

■ Conclusion

We have shown an association of the Q89R polymorphism in the LRP5 gene with a radiographic feature of spinal osteophytosis in postmenopausal Japanese women. The women with QQ genotypes had significantly lower osteophyte formation scores. The LRP5 genotyping might be beneficial in the prevention and management of spinal osteophytosis as well as osteoporosis. The present findings regarding the correlation of LRP5 polymorphism with spinal osteoarthritis provide a new promising direction for the clinical medicine of the spine disease, which leads us to the development of new diagnostic markers as well as therapeutic options based on the molecular target.

■ Key Points

- Wnt/β-catenin signaling pathway regulates bone and cartilage metabolism.
- The single-nucleotide polymorphism, causing an amino-acid change (Q89R) in LRP5 gene that encodes a Wnt coreceptor, was associated with spinal osteophytosis in Japanese postmenopausal women.
- We suggest that a genetic variation at the LRP5 gene locus is associated with spinal osteoarthritis.

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ORIGINAL ARTICLE

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Association of a single nucleotide polymorphism in the lipoxygenase *ALOX15* 5′-flanking region (−5229G/A) with bone mineral density

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Abstract The 12/15-lipoxygenase gene *Alox15* has been identified as a susceptibility gene for bone mineral density (BMD) in mice through combined genetic and genomic analyses. Here we studied the association between bone mineral density and an *ALOX15* gene single nucleotide polymorphism to assess the potential involvement of the human *ALOX15* gene in postmenopausal osteoporosis. Specifically, we examined the association between a single nucleotide polymorphism at −5299G/A in the *ALOX15* 5′-flanking region with BMD in 319 postmenopausal Japanese women (66.7 ± 8.9 years, mean ± SD). We found that subjects bearing at least one variant A allele (GA + AA; $n = 273$) had significantly lower Z scores for lumbar spine and total body bone mineral density than did subjects with no A allele (GG; $n = 46$) (lumbar spine, −0.25 ± 1.34 versus 0.48 ± 1.70; $P = 0.0014$; total body, 0.25 ± 1.01 vs 0.62 ± 1.11; $P = 0.048$). These findings suggest that the *ALOX15* gene is one of the genetic determinants of BMD in postmenopausal women. Accordingly, this polymorphism could be useful as a genetic marker for predicting the risk of osteoporosis.

Key words Adipogenesis · *ALOX15* · PPARγ · Osteoporosis · Bone mineral density · Polymorphism

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Introduction

Osteoporosis is characterized by low bone mineral density (BMD), increased bone fragility, and consequently increased risk of fracture [1]. Studies of twins and siblings have shown that BMD is under genetic control, with estimates of heritability ranging from 50% to 90% [2,3]. BMD is regulated by interaction of multiple environmental and genetic factors, each having modest effects on bone mass and bone turnover [4,5]. Polymorphisms of several genes have been investigated to clarify the determinants of BMD [6,7]. These genes of which polymorphisms were associated with BMD include those implicated in bone formation by regulation of osteoblast growth and function, such as *vitamin D receptor* gene [8], *transforming growth factor beta-1* (*TGFβ1*) gene, *collagen type Iα1* (*COLIA1*) gene [9], *peroxisome proliferator-activated receptor-γ* (*PPARγ*) gene [10], and *low-density lipoprotein receptor-related protein 5* (*LRP5*) gene [11]. Identification of candidate genes that affect bone mass will be useful for early detection of individuals who are at risk of osteoporosis for early institution of preventive measures.

The decrease in bone volume associated with osteoporosis is accompanied by an increase in marrow adipose tissues [12,13]. Indeed, an increase in marrow adipocytes is observed in several conditions that lead to bone loss, such as ovariectomy [14], immobilization [15], and treatment with glucocorticoids [16]. Recent studies have identified rodent quantitative trait locus associated with increased BMD in the mouse gene encoding 12/15-lipoxygenase [17], the enzyme that converts linoleic acid and arachidonic acid into endogenous ligands for the PPARγ [18–20]. Activation of this pathway in marrow-derived mesenchymal progenitors stimulates adipogenesis and inhibits osteoblastogenesis [21,22]. Mice that are deficient in this gene or have been treated with 12/15-lipoxygenase inhibitors demonstrate increased bone mass as compared with controls [17]. These findings suggest that genetic variants of the 12/15-lipoxygenase encoding gene may affect the BMD in humans as well as mice. The mouse 12/15-lipoxygenase enzyme

corresponds to at least three lipoxygenases in humans. 15-Lipoxygenase has two isoenzymes: type 1 (human *ALOX15*, encoded by a gene at chromosome 17p13.3) and type 2 (human *ALOX15B*, encoded by a separate gene at 17p13.1). 12-Lipoxygenase (human *ALOX12*, encoded by a gene at chromosome 17p13.1) is predominantly expressed in platelets and macrophages and is distinct from 15-lipoxygenase [23]. In the present study, we examined the possibility that there is an association between a polymorphism in the human *ALOX15* gene and BMD in Japanese women to investigate the possible contribution of the lipoxygenase to bone metabolism.

Subjects and methods

Subjects

We analyzed genotypes in DNA samples from 319 healthy postmenopausal Japanese women (66.7 ± 8.9 years, mean ± SD). We excluded women having endocrine disorders such as hyperthyroidism, hyperparathyroidism, diabetes mellitus, liver disease, and renal disease; those who used medications known to affect bone metabolism (e.g., corticosteroids, anticonvulsants, and heparin); and those with an unusual gynecological history. All subjects were unrelated volunteers. Each subject provided informed consent before entering the study.

Measurement of bone mineral density and biochemical markers

We measured the lumbar spine BMD and total body BMD of participants by dual-energy X-ray absorptiometry using the fast-scan mode (DPX-L; Lunar, Madison, WI, USA). The BMD data were recorded as *Z* scores, as the deviation from the weight-adjusted average BMD for each year of age, based on data from 20000 Japanese women. We also measured each subject's serum concentrations of alkaline phosphatase (ALP), intact osteocalcin (I-OC), intact parathyroid hormone (PTH), calcitonin, 1,25-$(OH)_2D_3$, total cholesterol (TC), and triglyceride (TG). We also measured urinary ratios of deoxypyridinoline (DPD) to creatinine using the high pressure liquid chromatography (HPLC) method.

Determination of a single nucleotide polymorphism in the ALOX15 gene

We extracted a polymorphic variation of the putative *ALOX15* gene promoter/enhancer region from the Assays-on-Deman SNP Genotyping Products database (Applied Biosystems, Foster City, CA, USA) and, according to its localization on the gene, denoted it −5299 G>A. We determined the −5299G/A polymorphism of the *ALOX15* gene using the TaqMan (Applied Biosystems) polymerase chain reaction (PCR) method [24]. To determine the *ALOX15* SNP we used Assays-on-Demand SNP Genotyping Products C_926671_10 (Applied BioSystems), which contains sequence-specific forward and reverse primers and two TaqMan MGB probes for detecting alleles. During the PCR cycle, two TaqMan probes competitively hybridize to a specific sequence of the target DNA and the reporter dye is separated from the quencher dye, resulting in an increase in fluorescence of the reporter dye. The fluorescence levels of the PCR products were measured with the ABI PRISM 7000 (Applied Biosystems), resulting in clear identification of three genotypes of the single nucleotide polymorphism (SNP).

Statistical analysis

We divided subjects into those having one or two chromosomes of the major A allele and those with only the minor G allele encoded at the same locus. Comparisons of *Z* scores and biochemical markers between these two groups were subjected to statistical analysis (Student's *t* test; StatView-J 4.5). A *P* value of less than 0.05 was considered statistically significant.

Results

Association of ALOX15 gene polymorphism with bone mineral density

Among our 319 subjects, 46 were GG homozygotes, 155 were GA heterozygotes, and 118 were AA homozygotes. Allelic frequencies were 0.613 for the A allele and 0.387 for the G allele in this population. The allelic frequencies of this SNP in the present study were in Hardy–Weinberg equilibrium.

We compared the 273 subjects bearing at least one chromosome with the A allele (genotype GA + AA) and the 46 subjects having no A allele (GG) with respect to their *Z* scores for lumbar spine and total body BMD. Those with the A allele had significantly lower *Z* scores for lumbar spine BMD (−0.25 ± 1.34 vs 0.48 ± 1.70; $P = 0.0014$) (Fig. 1A) and total body BMD (0.25 ± 1.01 vs 0.62 ± 1.11; $P = 0.048$) (Fig. 1B). As shown in Table 1, the background and biochemical data did not significantly differ between these groups.

Discussion

Various regulating elements have been identified within the *ALOX15* 5′-flanking promoter/enhancer region, including a site for binding with Sp1 [25], AP1 [25], and GATA [26], as well as sites for methylation [27] and acetylation [28,29] and a Stat6 response element [29], suggesting that 15-lipoxygenase expression is directly regulated through transcription regulation. In the present study, we observed a significant association between BMD and a G/A SNP at the

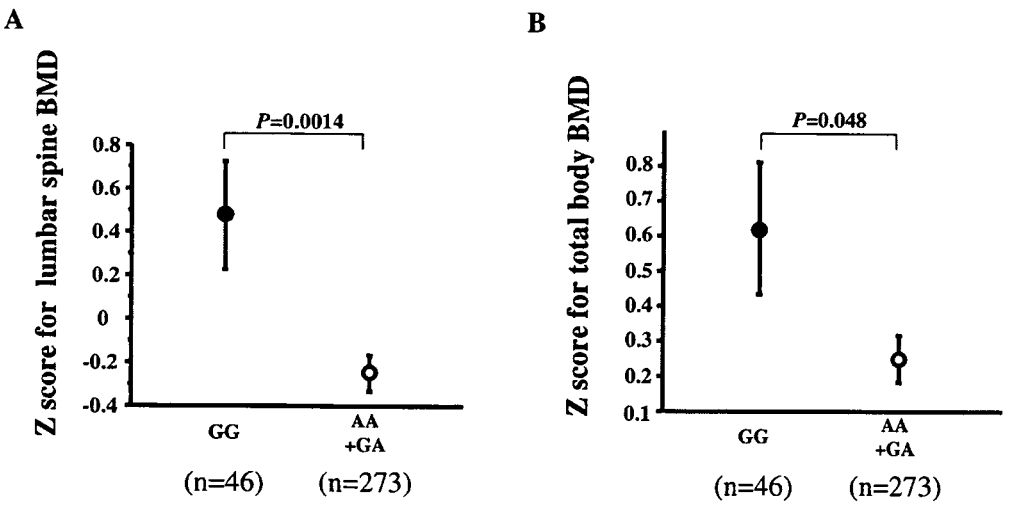

Fig. 1. *Z* scores of lumbar spine and total body bone mineral density (BMD) in subject groups with each genotype of *ALOX15* gene polymorphism in the 5′-flanking region (−5229G/A). **A** *Z* scores for lumbar spine BMD are shown for genotype AA + GA and genotype GG. Values are expressed as mean ± SE. Numbers of subjects are shown in *parentheses*. **B** *Z* scores for total body BMD are shown in the same manner as in **A**

Table 1. Comparison of background, BMD and biochemical data between subjects bearing at least one A allele (AA + GA) and subjects with no A allele (GG) in the *ALOX15* gene 5′-flanking region (−5299G/A)

Items	Genotype (mean ± SD)		*P* value[a]
	GG	GA + AA	
Number of subjects	46	273	
Age (years)	69.0 ± 8.9	66.3 ± 8.9	NS
Height (cm)	149.5 ± 6.9	150.3 ± 6.1	NS
Body weight (kg)	49.6 ± 8.6	50.3 ± 7.9	NS
Lumber spine BMD (*Z* score)	0.48 ± 1.70	−0.25 ± 1.37	0.0014
Total body BMD (*Z* score)	0.62 ± 1.12	0.25 ± 1.01	0.048
ALP (IU/l)	185.6 ± 63.9	193.4 ± 66.0	NS
I-OC (ng/ml)	8.2 ± 3.2	7.8 ± 3.6	NS
DPD (pmol/μmol Cr)	7.0 ± 3.0	7.6 ± 2.7	NS
Intact PTH (pg/ml)	38.6 ± 20.0	35.2 ± 15.1	NS
Calcitonin (pg/ml)	16.6 ± 4.5	23.1 ± 11.4	NS
$1,25\text{-}(OH)_2D_3$ (pg/ml)	33.0 ± 7.7	35.7 ± 11.8	NS
TC (mg/dl)	193.0 ± 45.0	199.4 ± 36.5	NS
TG (mg/dl)	142.5 ± 74.0	142.4 ± 81.8	NS
% Fat	32.1 ± 6.6	31.9 ± 7.7	NS
BMI	22.1 ± 3.0	22.1 ± 3.1	NS

BMD, bone mineral density; ALP, alkaline phosphatase; I-OC, intact-osteocalcin; DPD, deoxypyridinoline; Cr, creatinine; PTH, parathyroid hormone; TC, total cholesterol; TG, triglyceride; BMI, body mass index; NS, not significant

[a] Statistical analysis was performed according to the method described in the text

−5299 site in the *ALOX15* 5′-flanking region. This is the first report to our knowledge that a common SNP in the *ALOX15* gene affects BMD. One possible explanation for this effect is that this 5′-flanking region polymorphism may be involved in the newly defined transcriptional regulating element of the *ALOX15* promoter/enhancer. Alternatively, the 5′-flanking region polymorphism may have a linkage with another base of the *ALOX15* promoter/enhancer that may control transcription of the *ALOX15* gene. It is also possible that this SNP may be linked with mutation of the *ALOX15* exons or another unidentified gene adjacent to the *ALOX15* locus, which affect the bone mass.

Although there are three lipoxygenases in humans, *ALOX15*, *ALOX15B*, and *ALOX12*, that correspond to 12/15-lipoxygenase in mice [23], we know little of their roles in human bone metabolism. Our results suggest that the 15-lipoxygenase type 1, the *ALOX15*, may have a specific function in the regulation of bone mass in human. It should be required to determine how signals from 15-lipoxygenase can be transduced to the regulation of the bone metabolism.

Three major cellular events are involved in senile osteoporosis: declining levels of osteogenesis, increasing numbers of apoptotic osteoblasts and osteocytes, and increasing levels of bone marrow adipogenesis [30–32]. The bone marrow adipogenesis that occurs with aging may be due to alterations in cell differentiation, in part by PPARγ activation [33–35] and increasing lipid oxidation [36]. Previous

reports demonstrated that 12/15-lipoxygenases are involved in this system [17,18,37,38], suggesting that 12/15-lipoxygenase may increase with aging in progenitor cells and activate adipogenesis. It has been also shown that 12/15-lipoxygenase is increased in Alzheimer's disease, which is the most common neurodegenerative disorder of the elderly [39]. Therefore, it is tempting to speculate that 12/15-lipoxygenase is increased associated with aging and senile osteoporosis. To test this hypothesis, measurement of 12/15-lipoxygenase activity and association study between BMD and the *ALOX15* gene SNP in older subjects is desirable.

In conclusion, our finding suggests that the *ALOX15* gene may be a genetic determinant of BMD in postmenopausal women. Examining the variation in the *ALOX15* gene will hopefully enable us to elucidate one of the mechanisms of involutional osteoporosis. Furthermore, the variation may be a potential genetic susceptibility factor that need to be further evaluated with regard to the risk of other diseases in which 15-lipoxygenase have been clearly implicated, including atherosclerosis [40], asthma [41], cancer [42], and glomerulonephritis [43].

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ORIGINAL ARTICLE

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Association of a single nucleotide polymorphism in the WISP1 gene with spinal osteoarthritis in postmenopausal Japanese women

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Abstract The Wnt-β-catenin signaling pathway that regulates bone density is also involved in cartilage development and homeostasis in vivo. Here, we assumed that genetic variation in Wnt-β-catenin signaling genes can affect the pathogenesis of cartilage related diseases, such as osteoarthritis. Wnt-1-induced secreted protein 1 (WISP1) is a target of the Wnt pathway and directly regulated by β-catenin. In the present study, we analyzed the association of a single nucleotide polymorphism (SNP) in the WISP1 3′-UTR region with the development of radiographically observable osteoarthritis of the spine. For this purpose, we evaluated the presence of osteophytes, endplate sclerosis, and narrowing of disc spaces in 304 postmenopausal Japanese women. We compared those who carried the G allele (GG or GA, $n = 184$) with those who did not (AA, $n = 120$). We found that the subjects without the G allele (AA) were significantly over-represented in the subjects having higher endplate sclerosis score ($P = 0.0069$; odds ratio, 2.91; 95% confidence interval, 1.34–6.30 by logistic regression analysis). On the other hand, the occurrence of disc narrowing and osteophyte formation did not significantly differ between those with and without at least one G allele. Thus, we suggest that a genetic variation in the WISP1 gene locus is associated with spinal osteoarthritis, in line with the involvement of the Wnt-β-catenin-regulated gene in bone and cartilage metabolism.

Key words single nucleotide polymorphism (SNP) · Wnt-β-catenin signaling · WISP1 · osteoarthritis · endplate sclerosis

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Introduction

Spinal osteoarthritis is a highly prevalent musculoskeletal disorder and a major cause of back symptoms [1]. Vertebral osteophytes, endplate sclerosis, and intervertebral disc narrowing are recognized as characteristic features of spinal degeneration. Recent studies indicate that the appearance of these radiographic features is influenced by physical loading and other environmental factors [2,3]. Moreover, spinal osteoarthritis has been shown to have a familial component and in some studies to be influenced by specific genetic risk factors, mainly by investigating genes encoding structural proteins of the extracellular matrix of cartilage (e.g., collagen type II α1, cartilage matrix protein, and aminoguanidine) or genes playing a role in the regulation of bone density and mass (e.g., vitamin D receptor, insulin-like growth factor-I, and estrogen receptor-α) [4,5].

The Wnt (wingless-type MMTV integration site family) represents a large group of secreted signaling proteins that are involved in cell proliferation, differentiation, and morphogenesis [6]. The name 'Wnt' is derived from *wingless* gene in *Drosophila melanogaster* [7] and murine *int-1* oncogene identified in tumors induced by mouse mammary tumor virus [8]. It is also known that Wnt and bone morphogenetic protein (BMP) signals control apical ectodermal ridge (AER) formation and dorsoventral patterning during limb development [9,10]. Wnt proteins activate signal transduction through Frizzled, which act as receptors for

Wnt proteins [11] and induce stabilization of cytoplasmic β-catenin protein, which also regulates target gene expression as a transcriptional coactivator. The physiological role of the Wnt in the regulation of osteoblastogenesis has been studied in experimental models. Mice expressing Wnt10b transgene in bone marrow have shown high bone mass by simulating osteoblastogenesis [12]. It is also shown that activated β-catenin stimulates osteoblast differentiation [13]. Further, low-density lipoprotein (LDL)-receptor-related protein 5 and 6 (LRP5/6) were also found to be required for Wnt coreceptors [14,15]. Recent reports demonstrated that the Wnt/β-catenin signaling pathway regulates bone mineral density (BMD) through LRP5 [16–19]. Moreover, we and several groups reported that single nucleotide polymorphisms (SNPs) in the LRP5 gene predicted bone mass [20–23]. These findings indicate that the Wnt-β-catenin signaling pathway plays important roles in skeletal biology.

In addition to the regulation of limb development and bone metabolism, Wnt/β-catenin signaling may be involved in the maintenance and pathophysiology of cartilage. This possibility is indirectly supported by the observation that several Wnt proteins and Frizzled receptors are expressed in the synovial tissue of arthritic cartilage [24]. In addition, a secreted Frizzled-related protein (FrzB-2) that act as an antagonist for Frizzled receptor is strongly expressed in osteoarthritic cartilage and may regulate chondrocyte apoptosis [25]. It is also shown that chondrocytes express β-catenin at a low level and that an accumulation of β-catenin is sufficient to cause dedifferentiation of chondrocytes, suggesting that Wnt signaling is involved in cartilage metabolism [26].

Wnt-1-induced secreted protein 1 (WISP1) is a member of the CCN family growth factors, which includes connective tissue growth factor (CTGF), cysteine-rich 61 (Cyr61), nephroblastoma overexpressed (NOV), WISP2, and WISP3 [27–30]. WISP1 is a target of the Wnt/β-catenin pathway, and its expression is regulated by β-catenin [30,31]. WISP1 activity and availability are modulated by its interaction with decorin and biglycan, two extracellular matrix-associated proteoglycans found abundantly in bone and cartilage [32]. In mouse chondrocytic cell lines, WISP1 increased proliferation and saturation density but repressed chondrocytic representation [33]. These data suggest that WISP1 could play an important regulatory role in bone and cartilage homeostasis. In the present study, we examined an association between a polymorphism in the WISP1 gene and radiographic features of spinal osteoarthritis including osteophyte formation, endplate sclerosis, and disc space narrowing to investigate a possible contribution of WISP1 to human bone and cartilage metabolism.

Materials and methods

Subjects

Genotypes were analyzed in DNA samples obtained from 304 healthy postmenopausal Japanese women (mean age ± SD, 66.3 ± 9.0) living in the central area of Japan. Exclusion criteria included endocrine disorders such as hyperthyroidism, hyperparathyroidism, diabetes mellitus, liver disease, renal disease, use of medications known to affect the bone metabolism (e.g., corticosteroids, anticonvulsants, heparin sodium), or unusual gynecological history. Patients with severe hip and knee arthritis were excluded from the present study. The eligibility of subjects was determined by taking the history and physical examination. All were nonrelated volunteers and provided informed consent before this study. Ethical approval for the study was obtained from appropriate ethics committees.

Radiographic grading of spinal osteoarthritis

Conventional thoracic and lumbar spinal plain roentgenograms in lateral and anteroposterior projection were obtained from all participants. The severities of spinal degeneration including osteophyte formation, endplate sclerosis, and disc space narrowing were assessed semiquantitatively from T4–T5 to L4–L5 disc level or from T4 to L5 vertebrae by using the grading scale of Genant [34]. Briefly, osteophyte formation at a given disc was graded 0–3 degrees, endplate sclerosis at given vertebra was graded 0–2 degrees, and disc space narrowing was graded 0–1 degrees. Then, we defined the sum of each degree from T4–T5 to L4–L5 disc level for osteophyte formation on anteroposterior radiographs as a score of osteophyte formation. We also defined the sum of each degree from T4 to L4 vertebra for endplate sclerosis and that from T4–T5 to L4–L5 disc level for disc space narrowing on lateral radiographs as a score of endplate sclerosis and disc narrowing, respectively. These semiquantitaive gradings on radiographics were performed by two expert medical doctors.

Determination of a SNP in the WISP1 gene

We extracted a polymorphic variation in the WISP1 gene exon 5 3′-untrans lated region (UTR) from the Assays-on-Demand SNP Genotyping Products database (Applied Biosystems, Foster City, CA, USA) and, according to its localization on the gene, denoted it 2364A/G. We determined the 2364A/G polymorphism of the WISP1 gene using the TaqMan (Applied Biosystems) polymerase chain reaction (PCR) method [35]. To determine the WISP1 SNP, we used Assays-on-Demand SNP, Genotyping Products C_9086661_10 (Applied BioSystems) (rs2929970), which contains sequence-specific forward and reverse primers and two TaqMan MGB probes for detecting alleles. During the PCR cycle, two TaqMan probes competitively hybridize to a specific sequence of the target DNA and the reporter dye is separated from the quencher dye, resulting in an increase in fluorescence of the reporter dye. The fluorescence levels of the PCR products were measured with the ABI PRISM 7000, resulting in clear identification of three genotypes of the SNP.

Statistical analysis

Age, height, body weight, body mass index (BMI), and osteoarthritis parameters (number of osteophytes, endoplate sclerosis, and disc narrowing) in the groups of subjects classified by the WISP1 SNP genotypes were compared by analysis of variance (ANOVA) and Kruskal–Wallis test. Stepwise regression analysis was carried out to assess the independent effect of four variables (age, height, body weight, WISP 1 SNP genotypes) on endplate sclerosis score. We also divided subjects into those having one or two allele(s) of the minor G allele (AG + GG) and those with only the major A allele (AA) encoded at the same locus. Multivariate logistic regression was used to estimate odds ratios and 95% confidence intervals (95% CIs) for these two groups and the risk of endplate sclerosis. Analyses for the association of WISP1 2364A/G genotypes and radiographic spinal endplate sclerosis were performed with adjustment for age. P values less than 0.05 were considered significant. Analysis was performed using StatView-J 4.5 software (SAS Institute, Cary, NC, USA).

Results

We analyzed the genotypes for the SNP of WISP1 gene at the 3′-UTR region (2364 A > G) in 304 subjects, using the TaqMan method. Among these postmenopausal Japanese women, 120 were AA homozygotes, 149 were AG heterozygotes, and 35 were GG homozygotes (Table 1). The allelic frequencies of this SNP in the present study were in Hardy–Weinberg equilibrium.

The background data (age, height, body weight, BMI) were not statistically different among these groups (Table 1). On ANOVA analysis, we found significant associations between WISP1 2364A/G genotype and endplate sclerosis score (Table 1; $P = 0.0062$). On Kruskal–Wallis analysis, we also found significant associations between WISP1 2364A/G genotype and endplate sclerosis score (Table 1; $P = 0.024$). Women with the AA allele had a significantly higher endplate sclerosis score than did subjects bearing at least one G allele (AG + GG). On the other hand, the occurrence of disc narrowing and osteophytes did not significantly differ among those SNP genotypes (see Table 1).

Recent studies have shown that the physical and constitutional factors contribute to spinal osteoarthritis. Therefore, we carried out stepwise regression analysis to assess the independent effect of age, height, body weight, and WISP1 SNP genotypes on endplate sclerosis score. Among these factors, only age and WISP1 SNP genotypes correlated significantly with spinal endplate sclerosis score (Table 2). The standard regression coefficients were 0.261 for age and –0.166 for WISP1 SNP genotypes.

Last, we analyzed the association between the alleic frequency of WISP1 SNP genotypes and endplate sclerosis score after stratification by age. In these analyses, we divided subjects into two groups, those who carried the G allele (GG or GA, $n = 184$) and with those who did not (AA, $n = 120$). We found that the subjects without the G allele (AA) were significantly overrepresented in the subjects having a one or more endplate sclerosis score compared in the subjects having no endplate sclerosis after being age-adjusted (Table 3; $P = 0.044$; odds ratio 1.78; 95% confidence interval 1.01–3.13 by logistic regression analysis). We also found that the subjects with the genotype AA were significantly

Table 1. Comparison of background and clinical characteristics among subjects with single nucleotide polymorphism (SNP) genotypes (AA genotype, AG genotype and GG genotype) in the WISP1 gene 3′-UTR region (2364A/G)

Items	Genotype (mean ± SD)			P value (ANOVA)	P value (Kruskal–Wallis)
	AA	AG	GG		
Number of subjects	120	149	35		
Age (years)	66.1 ± 9.2	66.3 ± 8.5	67.1 ± 10.6	NS	NS
Height (cm)	150.7 ± 5.6	150.2 ± 6.8	150.0 ± 5.0	NS	NS
Body weight (kg)	50.3 ± 7.6	50.2 ± 8.3	48.0 ± 5.4	NS	NS
BMI	22.1 ± 2.9	22.2 ± 2.9	21.3 ± 3.3	NS	NS
Endplate sclerosis	0.58 ± 1.09	0.34 ± 0.74	0.09 ± 0.28	0.0062	0.024
Osteophyte	5.89 ± 3.93	5.72 ± 3.40	5.57 ± 4.08	NS	NS
Disk narrowing	2.21 ± 1.79	2.09 ± 2.00	2.03 ± 1.86	NS	NS

BMI, body mass index; NS, not significant

Table 2. Results of stepwise regression analysis of four factors for endplate sclerosis score

Factors	F value			r.c.	s.r.c.
	Step 0	Step 1	Step 2	Step 2	($R^2 = 0.094$)
Intercept	63.7	12.8	9.4	–1.106	–1.106
WISP1 SNP genotypes (AA = 0, AG, GG = 1)			9.1	–0.297	–0.166
Age (years)		21.5	22.7	0.025	0.261
Weight (kg)			Not selected		
Height (cm)			Not selected		

r.c., regression coefficient; s.r.c., standard regression coefficient

Table 3. Association of WISP1 SNP genotype (2364A/G) in subjects with spinal endplate sclerosis after stratifying age

Group compared	AA vs. AG + GG		
	OR	*P* value	95% CI
Endplate sclerosis (≧1) (n = 235) versus no endplate sclerosis (=0) (n = 69)	1.78	0.044	1.01–3.13
Higher endplate sclerosis (≧2) (n = 271) versus lower endplate sclerosis (≦0) (n = 33)	2.91	0.0069	1.34–6.30

OR, odds ratio; 95% CI, 95% confidence interval

over-represented in the subjects having a higher (two or more) endplate sclerosis score compared in the subjects having lower (one or no) endplate sclerosis score after being age-adjusted (Table 3; P = 0.0069; odds ratio 2.91; 95% confidence interval 1.34–6.30 by logistic regression analysis). Thus, we suggest that a genetic variation at the WISP1 gene locus is associated with spinal osteoarthritis, especially with endplate sclerosis, independently with background parameters.

Discussion

The present study is the first report that shows the influence of a SNP of the WISP1 gene on spinal osteoarthritis. The WISP1 is an osteogenic potentiating factor promoting mesenchymal cell proliferation and osteoblastic differentiation while repressing chondrocytic differentiation [33]. We demonstrated that Japanese postmenopausal women who had the AA genotype at the WISP1 2364A/G SNP showed a significantly higher endplate sclerosis score of the spine. Our findings might also be supported by genetic linkage scan for early-onset osteoarthritis and chondrocalcinosis susceptibility loci that showed a linkage to chromosome 8q [36], which includes the WISP1 gene locus on 8q24.

It has been recently shown that haplotype analysis in LRP5 gene revealed that there was a common haplotype that provided a 1.6-fold-increased risk of knee osteoarthritis [37]. We have revealed that a SNP (Q89R) in the LRP5 gene is associated with spinal osteoarthritis [38]. It is also reported that there was a significant association of a functional genetic variant of secreted frizzled-related protein 3 (sFRP3), which antagonizes Wnt signaling, with hip osteoarthritis in women [39]. Taken together, our results and the recent evidence suggest that the Wnt-β-catenin signaling pathway including WISP1 is important in the pathogenesis of skeletal abnormality including osteoarthritis.

WISP1 is a member of the CCN family of connective tissue growth factors, which also includes WISP2 and WISP3. Members of the CCN family have been implicated in developmental processes such as chondrogenesis, osteogenesis, and angiogenesis [27–29]. Specifically, mutations of WISP3 cause the rare skeletal syndrome, progressive pseudorheumatoid dysplasia (PPD) [40]. In affected individuals, symptoms develop between the age of 3 years and 8 years and consist of stiffness and swelling of multiple joints, motor weakness, and joint contractures. It has been also reported that WISP3 polymorphisms were associated with susceptibility to juvenile idiopathic arthritis [41]. Moreover, the WISP3 was shown to be expressed in chondrocytes derived from human cartilage and be able to regulate type II collagen and aggrecan expression [42]. On the other hand, the expression of the WISP2 was preferentially detected in rheumatoid arthritis synovium [43]. These data suggest that CCN family members play a critical role in cartilage homeostasis. In the present study, we investigated a possible contribution of WISP1 polymorphism to spinal osteoarthritis in Japanese women. Taken together, the CCN family gene polymorphisms may affect the pathogenesis of cartilage disease.

In the present study, we excluded subjects with severe hip or knee arthritis, because these joint diseases themselves may induce spinal deformity or malalignment. Therefore, we could not assess such joint arthritis here. Recent studies have shown that some SNPs in the sFRP3 and LRP5 genes, involved in Wnt signaling, were associated with hip and knee osteoarthritis, respectively [37,39]. Moreover, WISP3 polymorphisms are associated with juvenile idiopathic arthritis that affects multiple joints [41]. In this regard, it may be important to examine the association of the SNPs in the WISP1 gene with hip and knee arthritis in the future. Meanwhile, it would be better if we had also evaluated the facet joint, because spinal osteoarthritis is represented not only by the anterior elements such as disc narrowing, osteophytosis, or endplate sclerosis but also by the posterior elements, especially a facet joint lesion. However, we here evaluated only the anterior elements of thoracolumbar vertebral bodies, because a reproducible semiquantitative assessment for facet joint using anteroposterior (A-P) and lateral X-ray radiographs has not been well established.

In conclusion, we have shown an association of the polymorphism in the WISP1 gene with a radiographic feauture of spinal endplate sclerosis in postmenopausal Japanese women. The women with AA genotypes had significantly higher endplate sclerosis scores. WISP1 genotyping may be benefical in the prevention and management of spinal osteoarthritis. Thus, the WISP1 would be a useful molecular target for the development of new diagnostic markers as well as therapeutic options in osteoarthritis.

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Metabolic syndrome, insulin resistance, and atherosclerosis in Japanese type 2 diabetic patients

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Abstract

The aim of the present study was to investigate the relationships between metabolic syndrome and atherosclerosis in 57 Japanese type 2 diabetic patients. Metabolic syndrome was diagnosed based on the criteria raised by the Japan Internal Medicine Society. Insulin resistance was estimated by the insulin resistance index of homeostasis model assessment. Ultrasonographically measured carotid atherosclerosis, brachial-ankle pulse wave velocity (ba-PWV), and ankle brachial index (ABI) were used to assess the degree of atherosclerosis. Of 57 patients, 25 were diagnosed as having metabolic syndrome. The patients with metabolic syndrome had significantly higher levels of waist circumference, insulin, insulin resistance index of homeostasis model assessment, systolic and diastolic blood pressures, and serum triglycerides, and lower concentrations of adiponectin. However, there was no significant difference in age, sex, glycosylated hemoglobin (hemoglobin A_{1c}), fasting glucose, leptin, and tumor necrosis factor system activities including tumor necrosis factor α between the 2 groups. Furthermore, no significant difference was observed in the degree of carotid atherosclerosis (intimal-medial thickness in plaque-free segments: 0.72 ± 0.03 vs 0.72 ± 0.02 mm, $P = .435$; carotid stenosis in plaque segments: 6.6% ± 3.0% vs 6.6% ± 1.7%, $P = .497$), ba-PWV (1676 ± 56 vs 1654 ± 44, $P = .380$), and ABI (1.16 ± 0.01 vs 1.15 ± 0.01, $P = .245$) between the 2 groups. From these results, it can be suggested that metabolic syndrome, an insulin-resistant state, is not associated with carotid atherosclerosis, ba-PWV, or ABI in Japanese type 2 diabetic patients.

1. Introduction

Type 2 diabetes mellitus is a heterogeneous syndrome characterized by insulin resistance and/or defective insulin secretion [1]. There seems to be ethnic difference in insulin resistance in type 2 diabetes mellitus. Using a minimal model approach shown by Bergman [2] and Welch et al [3], we previously demonstrated that 40% of type 2 diabetic patients are insulin resistant in Japanese populations [4-6]. In contrast, Haffner et al [7] used this approach and found that 92% of type 2 diabetic patients are insulin resistant in white populations. Moreover, mean body mass index (BMI) in representative epidemiological studies of Japanese type 2 diabetic patients were 23 to 25 kg/m^2, lower than that found in the studies of the whites [8]. Whereas it is well recognized that BMI is one of the most important factors contributing to insulin resistance in diabetic patients, this unique feature of Japanese type 2 diabetic patients allows us to explore other factors related to insulin resistance.

Taking into account these fascinating features, we previously demonstrated that serum triglycerides is independently associated with insulin resistance in Japanese type 2 diabetic patients [9,10]. Thereafter, we found that

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