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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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