Single-nucleotide polymorphism in the hyaluronan and proteoglycan link protein 1 (*HAPLN1*) gene is associated with spinal osteophyte formation and disc degeneration in Japanese women

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Abstract Spinal osteoarthritis including disc degeneration is a very common condition in the axial skeletons of aged people. Recently, spinal osteoarthritis has been shown to be influenced by specific genetic risk factors. Vertebral osteophytes, endplate sclerosis, and intervertebral disc narrowing are recognized as radiographic features of spinal disc degeneration. *HAPLN1* is a key component of the cartilage extracellular matrix; thus, variations in this gene may affect the pathogenesis of cartilage-related diseases such as spinal degeneration. Here, we examine the association between an *HAPLN1* gene polymorphism and the radiographic features

of spinal degeneration. We evaluated the degree of endplate sclerosis, osteophyte formation, and disc space narrowing in 622 Japanese postmenopausal women. Four SNPs in the *HAPLN1* gene—in the 5' flanking region, intron 1, intron 2, and intron 4—were analyzed using the TaqMan polymerase chain reaction method. We found that compared to subjects with the CC or CT genotype, those with the TT genotype for an SNP at intron 2 (rs179851) were significantly overrepresented among the subjects with higher scores for osteophyte formation ($P = 0.0001$; odds ratio 2.12; 95% confidence interval 1.45–3.11, as determined by logistic regression analysis) and disc space narrowing ($P = 0.0057$; odds ratio 1.83; 95% confidence interval 1.19–2.83). Consistent with the involvement of the *HAPLN1* gene in cartilage metabolism, a variation in a specific *HAPLN1* gene locus may be associated with spinal degeneration.

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Introduction

Spinal osteoarthritis with spinal degeneration is a very common condition affecting the axial skeleton of elderly people [3]. Vertebral osteophytosis, endplate sclerosis, and intervertebral disc space narrowing are recognized as characteristic features of spinal degeneration. Recent studies indicate that the appearance of these radiographic features is influenced by genetic factors or physical loading and other environmental factors [7, 19]. Genetic association studies have been performed considering various definitions of osteoarthritis [7, 11, 19, 20]. Several studies have shown that the Tt and tt genotypes of *Taq I* polymorphism of the vitamin

D receptor gene are associated with disc degeneration in various populations [2, 8, 9, 27, 28].

Hyaluronan and proteoglycan link protein 1 (HAPLN1), also called cartilage link protein 1 (CRTL1), is a key component of the cartilage extracellular matrix [6, 14, 16, 17]. HAPLN1 stabilizes aggregates of aggrecan and hyaluronic acid [6, 14, 16] by binding to aggrecan along the hyaluronic acid chain. The resulting aggregates have a stable macromolecular structure that contributes to compression resistance and shock absorption in the joints. Hapln1-null mice develop perinatally lethal chondrodysplasia [29]. The limb and vertebral cartilage of Hapln1-null mice is significantly deficient in aggrecan deposits in the hypertrophic zone and contains a reduced number of prehypertrophic and hypertrophic chondrocytes [29, 30]. Chondrocytes of the vertebral bodies are disorganized in Hapln1-null mice [29, 30]. Cartilage-specific expression of the Hapln1 transgene can completely prevent perinatal mortality in Hapln1-null mice and can dose dependently correct skeletal abnormalities [4]. In addition, a synthetic peptide of HAPLN1 stimulates the biosynthesis of collagens II, IX, and proteoglycan in the intervertebral disc cells [15]. HAPLN1 may function as a growth factor that upregulates the synthesis of aggrecan and type II collagen in cartilage [12]. HAPLN1 mRNA expression is upregulated by SOX9, a key regulator of cartilage matrix genes and chondrogenesis, and is downregulated in osteoarthritic cartilage [10, 22]. Thus, HAPLN1 seems to be an important regulator of cartilage homeostasis and may contribute to the pathogenesis of osteoarthritis with spinal degeneration. In the present study, we examine the association between an *HAPLN1* gene polymorphism and the radiographic features of spinal degeneration, including osteophyte formation, endplate sclerosis, and disc space narrowing, to elucidate how HAPLN1 contributes to metabolism in human cartilage.

Materials and methods

Subjects

DNA samples were collected from 622 healthy Japanese postmenopausal women (mean age + SD; 67.1 ± 7.8 years) living in central Japan, and the genotypes were analyzed. The exclusion criteria included endocrine disorders such as hyperthyroidism, hyperparathyroidism, or diabetes mellitus; liver or kidney disease; use of medications known to affect bone metabolism (e.g., bisphosphonates, hormone replacement therapies, corticosteroids, anticonvulsants, and heparin sodium), or an unusual gynecologic history. Patients with severe hip or knee arthritis were excluded from the study. The eligibility of

the subjects was determined by history taking and physical examination. All the subjects gave informed consent before the study. This study was approved by the Ethics Committees of the University of Tokyo Hospital and the participating clinical institutes.

Radiographic grading of spinal disc degeneration

Conventional plain roentgenograms of the thoracic and lumbar spine in the lateral and anteroposterior (A–P) projections were obtained from all participants. Both lateral projections in the lateral decubitus position and A–P projections in the supine position were obtained at a constant focus–film distance of 1.5 m. The severity of spinal degeneration, including osteophyte formation, endplate sclerosis, and disc space narrowing, was semiquantitatively assessed from the T4/5 to L4/5 disc level or from the T4 to L4 level using the grading scale described by Genant [31]. Next, we radiographically assessed spinal disc degeneration by using a previously reported scoring system [23–26]. In brief, osteophyte formation at a particular disc level was graded 0–3 degrees; endplate sclerosis, 0–2 degrees; disc space narrowing, 0–1 degree. We defined the sum of the degrees of osteophyte formation from the T4/5 to L4/5 level, as determined from A–P radiographs, as the score for osteophyte formation. Further, we defined the sum of the degrees of endplate sclerosis from the T4 to L4 level and the sum of the degrees of disc space narrowing from the T4/5 to L4/5 level, as determined from lateral radiographs, as the scores for endplate sclerosis and disc space narrowing, respectively.

Identification of a single-nucleotide polymorphism in the *HAPLN1* gene

By searching through the Assays-on-Demand single-nucleotide polymorphism (SNP) genotyping products database (Applied Biosystems, Foster City, CA), we extracted four polymorphic variations in the *HAPLN1* gene—in the 5′ flanking region (rs975563), intron 1 (rs10942332), intron 2 (rs179851), and intron 4 (rs4703570). We identified these four SNPs in the *HAPLN1* gene by using the TaqMan (Applied Biosystems) polymerase chain reaction (PCR) method [23–26]. To identify an SNP in *HAPLN1*, we used Assays-on-Demand SNP genotyping products, which include sequence-specific forward and reverse primers and two TaqMan MGB probes for detecting alleles. The TaqMan PCR method utilizes (a) two kinds of TaqMan probes that correspond to a DNA fragment containing the target SNP site with different alleles and (b) the 5′–3′ nuclease activity of *Taq* polymerase, which is essential for PCR. TaqMan probes contain a fluorescence dye at the 5′ end and a quencher at the 3′ end. During PCR, these probes anneal to

the target DNA and are excised through the 5′–3′ nuclease activity of *Taq* polymerase, provided there is no mismatch between the probes and target sequences. Next, the fluorescent dyes are released from the probes, and the fluorescence intensity can be monitored using the ABI PRISM 7500 detector (Applied Biosystems). We found that the allele frequencies of four SNPs did not significantly deviate from the Hardy–Weinberg equilibrium. Since the Hardy–Weinberg equilibrium is based on assumptions such as no genetic drift, no gene flow, no natural selection, negligible mutations, and random mating, the population exhibiting the equilibrium does not evolve, and its genotype and allele frequencies are predicted to remain constant over successive generations. Thus, we considered our subjects eligible for the correlation study.

Statistical analysis

We classified the subjects as those with one or two minor alleles and those with only the major allele encoded at the same locus. The age, height, body weight, body mass index (BMI), and parameters indicative of spinal degeneration (number of osteophytes, endplate sclerosis, and disc space narrowing) in the two groups of subjects were compared using an unpaired *t* test. A stepwise regression analysis was performed to assess the independent effects of four variables (age, height, body weight, and *HAPLN1* SNP genotypes) on osteophyte formation and the score for disc space narrowing. Multivariate logistic regression was used to estimate the odds ratios and 95% confidence intervals (95% CIs) for the two groups, and the risk of osteophyte formation and disc space narrowing. The association between *HAPLN1* genotypes and radiographically detected spinal endplate sclerosis was analyzed with adjustment for age. *P* values <0.05 were considered to be significant. The analyses were performed using the StatView-J 4.5 software (SAS Institute Inc., Cary, NC). Linkage disequilibrium (r^2) between adjacent *HAPLN1* SNPs was examined using the Haploview software [1].

Results

We genotyped four *HAPLN1* gene SNPs—in the 5′ flanking region (rs975563), intron 1 (rs10942332), intron 2 (rs179851), and intron 4 (rs4703570)—in 622 subjects. The allele frequencies of the four SNPs were found to be in Hardy–Weinberg equilibrium. Further, we found that none of the four SNPs were in linkage disequilibrium ($r^2 = 0–0.45$) (Fig. 1).

The distribution of age, body weight, and height did not significantly differ between the four SNPs (data not shown). We compared the scores for spinal degeneration,

Fig. 1 Genomic organization and linkage disequilibrium mapping of single-nucleotide polymorphisms (SNPs) in the *HAPLN1* gene. The *HAPLN1* gene structure and relative physical position of four SNPs are shown in the upper diagram. The pairwise linkage disequilibrium (r^2) between the SNPs is shown below each SNP combination

Table 1 The association of each genotype in and near the *HAPLN1* gene with parameters indicative of spinal degeneration

	Osteophyte formation	Endplate sclerosis	Disc space narrowing
rs975563	NS	NS	NS
rs10942332	NS	NS	NS
rs179851	$P < 0.0001$	NS	$P = 0.0032$
rs4703570	NS	NS	NS

The association of each genotype groups with spinal degeneration parameters was determined by unpaired *t* test

NS not significant

including osteophyte formation, endplate sclerosis, and disc space narrowing, between the subjects with one or two minor alleles and those with two major alleles. A significant intergroup difference for both osteophyte formation and disc space narrowing was observed only among the subjects with the rs179851 SNP (Table 1). Among the postmenopausal Japanese women with this SNP, 166 were TT homozygotes, 294 were CT heterozygotes, and 162 were CC homozygotes (Table 2). Subjects in the TT group had significantly higher scores for osteophyte formation (CC + CT vs. TT $7.67 + 6.08$ vs. $9.99 + 7.46$; $P < 0.0001$) (Fig. 2a, Table 2) and disc space narrowing (CC + CT vs. TT $1.94 + 1.72$ vs. $2.46 + 2.16$; $P = 0.0032$) (Fig. 2c, Table 2) than those in the CC + CT group. The rs179851

Table 2 Comparison of the background and clinical characteristics of subjects with different genotypes (CC + CT genotype and TT genotype) for a single-nucleotide polymorphism (SNP) at intron 2 (rs179851) of the HAPLN1 gene

Items	Genotype (mean $\pm$ SD)		<i>P</i> value
	CC + CT	TT	
Number of subjects	456	166	
Age (years)	67.0 $\pm$ 7.9	67.3 $\pm$ 7.5	NS
Height (cm)	150.5 $\pm$ 5.8	150.8 $\pm$ 5.6	NS
Body weight (kg)	50.8 $\pm$ 7.5	51.3 $\pm$ 8.3	NS
Osteophyte formation	7.67 $\pm$ 6.08	9.99 $\pm$ 7.46	<0.0001
Endplate sclerosis	0.35 $\pm$ 0.80	0.41 $\pm$ 0.80	NS
Disc space narrowing	1.94 $\pm$ 1.72	2.46 $\pm$ 2.16	0.0032

NS not significant

SNP was also found to be significant for both osteophyte formation and disc narrowing, even when the most conservative Bonferroni correction was applied. The scores for endplate sclerosis and background data were not significantly different between the two groups (Fig. 2b, Table 1).

Finally, we analyzed the association of the allele frequency of rs179851 SNP genotypes with osteophyte formation and the disc narrowing score after stratification by age. We found that subjects with the TT genotype were significantly overrepresented among those with higher scores (≥ 11) for osteophyte formation than among those with lower scores (<10) after adjustment for age ($P = 0.0001$; odds ratio 2.12; 95% CI 1.45–3.11, as determined by logistic regression analysis) (Table 3). We also found that the subjects with the TT genotype were significantly overrepresented among those with higher scores (≥ 4) for disc space narrowing than among those with lower scores (<3) after adjustment for age ($P = 0.0057$; odds ratio 1.84; 95% CI 1.19–2.83, as determined by logistic regression

analysis) (Table 4). Thus, we suggest that a variation in a specific *HAPLN1* gene locus is associated with spinal degeneration, especially with osteophyte formation and disc space narrowing.

Discussion

HAPLN1 is a member of the *HAPLN* gene family and is also known as *Crtl1* [17]. Other genes of this family include: the brain/central nervous system-specific gene *HAPLN2* (also known as *Bral1*); the widely expressed gene *HAPLN3* (also known as *Lp3*), which is expressed in a variety of smooth muscle cells; and *HAPLN4* (also known as *Bral2*) [21]. HAPLN proteins stabilize the binding interaction between hyaluronan and chondroitin sulfate proteoglycans [21]. HAPLN1 is known for its role in cartilage formation, where it stabilizes the hyaluronan/aggre-can and hyaluronan/versican complexes [5, 6, 14, 16, 17]. Its importance in cartilage formation has previously been demonstrated in studies on Hapln1-deficient mice [4, 29]. These studies have reported that Hapln1-deficient mice exhibit defective cartilage development and delayed bone formation and therefore have short limbs and craniofacial anomalies. HAPLN1 mRNA expression is upregulated by SOX9, a key regulator of cartilage matrix genes and chondrogenesis, and is downregulated in osteoarthritic cartilage [10, 22]. These reports have highlighted the importance of HAPLN1 in promoting cartilage growth and development, implying the potential involvement of HAPLN1 in the etiology of osteoarthritis.

To the best of our knowledge, the present study is the first to demonstrate the influence of an SNP of the *HAPLN1* gene on spinal degeneration. Attempting to elucidate the pathogenesis of lower back pain, we previously investigated the association of various genetic factors with spinal

Fig. 2 Scores of spinal degeneration between the SNP genotypes (CC + CT genotype and TT genotype) at intron 2 (rs179851) of the HAPLN1 gene. **a** The scores for osteophyte formation are shown for the CC + CT and GG genotypes. The scores are expressed as the mean $\pm$ SE. The number of subjects is shown in parentheses. **b** Scores for endplate sclerosis. **c** Scores for disc space narrowing. The association of the genotype groups with parameters indicative of spinal degeneration was determined using an unpaired *t* test

Table 3 Association of the *HAPLN1* SNP (rs179851) genotype [CC + CT ($n = 456$) versus TT ($n = 166$)] with the score for osteophyte formation after stratification by age (logistic regression analysis)

Severity of osteophytes	OR	95% CI	<i>P</i> value
Eight or more osteophytes ($n = 433$) versus less (<7) osteophytes ($n = 189$)	1.75	1.21–2.53	0.0028
Nine or more osteophytes ($n = 358$) versus less (<8) osteophytes ($n = 264$)	1.78	1.24–2.58	0.0019
Ten or more osteophytes ($n = 295$) versus less (<9) osteophytes ($n = 327$)	1.74	1.20–2.53	0.0038
Eleven or more osteophytes ($n = 237$) versus less (<10) osteophytes ($n = 385$)	2.12	1.45–3.11	0.0001

OR odds ratio, 95% CI 95% confidence intervals

Table 4 Association of the *HAPLN1* SNP genotype [CC + CT ($n = 456$) vs. TT ($n = 166$)] with the score for disc space narrowing after stratification by age (logistic regression analysis)

Severity of disc narrowing	OR	95% CI	<i>P</i> value
One or more disc narrowing ($n = 493$) versus no disc narrowing ($n = 129$)	1.55	0.96–2.50	0.070
Two or more disc narrowing ($n = 328$) versus less (<1) disc narrowing ($n = 294$)	1.36	0.94–1.96	0.101
Three or more disc narrowing ($n = 206$) versus less (<2) disc narrowing ($n = 416$)	1.39	0.95–2.03	0.095
Four or more disc narrowing ($n = 119$) versus less (<3) disc narrowing ($n = 503$)	1.84	1.19–2.83	0.0057

OR odds ratio, 95% CI 95% confidence intervals

degeneration [23–26]. In our previous studies on the common genetic variants associated with spinal degeneration, we found that polymorphism of the *LRP5* gene correlated with osteophyte formation [26], while that of the *IGF1R* correlated with disc space narrowing [25]. Here, we demonstrated that Japanese postmenopausal women with two T alleles at intron 2 of the *HAPLN1* gene had significantly higher scores for both osteophyte formation and disc space narrowing. Our previous study has demonstrated that the degree of spinal osteophyte formation and disc space narrowing did not relate with each other [18]. These data suggest that the *HAPLN1* gene may affect the independent parameters of disc degeneration. However, it remains unclear as to how this intronic polymorphism in the *HAPLN1* gene affects spinal degeneration. We propose the following three hypotheses to explain the same: (1) the intronic polymorphism may directly affect the transcriptional regulation of the *HAPLN1* gene; (2) the intronic polymorphism may be linked with exon mutations of the *HAPLN1* gene or adjacent genes and alter protein functions; and (3) this polymorphism may be linked with mutations in regulatory elements of the *HAPLN1* gene or adjacent genes and may affect their expression levels through transcriptional regulation.

In the present study, we excluded subjects with severe knee or hip arthritis because these joint diseases themselves may cause spinal deformities or misalignment. In the Rotterdam study, no association was found between *HAPLN1* gene polymorphism and radiographically evident osteoarthritis of the knee or hip [13]. However, the above-mentioned study assessed the CA-repeat polymorphism in the promoter region of the *HAPLN1* gene, which is

different from the rs17985 SNP assessed in the present study. Another study has reported that *HAPLN1* gene expression was lower in the affected cartilage of patients with knee osteoarthritis than in the cartilage of age-matched controls, suggesting that the *HAPLN1* gene may be involved in the pathogenesis of knee arthritis [22]. In this regard, future studies should aim to examine the association between the rs17985 SNP at intron 2 of the *HAPLN1* gene and knee or hip arthritis. In the present study, we have focused on identifying SNPs associated with disc degeneration by the candidate gene approach in women. Replication of association studies between the *HAPLN1* SNPs and disc degeneration will be required in other populations, including men. In conclusion, we have demonstrated that an SNP (rs179851) at intron 2 of the *HAPLN1* gene is associated with the radiographic features of spinal osteophyte formation and disc space narrowing in Japanese postmenopausal women. Women with the TT genotype had significantly higher scores for osteophyte formation and disc space narrowing. Thus, *HAPLN1* genotyping could be beneficial for the prevention and management of spinal degeneration. The present findings regarding the correlation between *HAPLN1* polymorphism and spinal degeneration provide a new promising direction to the clinical management of spinal disease. These findings may lead to the development of new diagnostic markers and therapeutic strategies based on specific molecular targets.

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References

- Barrett JC, Fry B, Maller J (2005) Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* 21:263–265
- Cheung KM, Chan D, Karppinen J, Chen Y, Jim JJ, Yip SP, Ott J, Wong KK, Sham P, Luk KD, Cheah KS, Leong JC, Song YQ (2006) Association of the Taq I allele in vitamin D receptor with degenerative disc disease and disc bulge in a Chinese population. *Spine* 31:1143–1148
- Creamer P, Hochberg MC (1997) Osteoarthritis. *Lancet* 350:503–508
- Czipri M, Otto JM, Cs-Szabó G, Kamath RV, Vermes C, Firneisz G, Kolman KJ, Watanabe H, Li Y, Roughley PJ, Yamada Y, Olsen BR, Glant TT (2003) Genetic rescue of chondrodysplasia and the perinatal lethal effect of cartilage link protein deficiency. *J Biol Chem* 278:39214–39223
- Dudhia J (2005) Aggrecan, aging and assembly in articular cartilage. *Cell Mol Life Sci* 62:2241–2256
- Dudhia J, Bayliss MT, Hardingham TE (1994) Human link protein gene: structure and transcription pattern in chondrocytes. *Biochem J* 303:329–333
- Lane NE, Nevitt MC, Genant HK, Hochberg MC (1993) Reliability of new indices of radiographic osteoarthritis of the hand and hip and lumbar disc degeneration. *J Rheumatol* 20:1911–1918
- Jones G, White C, Sambrook P, Eisman J (1998) Allelic variation in the vitamin D receptor, lifestyle factors and lumbar spinal degenerative disease. *Ann Rheum Dis* 57:94–99
- Kawaguchi Y, Kanamori M, Ishihara H, Ohmori K, Matsui H, Kimura T (2002) The association of lumbar disc disease with vitamin-D receptor gene polymorphism. *J Bone Joint Surg Am* 84:2022–2028
- Kou I, Ikegawa S (2004) SOX9-dependent and -independent transcriptional regulation of human cartilage link protein. *J Biol Chem* 279:50942–50948
- Loughlin J (2003) Genetics of osteoarthritis and potential for drug development. *Curr Opin Pharmacol* 3:295–299
- McKenna LA, Liu H, Sansom PA, Dean MF (1998) An N-terminal peptide from link protein stimulates proteoglycan biosynthesis in human articular cartilage in vitro. *Arthritis Rheum* 41:157–162
- Meulenbelt I, Bijkerk C, de Wildt SC, Miedema HS, Valkenburg HA, Breedveld FC, Pols HA, Te Koppele JM, Sloos VF, Hofman A, Slagboom PE, van Duijn CM (1997) Investigation of the association of the CRTM and CRTLI genes with radiographically evident osteoarthritis in subjects from the Rotterdam study. *Arthritis Rheum* 40:1760–1765
- Mörgelin M, Paulsson M, Hardingham TE, Heinegård D, Engel J (1988) Cartilage proteoglycans. Assembly with hyaluronate and link protein as studied by electron microscopy. *Biochem J* 253:175–185
- Mwale F, Demers CN, Petit A, Roughley P, Poole AR, Steffen T, Aebi M, Antoniou J (2003) A synthetic peptide of link protein stimulates the biosynthesis of collagens II, IX and proteoglycan by cells of the intervertebral disc. *J Cell Biochem* 88:1202–1213
- Neame PJ, Barry FP (1993) The link proteins. *Experientia* 49:393–402
- Neame PJ, Christner JE, Baker JR (1986) The primary structure of link protein from rat chondrosarcoma proteoglycan aggregate. *J Biol Chem* 261:3519–3535
- Oishi Y, Shimizu K, Katoh T, Nakao H, Yamaura M, Furuko T, Narusawa K, Nakamura T (2003) Lack of association between lumbar disc degeneration and osteophyte formation in elderly Japanese women with back pain. *Bone* 32(4):405–411
- O'Neill TW, McCloskey EV, Kanis JA, Bhalla AK, Reeve J, Reid DM, Todd C, Woolf AD, Silman AJ (1999) The distribution, determinants, and clinical correlates of vertebral osteophytosis: a population based survey. *J Rheumatol* 26:842–848
- Spector TD, MacGregor AJ (2004) Risk factors for osteoarthritis: genetics. *Osteoarthritis Cartilage* 12:S39–S44
- Spicer AP, Joo A, Bowling RA Jr (2003) A hyaluronan binding link protein gene family whose members are physically linked adjacent to chondroitin sulfate proteoglycan core protein genes: the missing links. *J Biol Chem* 278:21083–21091
- Tew SR, Clegg PD, Brew CJ, Redmond CM, Hardingham TE (2007) SOX9 transduction of a human chondrocytic cell line identifies novel genes regulated in primary human chondrocytes and in osteoarthritis. *Arthritis Res Ther* 9:R107
- Urano T, Narusawa K, Kobayashi S, Shiraki M, Horie-Inoue K, Sasaki N, Hosoi T, Ouchi Y, Nakamura T, Inoue S (2010) Association of HTRA1 promoter polymorphism with spinal disc degeneration in Japanese women. *J Bone Miner Metab* 28:220–226
- Urano T, Narusawa K, Shiraki M, Usui T, Sasaki N, Hosoi T, Ouchi Y, Nakamura T, Inoue S (2007) Association of a single nucleotide polymorphism in the WISP1 gene with spinal osteoarthritis in postmenopausal Japanese women. *J Bone Miner Metab* 25:253–258
- Urano T, Narusawa K, Shiraki M, Usui T, Sasaki N, Hosoi T, Ouchi Y, Nakamura T, Inoue S (2008) Association of a single nucleotide polymorphism in the insulin-like growth factor-1 receptor gene with spinal disc degeneration in postmenopausal Japanese women. *Spine* 33:1256–1261
- Urano T, Shiraki M, Narusawa K, Usui T, Sasaki N, Hosoi T, Ouchi Y, Nakamura T, Inoue S (2007) Q89R polymorphism in the LDL receptor-related protein 5 gene is associated with spinal osteoarthritis in postmenopausal Japanese women. *Spine* 32:25–29
- Videman T, Gibbons LE, Battié MC, Maravilla K, Vanninen E, Leppävuori J, Kaprio J, Peltonen L (2001) The relative roles of intragenic polymorphisms of the vitamin D receptor gene in lumbar spine degeneration and bone density. *Spine* 26:E7–E12
- Videman T, Leppävuori J, Kaprio J, Battié MC, Gibbons LE, Peltonen L, Koskenvuo M (1998) Intragenic polymorphisms of the vitamin D receptor gene associated with intervertebral disc degeneration. *Spine* 23:2477–2485
- Watanabe H, Yamada Y (1999) Mice lacking link protein develop dwarfism and craniofacial abnormalities. *Nat Genet* 21:225–229
- Watanabe H, Yamada Y (2002) Chondrodysplasia of gene knockout mice for aggrecan and link protein. *Glycoconj J* 19:269–273
- Yu W, Glüer CC, Fuerst T, Grampp S, Li J, Lu Y, Genant HK (1995) Influence of degenerative joint disease on spinal bone mineral measurements in postmenopausal women. *Calcif Tissue Int* 57:169–174