A Functional Polymorphism in *COL11A1,* Which Encodes the α1 Chain of Type XI Collagen, Is Associated with Susceptibility to Lumbar Disc Herniation

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Lumbar disc herniation (LDH), degeneration and herniation of the nucleus pulposus of the intervertebral disc (IVD) of the lumbar spine, is one of the most common musculoskeletal diseases. Its etiology and pathogenesis, however, remain unclear. Type XI collagen is important for cartilage collagen formation and for organization of the extracellular matrix. We identified an association between one of the type XI collagen genes, *COL11A1,* and LDH in Japanese populations. *COL11A1,* which encodes the α1 chain of type XI collagen, was highly expressed in IVD, but its expression was decreased in the IVD of patients with LDH. The expression level was inversely correlated with the severity of disc degeneration. A single-nucleotide polymorphism (c.4603C→T [*rs1676486*]) had the most significant association with LDH ($P = 3.3 \times 10^{-6}$), and the transcript containing the disease-associated allele was decreased because of its decreased stability. These observations indicate that type XI collagen is critical for IVD metabolism and that its decrease is related to LDH.

Lumbar disc herniation (LDH), degeneration and herniation of the nucleus pulposus of intervertebral disc (IVD) of the lumbar spine, is one of the most common musculoskeletal diseases.[1–3] Its etiology and pathogenesis, however, remain unclear. Genetic factors have been implicated in the etiology of lumbar disc degeneration.[4,5] Genetic abnormalities of the extracellular matrix (ECM) are implicated in disc degeneration and LDH. Phenotypes of transgenic mice and human mutations underscore the candidacy of ECM genes as susceptibility genes for LDH.[6,7] Several researchers have reported the association of ECM protein genes, including genes for type IX collagen[8,9] and aggrecan,[10] with lumbar disc disease (LDD). We reported elsewhere that cartilage intermediate layer protein and asporin—ECM proteins highly expressed in IVD, as well as articular cartilage—are implicated in LDD.[11,12]

Type XI collagen is a cartilage-specific ECM protein important for cartilage collagen fibril formation and for ECM organization.[13–16] Type XI collagen is composed of three α-chains, α1(XI), α2(XI), and α3(II), which are encoded by *COL11A1, COL11A2,* and *COL2A1,* respectively. The three chains fold into triple-helical heterotrimers to form procollagen, which is secreted into the ECM, where it participates in fibril formation with other cartilage-specific collagens, type II and IX collagens.[13] Type XI collagen regulates the diameter of cartilage collagen fibrils. Its N-terminal noncollagenous region limits the appositional lateral growth of the fibril by blocking further accretion of type II collagen.[14,15] Chondrodysplasia in mouse (*cho*) is an autosomal recessive disorder due to a frame-shift mutation of *COL11A1*.[16] The collagen fibrils of *cho* mice are much thicker than normal.[16,17] Thus, type XI collagen has a critical role in the organization of the supramolecular architecture of cartilage collagen.

Type XI collagen is present in IVD, both in the annulus fibrosus and nucleus pulposus,[18] but its significance in LDH is not known. Type XI collagen is a quantitatively minor component of cartilage collagen fibrils, but it is essential for the interaction between proteoglycan (PG) aggregates and collagens. It binds with high affinity to PG, which is important in vivo for anchoring cartilage PG to the collagen fibrillar network.[19] Mutations in type XI collagen cause various types of chondrodysplasias in human, including Stickler syndrome type II (MIM #604841), Marshall syndrome (MIM #154780), and oto-spondylo-mega-epiphyseal dysplasia (MIM #215150). These disorders are collectively termed "type XI collagenopathies,"[20] and all are complicated by abnormalities of the spine, including narrowing of the IVD. In particular, patients with Stickler syndrome have spondylar abnormalities and Schmorl's node (disc herniation into the vertebral body).[21] These human mutations are in vivo evidence that type XI collagen is critical for IVD integrity; thus, the type XI collagen genes are good candidates for the gene that causes LDH.

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Received June 26, 2007; accepted for publication August 9, 2007; electronically published October 16, 2007.

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Am. J. Hum. Genet. 2007;81:1271–1277. 0002-9297/2007/8106-0013$15.00
DOI: 10.1086/522377

Table 1. Clinical Characteristics of Subjects

Screening and Group	No. of Subjects	Age (years) Mean ± SD	Range	Male (%)	BMI[a]
1st:					
Case:					
LDD[b]	188	26.5 ± 10.4	13–74	40.0	21.0
LDH only	130	25.5 ± 6.9	13–66	54.0	21.1
Control	179	58.7 ± 11.7	23–81	6.0	23.0
2nd[c]:					
Case	359	41.4 ± 14.6	15–77	62.4	23.1
Control	286	69.6 ± 9.2	38–87	58.4	24.3
3rd[c]:					
Case	334	41.8 ± 15.1	11–83	61.3	23.4
Control	376	53.9 ± 9.7	13–86	47.6	22.2

[a] BMI calculated as body weight in kilograms divided by the square of height in meters.
[b] Includes disc degeneration only and LDH.
[c] Case group in the 2nd and 3rd screenings has LDH only.

Here, we present evidence that *COL11A1,* one of the type XI collagen genes, contributes to the genetic risk of LDH in Japanese. We have observed significant association between LDH and a functional SNP in *COL11A1* in independent Japanese populations. *COL11A1* was highly expressed in IVD, but its expression was decreased in the IVD of patients with LDH. *COL11A1* expression level was inversely correlated with the severity of disc degeneration in patients with LDH, and the transcript containing the disease-associated allele of the SNP was decreased.

Material and Methods

Study Population

All subjects were Japanese who were living in the middle part of the Honshyu island in Japan (table 1). They visited the participating hospitals and received medical examinations. For the initial screening, we recruited 188 case patients with LDD and 179 control subjects. The mean ages of the case and control groups were 26.5 and 58.7 years, respectively. The case group included 58 patients who had no herniation (disc degeneration only) and 130 patients with LDH. The mean age of the LDH case patients was 25.5 years. For the second screening (replication study), we recruited 359 patients with LDH and 286 control subjects. The mean ages of the case and control groups were 41.4 and 69.6 years, respectively. For the third screening, we recruited 334 patients with LDH and 376 control subjects. The mean ages of the case and control groups were 41.8 and 53.9 years, respectively. Subjects for the initial, second, and third screenings were recruited at the participating hospitals in the Toyama, Tokyo, and Kyoto areas, respectively. All LDH case patients had unilateral pain radiating from the back along the femoral or sciatic nerve to the corresponding dermatome of the nerve root with duration of >3 mo. Radiographic examination, including functional four-direction images and magnetic resonance imaging (MRI) (sagittal and axial images obtained with a 1.5-T imaging system), revealed positive findings indicating disc herniation. The degree of disc degeneration was evaluated by MRI and was scored according to Schneiderman's classification.[22] Of the affected individuals, 787 case patients underwent surgical treatment, and the other individuals with LDH were treated conservatively. All were followed up for >1 year. We excluded from the study individuals with spinal canal stenosis, spondylolisthesis, spondylosis, synovial cysts, spinal tumor, and trauma. We also excluded those who had occupational and/or habitual risk factors, such as heavy manual laborers, occupational drivers, and heavy smokers. We obtained informed consent from each subject, as approved by the ethical committees at the SNP Research Center of RIKEN and the participating hospitals.

Genotyping

We selected sequence variations of the type XI collagen genes (*COL11A1, COL11A2,* and *COL2A1*) for the first screening from the International HapMap Project database and JSNP Database. The SNPs covered >90% of the alleles with an r^2 value ≥ 0.8. We identified additional sequence variations in *COL11A1* by direct sequencing of a 230-kb region of DNA from 24 case patients. We extracted genomic DNA for genotyping from peripheral blood leukocytes of the subjects and genotyped SNPs as described elsewhere.[11,12]

Haplotype Structure and Statistical Analyses

We estimated haplotype frequencies, using the expectation-maximization algorithm and pairwise linkage-disequilibrium index (D' and Δ in 465 control individuals, as described elsewhere).[23] χ^2 tests were used to compare cases with controls for allelic and genotypic frequencies; the odds ratio (OR) and its 95% CI were calculated. We used a permutation test to adjust significance in the analysis of association between the *COL11A1* SNPs and LDH.[24] We performed 10^7 permutations of the cases and the controls. Bonferroni correction was applied when significance was adjusted for the number of SNPs genotyped. MRI data, real-time PCR data, and mRNA stability data were tested using Student's *t* test.

Analysis of COL11A1 *Expression*

We extracted and purified total RNAs and synthesized randomly primed cDNAs, using Multiscribe reverse transcriptase (PE Ap-

Table 2. Association between LDH and c.4603C→T (*rs1676486*) in *COL11A1*

Screening and Case Group	No. of Cases with Genotype CC	CT	TT	Total No. of Cases	No. of Controls with Genotype CC	CT	TT	Total No. of Controls	T Allele Frequency Case	Control	*P*	OR (95% CI)
1st:												
LDD[a]	85	86	17	188	99	67	13	179	.31	.26	.076	1.34 (.97–1.84)
LDH only	55	60	15	130	99	67	13	179	.34	.26	.020	1.51 (1.07–2.14)
2nd:												
LDH only	149	163	47	359	154	108	21	283	.35	.26	.00038	1.55 (1.21–1.97)

[a] Includes disc degeneration only and LDH.

Table 3. Polymorphisms in *COL11A1* and Their Association with LDH

Location in *COL11A1* and Nucleotide Sequence Change	Amino Acid Change	dbSNP	No. in the Three Genotype Groups[a]		Allelic Frequency		*P*[b]		OR (95% CI)[c]
			Case	Control	Case	Control	Allele	Genotype[d]	
IVS1:									
9284T→C	...	*rs1415359*	423/63/1	422/42/1	.07	.05	.068	.16	.69 (.47–1.03)
IVS6:									
82274A→C	...	...	437/49/1	424/38/1	.05	.04	.35	.61	.82 (.53–1.25)
IVS10:									
90221G→A	...	*rs945748*	426/62/1	414/48/1	.07	.05	.29	.54	.82 (.56–1.19)
IVS11:									
90406A→G	...	*rs3767272*	396/76/3	401/55/0	.09	.06	.032	.049	1.47 (1.03–2.10)
IVS20:									
104122A→T	...	*rs2622877*	438/47/2	400/46/0	.05	.05	.94	.38	.98 (.65–1.48)
IVS26:									
111262T→C	...	*rs2786125*	428/49/1	429/33/1	.05	.04	.11	.24	.70 (.45–1.08)
IVS41:									
146354T→C	...	*rs1012282*	425/62/1	415/47/1	.07	.05	.24	.47	1.26 (.86–1.84)
IVS42:									
165864A→C	...	*rs1841838*	381/104/3	374/84/6	.11	.1	.52	.27	1.10 (.82–1.47)
IVS44:									
169351A→G	...	*rs2126643*	378/100/3	373/79/6	.11	.1	.44	.23	1.12 (.84–1.51)
172702C→G	...	*rs3767273*	382/103/3	372/84/4	.11	.1	.41	.5	.88 (.66–1.19)
IVS50:									
192606G→A	...	*rs4908273*	231/211/43	271/167/23	.31	.23	.00023	.001	1.47 (1.20–1.80)
Exon 52:									
193817(c.3968)T→C	L1323P	*rs3753841*	193/230/65	238/187/38	.37	.28	.000081	.00041	1.47 (1.21–1.79)
IVS52:									
194187T→C	...	...	218/214/48	258/178/26	.32	.25	.00038	.0016	.69 (.57–0.85)
IVS54:									
200918A→G	...	*rs3767274*	399/73/4	367/86/5	.09	.1	.15	.34	.79 (.58–1.08)
206255G→T	...	*rs3767275*	457/30/0	442/15/1	.03	.02	.088	.068	.60 (.33–1.09)
208970T→A	...	*rs1676500*	443/45/1	425/33/1	.05	.04	.29	.53	1.27 (.81–1.99)
IVS58:									
218282C→G	...	...	431/46/1	430/32/1	.05	.04	.15	.32	.72 (.46–1.13)
Exon 62:									
219597(c.4603)C→T	P1535S	*rs1676486*	204/223/62	252/177/33	.35	.26	.000015	.000099	1.54 (1.27–1.88)
Exon 63:									
221284(c.4770)C→T	I1590I	*rs2229783*	169/236/83	214/201/47	.41	.32	.000028	.00017	1.49 (1.24–1.80)
IVS63:									
221659G→A	...	*rs1463048*	169/235/83	212/199/50	.41	.32	.000081	.00047	1.46 (1.21–1.76)
IVS65:									
225275T→A	...	*rs3753844*	207/223/55	239/186/33	.34	.28	.0014	.0056	1.38 (1.13–1.68)
Exon 67 (3′ UTR):									
230265C→T	...	*rs1031820*	443/45/1	430/33/1	.05	.04	.27	.5	.78 (.50–1.21)
230461A→G	...	...	439/45/1	429/33/0	.05	.04	.17	.3	.73 (.46–1.15)

NOTE.—The cDNA (accession number NM001854.2) and genomic DNA (accession numbers AC093150.4, AL627203.7, and AC099567.2) sequences of *COL11A1* are based on data from GenBank. The A of the ATG translation initiation codon in the reference sequence corresponds to position +1.

[a] Homozygote of the major allele/heterozygote/homozygote of the minor allele.

[b] By the χ^2 test.

[c] Calculated for the alleles.

[d] Calculated for the homozygotes of the major allele versus the heterozygotes and the homozygotes of the minor allele.

plied Biosystems). We performed quantitative real-time PCR using the ABI PRISM 7700 (Applied Biosystems) and QuantiTect SYBR Green PCR (QIAGEN) according to the manufacturer's instructions.

RNA Stability Assay

We amplified by PCR ~1,700-bp of *COL11A1* cDNA that contained the entire ORF. We cloned the *COL11A1* cDNA containing the associated SNP c.4603C→T into pCR-Blunt II-TOPO (Invitrogen) and confirmed the sequence of the inserts. Vectors were

Table 4. Correlation between Age and Genotype at c.4603C→T (*rs1676486*) in *COL11A1*

Population	Mean ± SD Age (in years) for Genotype			*P*[a]
	CC	CT	TT	
Case	36.8 ± 15.0	36.9 ± 14.5	36.8 ± 14.5	.58
Control	64.8 ± 12.1	63.9 ± 11.1	63.1 ± 13.1	.54

[a] *P* value was calculated using the Kruskal-Wallis test.

Table 5. Genotype at c.4603C→T (*rs1676486*) in *COL11A1*, Stratified by Sex

Measure	Male			Female		
	Case	Control	Total	Case	Control	Total
No. of subjects:						
All	298	177	475	191	285	476
CC	116	98	214	88	154	242
CT	144	65	209	79	112	191
TT	38	14	52	24	19	43
T allele frequency (%)	.37	.26	.33	.33	.26	.29
P value[a]	...	...	.00074	...	...	.021

[a] *P* value for allelic difference between the patients with LDH and the control groups for each sex, by the χ^2 test.

digested using *Hin*dIII, and *COL11A1* mRNAs were transcribed using RiboMax Large Scale RNA Production System-T7 (Promega) and were purified by SV Total RNA Isolation System (Promega). The whole-cell extract was prepared by washing OUMS-27 cells in PBS and resuspending them in an extraction buffer. After incubation on ice for 30 min and microcentrifugation at 4°C, we transferred supernatants to new tubes and stored them at −80°C until use. We mixed and incubated each 5 μg of synthesized RNA and the diluted (1:1,000) whole-cell extract at room temperature for the tested time (5 or 10 min). We stopped the reaction with addition of a formamide dye. The samples were then heated at 95°C for 5 min and were placed on ice immediately. We detected *COL11A1* mRNAs of the samples by northern-blot analysis and quantified their signal intensities, using the Esper-Scanner (Epson) and Adobe Photoshop 6.0.

Immunohistochemistry for Type XI Collagen

We processed and embedded tissue samples in paraffin by the AMeX method. We predigested the tissue sections with 500 U/ml of testicular hyaluronidase (Sigma) for 30 min at 37°C. For immunofluorescent visualization, we blocked nonspecific labeling with blocking reagent (DakoCytomation) for 10 min at room temperature and then incubated the sections with the rabbit polyclonal antibody against bovine type XI collagen (1:500) at 4°C overnight. For the staining of the negative control, we applied nonimmune rabbit IgG (DakoCytomation) to the section instead of primary antibody. After washing them with Tris-buffered saline, we incubated the sections with secondary antibody conjugated to horseradish peroxidase–labeled polymer (Envision+ [DakoCytomation]) for 30 min at room temperature. We visualized the immunoreactive products using a diaminobenzidine reagent and counterstained them with hematoxylin.

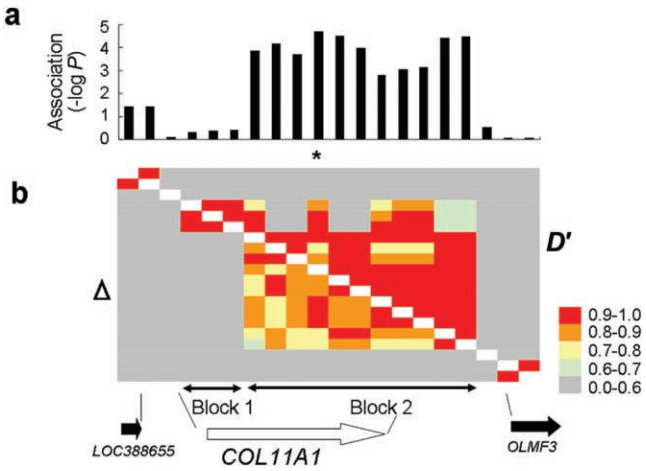

Figure 1. Case-control association study and linkage-disequilibrium mapping. *a,* Association of *COL11A1* with LDH. The $-\log_{10}$ transformation of the corrected *P* value (allele 1 vs. allele 2) was plotted on the *Y*-axis. The asterisk (*) indicates c.4603C→T. *b,* Pairwise linkage disequilibrium between SNPs in and around *COL11A1* measured by *D′* and Δ in 465 controls. The *COL11A1* region is divided into two linkage-disequilibrium blocks.

Results

We first examined the association of the type XI collagen genes (*COL11A1, COL11A2,* and *COL2A1*) with LDD, which included patients with and without LDH. We tested tag SNPs that were selected from the JSNP Database and the International HapMap Project database. A comparison of 188 LDD cases and 179 controls revealed no association with any of the SNPs; however, there was a significant association with *COL11A1* when cases were stratified on the basis of the presence or absence of LDH (table 2). In a comparison of 130 patients with LDH with 179 controls, one SNP (c.4603C→T [*rs1676486*]) had a significant association. To confirm the association, we examined another 359 LDH cases and 286 controls for the *COL11A1* SNP. Again, we identified the significant association between the SNP and LDH (table 2). Adjusted $P = .00030$ was obtained by 10^7 permutations

To identify the disease-causing sequence variation, we examined sequence variations in *COL11A1* exons and their flanking regions from a public database and by resequencing 24 patients with LDH. A total of 23 sequence variations were identified and were tested for association. SNP c.4603C→T had the most significant association (table 3), which remained significant after Bonferroni correction for multiple testing. We examined whether confounding effects, such as age and sex, affect the associations with LDH and found no relationship between the genotype and

Table 6. Haplotype Association Analysis of *COL11A1* with LDH

Haplotype	Frequency		*P*[a]
	Case	Control	
H1	.527	.616	.000154
H2	.302	.222	.000150
H3	.038	.039	.90
H4	.041	.037	.63
H5	.045	.034	.27
H6	.014	.014	.91
H7	.011	.008	.50

NOTE.—Results are for the haplotypes of block 2 that contained the susceptibility SNP, c.4603C→T.

[a] By the χ^2 test.

Table 7. Association between Genotype at c.4603C→T (*rs1676486*) in *COL11A1* and LDH in the Japanese Population

Group	No. with Genotype			Allelic Frequency	*P*	OR (95% CI)
	CC	CT	TT			
Case	360	367	96	.34	.0000033	1.42 (1.23–1.65)
Control	453	325	60	.265		

these factors (table 4). The association was positive in both sexes (table 5).

Using the 20 SNPs in and around *COL11A1* that had a minor-allele frequency >10%, we analyzed the linkage-disequilibrium structure of the region and found highly structured linkage-disequilibrium blocks (fig. 1). *COL11A1* was covered by two blocks, and the SNP with a significant association (c.4603C→T) was contained in block 2. We further analyzed the haplotype structure of block 2 and identified seven haplotypes with frequencies >0.01 that covered >97% of both the case and control groups (table 6). The association was weaker than that of c.4603C→T alone, suggesting the absence of a hidden causal SNP. We further examined the association of the SNP, using an additional 334 patients with LDH and 376 controls. Our findings of the association between this SNP and LDH were replicated ($P = .044$; OR 1.27 [95% CI 1.01–1.59]. Therefore, this SNP is strongly associated with LDH (combined $P = 3.3 \times 10^{-6}$ in allelic frequency) (table 7).

To clarify the functional impact of c.4603C→T, we quantified the allelic difference of the mRNA expression by real-time RT-PCR. The expression level of the susceptibility allele c.4603T was significantly lower than that of the c.4603C allele (fig. 2*a*). We hypothesized that this SNP affects *COL11A1* transcription by altering mRNA stability and examined the stability of *COL11A1* mRNA containing the SNP. We mixed mRNAs produced by in vitro transcription with cell lysate and assessed mRNA degradation by endogenous components of the cells, using northern-blot analysis. The transcript containing the susceptible allele degraded faster (fig. 2*b* and 2*c*).

To gain insight into the role of type XI collagen in LDH, we examined *COL11A1* expression in tissues and cells by quantitative real-time PCR. *COL11A1* mRNA was predominantly expressed in IVD (fig. 3*a*). We investigated the correlation between the *COL11A1* mRNA expression level and a variety of LDH phenotypes and found that severity of disc degeneration evaluated by MRI was inversely correlated with *COL11A1* expression in IVDs of patients with LDH (fig. 3*b*). We further analyzed the expression and localization of type XI collagen in IVD by immunohistochemistry. Normal discs had a highly uniform ECM structure, with intense immunostaining of type XI collagen in the nucleus pulposus cells and ECM (fig. 3*c*). In degenerative discs, however, we observed weak immunostaining of type XI collagen around the nucleus pulposus cells (fig. 3*d*). These findings implicate a decrease of type XI collagen in the pathogenesis of LDH.

Discussion

Through a case-control association study focusing on type XI collagen, we identified *COL11A1* as a susceptibility gene for LDH. *COL11A1* mRNA was substantially ex-

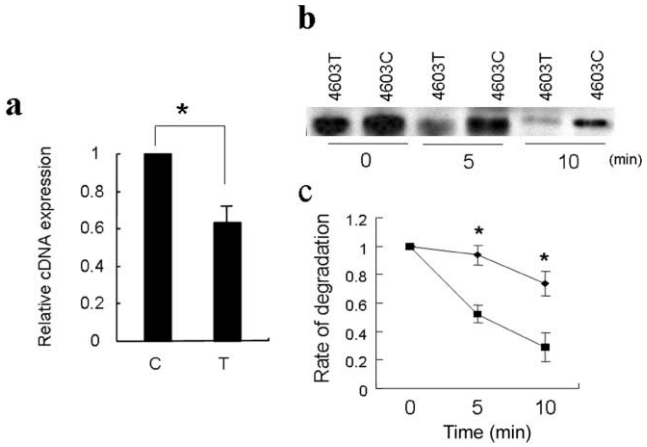

Figure 2. Difference in transcription and stability of *COL11A1* mRNA containing the LDH-associated SNP. *a,* Relative cDNA expression of c.4603C→T evaluated by real-time PCR. Data represent the ratios of cDNA to genomic DNA, and expression of the C allele is converted to 1 (an asterisk [*] indicates $P < .05$, by Student's *t* test). Data represent the mean ± SD in triplicate assays. *b,* Sequential change of *COL11A1* mRNA analyzed by northern blotting. "4603C" and "4603T" indicate *COL11A1* mRNA produced by in vitro transcription with c.4603C and c.4603T, respectively. *c,* Rate of degradation of the transcripts. Diamonds indicate the transcript with c.4603C; squares indicate the transcript with c.4603T. The difference of the rate of degradation was significant at both 5 min and 10 min after the reaction (an asterisk (*) indicates $P < .05$, by Student's *t* test). Data represent the mean ± SD in triplicate assays.

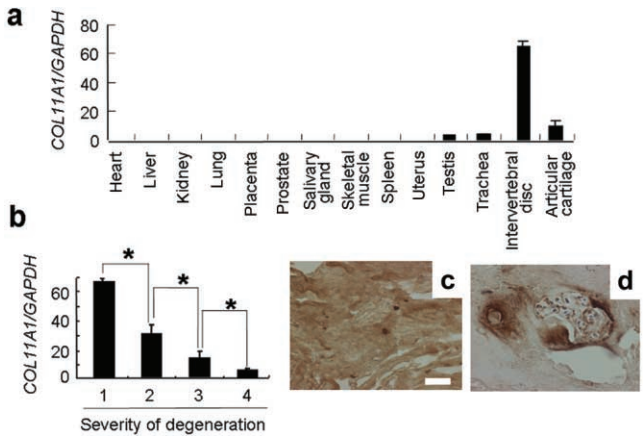

Figure 3. Type XI collagen expression in human. *a, COL11A1* expression in different tissues. *COL11A1* mRNA was predominantly expressed in IVD. *b,* Inverse correlation between *COL11A1* expression and severity of degeneration of IVD in patients with LDH (an asterisk [*] indicates $P < .05$, by Student's *t* test). The degree of disc degeneration is evaluated by MRI and is scored according to the classification of Schneiderman. *c* and *d,* Immunostaining of type XI collagen in IVDs from an unaffected individual (*c*) and a patient with LDH (Schneiderman's grade 3) (*d*). Ubiquitous and intense staining was found in the normal disc. In contrast, the staining was found only in and around the territorial matrices of clustered cells in the degenerative disc. The white scale bar indicates 50 nm.

pressed in IVD, and the expression in patients with LDH was decreased according to the severity of degeneration. Our findings further indicate that the susceptibility SNP produces unstable *COL11A1* transcripts. A few *cis*-elements have been implicated in mRNA stabilization.[25] The 4856–4865 nucleotides (caaaaaatct) in *COL11A1* mRNA closely match the consensus for a mRNA stability motif, "g/tanaaaag/tcc/t."[26] The sequence variation might affect the mRNA stability motif and disrupt the *cis*-element critical for mRNA stability, although they are >200 bp apart. Alternatively, the sequence variation might induce a conformational change in the mRNA that would decrease mRNA stability or increase the sensitivity to RNase. The decrease of the *COL11A1* transcript would lead to a decrease in type XI collagen in the ECM of IVD.

IVD has a highly structured ECM to resist mechanical forces. The highly oriented network of the fibrillar collagens offers tensile strength,[27,28] and highly hydrated aggregating PG resists comprehensive forces. They form a mesh suited to holding water molecules, which further increases their ability to withstand mechanical forces. Therefore, the structural integrity of ECM and the physiologic balance of its components are critical to IVD function. Perturbation of ECM metabolism would increase the mechanical load of the IVD, leading to its degeneration. The reduction in type XI collagen, the critical organizer of ECM, ultimately causes disintegration of ECM and hence IVD degeneration, although it could occur as a secondary event of LDH. The present study underscores the importance of ECM proteins in the pathogenesis of common bone and joint diseases, including LDH. Our results should lead to a better understanding of the pathogenic mechanisms of LDH and suggest promising targets for a novel treatment strategy for LDH.

Acknowledgments

We thank Drs. S. Seki, T. Koyanagi, Y. Fukui, T. Kono, H. Kono, H. Hirabayashi, K. Kono, M. Ishikawa, M. Tamura, K. Nojiri, H. Morisue, and N. Hosogane, for help in collecting samples and performing the experimental study, and Y. Takanashi, for technical assistance. This work was supported by grants-in-aid from Ministry of Education, Culture, Sports and Science of Japan (grant 19209049 [to S.I.]).

Web Resources

Accession numbers and URLs for data presented herein are as follows:

Applied Biosystems, http://www.appliedbiosystems.com/index.cfm
GenBank, http://www.ncbi.nlm.nih.gov/Genbank/ (for *COL11A1* sequences [accession numbers NM001854.2, AC093150.4, AL627203.7, and AC099567.2])
International HapMap Project, http://hapmap.org/
JSNP Database, http://snp.ims.u-tokyo.ac.jp/index.html
Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for Stickler syndrome type II, Marshall syndrome, and oto-spondylo-mega-epiphyseal dysplasia)

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