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A Functional Polymorphism in *THBS2* that Affects Alternative Splicing and MMP Binding Is Associated with Lumbar-Disc Herniation

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Lumbar-disc herniation (LDH), one of the most common musculoskeletal diseases, has strong genetic determinants. Recently, several genes that encode extracellular matrix (ECM) proteins in the intervertebral disc have been reported to associate with LDH. Thrombospondins (THBSs) 1 and 2 are good candidates for the LDH susceptibility gene: They are intervertebral disc ECM proteins that regulate the effective levels of matrix metalloproteinases (MMPs) 2 and 9, which are key effectors of ECM remodeling. Here, we report that *THBS2* is associated with LDH in Japanese populations. An intronic SNP in *THBS2* (IVS10-8C → T; rs9406328) showed significant association ($p = 0.0000028$) with LDH in two independent Japanese populations. This SNP, located in a polypyrimidine tract upstream of the 3′ splice site of intron 10, exerts allelic differences on exon 11 skipping rates in vivo, with the susceptibility allele showing increased skipping. Skipping of exon 11 results in decreased THBS2 interaction with MMP2 and MMP9. Further, a missense SNP in *MMP9* (Q279R; rs17576) is also strongly associated with LDH in the Japanese population ($p = 0.00049$) and shows a combinatorial effect with *THBS2* (odds ratio 3.03, 95% confidence interval 1.58–5.77). Thus, a splicing-affecting SNP in *THBS2* and a missense SNP in *MMP9* are associated with susceptibility to LDH. Our data indicate that regulation of intervertebral disc ECM metabolism by the THBS2-MMP system plays an essential role in the etiology and pathogenesis of LDH.

Introduction

Lumbar-disc herniation (LDH) is a predominant cause of low-back pain and unilateral leg pain. Low-back pain affects 70%–85% of all people during their lifetime, and LDH is the most common cause of activity limitation in individuals younger than 45 years of age.[1] Twenty percent of individuals with LDH require surgical treatment to relieve prolonged or aggravated leg pain.[2] Although many risk factors have been reported for LDH, its etiology and pathogenesis are for the most part unknown. The strong familial predisposition for lumbar-disc degeneration has been established through a number of family and twin studies.[3–5] Recently, several genes have been reported to associate with lumbar disc disease. Most encode extracellular matrix (ECM) proteins in the intervertebral disc, suggesting the importance of ECM metabolism in LDH.[6,7]

The thrombospondins (THBSs) are a family of five secreted, modular glycoproteins whose functions in the ECM are diverse and poorly understood.[8,9] THBS1 (MIM 188060) and THBS2 (MIM 188061) are structurally more similar to each other than to THBS3–THBS5 and are, therefore, considered to constitute a subfamily.[10] Both THBS1 and THBS2 interact with matrix metalloproteinase 2 (MMP2 [MIM 120360]) and 9 (MMP9 [MIM 120361]) and regulate their effective levels in the pericellular ECM.[11,12] Mice with deficiencies in either *Thbs1* or *Thbs2* show abnormal spine curvature.[13,14] *Thbs2* knockout mice also exhibit increased levels of MMP2 after injury.[15] A more recent study has shown immunolocalization of THBSs in the human intervertebral disc.[16] These observations prompted us to examine *THBS1* and *THBS2* as candidate genes for LDH.

We have identified a significant association between *THBS2* and LDH in Japanese populations. We show that an associated SNP in *THBS2* affects the splicing and consequently alters the binding affinity to MMP2 and MMP9. We further show *MMP9* is also associated with LDH. Our findings indicate that THBS2 is involved in LDH through the regulation of MMP activity in intervertebral-disc ECM.

Material and Methods

Subjects

For the case-control association analysis of LDH, we recruited 525 cases (34.7% female; mean age ± standard deviation [SD] = 41.5 ± 14.9; mean body mass index (BMI) ± SD = 23.1 ± 3.2) and 564 controls (42.4% female; mean age ± SD = 62.4 ± 9.9; mean BMI ± SD = 23.6 ± 3.3) for the first screen (Japanese A

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population), and 322 cases (36.6% female; mean age ± SD = 35.7 ± 15.2; mean BMI ± SD = 22.6 ± 3.5) and 332 controls (36.4% female; mean age ± SD = 60.9 ± 14.1; mean BMI ± SD = 23.4 ± 2.9) for the replication study (Japanese B population). Affected individuals with LDH were recruited from 19 collaborating hospitals between November 2001 and February 2007. The diagnosis of LDH required the following three criteria: (1) diagnosis of LDH by magnetic resonance imaging (sagittal and axial images obtained with a 1.5-T imaging system), (2) treatment and monitoring for more than 1 year by orthopedic surgeons, and (3) a history of unilateral pain radiating from the back along the femoral or sciatic nerve to the corresponding dermatome of the nerve root for a longer than 3 months. Primary exclusion criteria included synovial cyst, spinal tumor, spondylolisthesis, spondylosis, trauma, and inflammatory disease. From a total of 847 individuals with LDH, 811 underwent surgery for LDH, including posterior discectomy, anterior lumbar interbody fusion, microendoscopic discectomy, and percutaneous nucleotomy. We extracted genomic DNA from peripheral blood leukocytes of affected individuals and controls by using standard protocols. We obtained normal intervertebral disc tissue from individuals with idiopathic scoliosis during surgery (seven samples). Written informed consent was obtained from each subject as approved by the ethical committees of the SNP Research Center at RIKEN and participating clinical institutes.

Genotyping

We genotyped SNPs by using the multiplex polymerase chain reaction (PCR)-based Invader assay (Third Wave Technologies),[17] TaqMan SNP genotyping assays (Applied Biosystems), or by direct sequencing of PCR products with ABI 3700 DNA analyzers (Applied Biosystems) according to the manufacturers' protocols.

Statistical Analysis

We assessed association and Hardy-Weinberg equilibrium by using the χ^2 test. We estimated haplotype frequencies with the expectation-maximization algorithm.[18] Linkage disequilibrium coefficients (D' and r^2) were calculated as described previously.[7] We used the permutation test to adjust significance in the analysis of association between the *THBS2* SNPs and LDH.[19] We performed 1,000,000 permutations of the cases and the controls. Detection of significance of stratification (p) and estimation of quantitative assessment of population stratification (λ) were done according to previous reports.[20,21] We assessed the relationship between the clinical profiles and the genotype information by the Kruskal-Wallis test and the χ^2 test. Combinatorial effects of *THBS2* and *MMP9* with regard to LDH susceptibility were also examined as described previously.[22] The odds ratio was defined against the genotype consisting of homozygotes of nonsusceptibility alleles from both loci.

Cell Culture and RNA Extraction

Fibroblast cells and the human chondrosarcoma cell lines OUMS-27[23] and CS-OKB[24] were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS) at 37°C under 5% CO_2. Total RNA was extracted from tissues and cells with Isogen (Nippongene) and purified with SV Total RNA Isolation System (Promega), according to the manufacturers' instructions.

RT-PCR and Real-Time PCR

We obtained complementary DNA (cDNA) from all tissues other than intervertebral disc from Multiple Tissue cDNA Panels (Clontech). For the intervertebral disc tissue and cell line, cDNA was synthesized from total RNA using Multiscribe reverse transcriptase and oligo-dT primer (Applied Biosystems). We performed quantitative real-time PCR with an ABI PRISM 7700 sequence detector with the Quantitect SYBR Green PCR Kit (QIAGEN) in accordance with the manufacturers' instructions. Primer sequences for reverse transcriptase (RT)-PCR and real-time PCR are available on request.

Expression of Recombinant Partial Human THBS2

We cloned a cDNA encoding wild-type (WT-THBS2) and exon-11-skipped partial human THBS2 (Skip-THBS2) into pET42b expression vectors that had both S tag and GST tag and expressed them in the *E. coli* Rosetta (DE3) pLys strain. Recombinant proteins were solubilized from inclusion bodies, renatured with the Protein Refolding Kit (Novagen), and used for pull-down and solid-phase binding assays.

Western Blotting

Membranes were blocked with 3% bovine serum albumin in Tris-buffered saline Tween 20 (TBST). We used polyclonal antibodies against MMP2 and MMP9 (R&D Systems) at a 1:1000 dilution in TBST with 1% bovine serum albumin. We used a horseradish peroxidase (HRP)-conjugated anti-goat immunoglobulin G (IgG) (Santa Cruz) as the secondary antibody at a 1:7000 dilution in TBST with 1% bovine serum albumin.

S Protein Pull-down Assay

We incubated S-tagged recombinant THBS2 (5 μM, 20 μl) with 1 μg of purified human MMP2 or MMP9 (CHEMICON) for 2 hr at 4°C in 0.3 ml of binding buffer (20 mM Tris-HCl [pH 7.5], 250 mM NaCl, 1% Triton X-100, and Complete protease inhibitor cocktail [Roche]). Twelve and a half microliters of S protein agarose (Novagen) was added to the reaction and incubated for 30 min at room temperature. Precipitates were washed three times with binding buffer and subjected to 12.5% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). We detected coprecipitated proteins by Western blotting with antibodies to MMP2 or MMP9 (R&D Systems) and S protein HRP conjugate (Novagen).

Solid-Phase Binding Assay

We coated Maxisorp enzyme-linked immunosorbent assay (ELISA) plate (Nunc) wells with 50 μl of 0.2 μM GST-tagged recombinant THBS2 in 100 mM Tris-HCl (pH 7.2) at 4°C overnight. We then blocked the wells with 300 μl of blocking buffer (100 mM Tris-HCl [pH 7.2], 5 mM $CaCl_2$, 1% bovine serum albumin) for 1 hr at room temperature, added 50 μl of 4 μg/ml purified human MMP2 or MMP9 (CHEMICON) to the wells, and incubated them for 2 hr at room temperature. Wells were washed twice with 100 mM Tris-HCl (pH 7.2) and incubated with antibodies to MMP2 or MMP9 (R&D Systems). Next, we washed the wells three times with 100 mM Tris-HCl (pH 7.2) and incubated them with alkaline-phosphatase-conjugated antibody to goat IgG (ZYMED). After washing the wells three times with 100 mM Tris-HCl (pH 7.2), we assayed bound phosphatase activity with the Alkaline Phosphatase Substrate Kit (Bio-Rad) and detection at 405 nm.

Results

To confirm the candidacy of *THBS1* and *THBS2* for LDH, we examined *THBS1* and *THBS2* expression in various

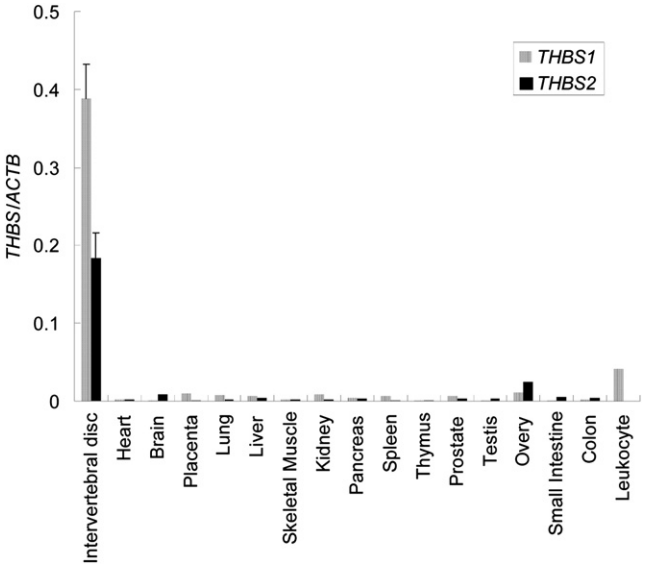

Figure 1. Real-Time PCR Analysis of *THBS1* and *THBS2* mRNAs in Various Human Tissues

THBS1 and *THBS2* mRNAs are abundantly expressed in the intervertebral disc. Data represent the ratio of *THBS* mRNAs to beta-actin (*ACTB*) mRNA. Error bars represent ± standard error of the mean (SEM) (n = 7).

human tissues by using real-time RT-PCR. We detected specific and high expression levels of both genes in intervertebral disc tissue (Figure 1), which further supported the candidacy of these genes for the LDH-susceptibility gene.

On the basis of information in the HapMap phase II database (International HapMap Consortium), we selected SNPs with minor allele frequencies of more than 10% that covered most haplotypes in the *THBS1* and *THBS2* genes. We selected 11 tag SNPs, two in *THBS1* and nine in *THBS2*. Together, these SNPs covered more than 80% of the alleles in each gene that had an r^2 value of 0.8 or greater. We first genotyped the tag SNPs in 188 individuals with LDH and 188 control individuals. We performed χ^2 tests for genotype, dominant, recessive, and allele-frequency models and identified three SNPs in *THBS2* showing nominal p values of less than 0.05 in any of the four models (data available from authors). We found no association in *THBS1* tag SNPs. Next, we genotyped the nine *THBS2* tag SNPs in additional 337 LDH cases and 376 controls. In a total of 525 cases and 564 controls (Japanese A population), we found one SNP (rs6422747) that was significantly associated with LDH (Table 1).

To locate the disease-causing sequence variation in *THBS2*, we resequenced in and around all exons of the gene and its promoter sequence (approximately 1 kb) in 24 LDH individuals and identified a total of 15 sequence variations other than two tag SNPs. We selected five of these by excluding SNPs that were in linkage disequilibrium (LD) with the landmark SNP (rs6422747), having an r^2 value of less than 0.1. We genotyped them for the Japanese A population and identified one SNP (IVS10-8C → T, rs9406328) that had the most significant association with LDH (χ^2 = 16.85, p = 0.000040; comparison of allele frequency, Table 1). The association was significant even after Bonferroni's correction of multiple testing (corrected p = 0.00064).

We estimated LD across the *THBS2* region by using genotyping data from control samples (n = 564, data available from authors). We then examined haplotypes based on the seven SNPs containing the SNP IVS10-8C → T. Six haplotypes with frequencies of more than 0.01 represented more than 95% of both the case and control populations. The haplotype associations were far less significant than that of the SNP IVS10-8C → T alone (Table 2). Therefore,

Table 1. Association of SNPs in *THBS2* with Lumbar-Disc Herniation

			LDH				Control				Test for Allele Frequency	
dbSNP ID	Assay[a]	Location	11	12	22	Sum	11	12	22	Sum	p Value[b]	Odds Ratio[c] (95% CI)
rs7382358	T	IVS4	367	142	16	525	342	201	21	564	0.0032	1.38 (1.11–1.72)
rs9379341	T	IVS5	266	212	47	525	251	246	67	564	0.023	1.24 (1.03–1.48)
rs13192849	D	IVS6	398	114	13	525	377	167	19	563	0.0019	1.45 (1.15–1.83)
rs9283850	T	IVS9	396	124	5	525	437	121	5	563	0.42	0.90 (0.70–1.16)
rs9406328	D	IVS10	203	243	79	525	164	271	129	564	0.000040	1.43 (1.20–1.70)
rs6940420	T	IVS14	360	146	19	525	362	181	19	562	0.24	1.14 (0.92–1.41)
rs6422748	D	IVS14	130	266	128	524	195	261	107	563	0.00036	0.74 (0.62–0.87)
rs9505891	D	IVS14	275	209	41	525	236	255	73	564	0.000087	1.44 (1.20–1.73)
rs6422747	T	IVS14	130	266	128	524	195	261	108	564	0.00043	0.74 (0.62–0.87)
rs11966235	T	IVS16	367	140	17	524	390	157	17	564	0.84	1.02 (0.82–1.28)
rs12665573	T	IVS17	422	99	4	525	437	117	10	564	0.15	1.22 (0.93–1.59)
rs10945405	T	IVS21	162	267	96	525	148	286	128	562	0.035	1.20 (1.01–1.42)
rs9393165	D	IVS22	161	267	97	525	148	286	128	562	0.044	1.19 (1.00–1.41)
rs8089	T	3′-UTR	424	98	3	525	453	105	5	563	0.81	1.03 (0.78–1.37)

LDH, lumbar-disc herniation; CI, confidence interval. Allele 1 and allele 2 indicate the major and minor allele, respectively.

[a] T, Tag SNPs; D, SNPs discovered by resequencing.

[b] Not corrected for multiple testing.

[c] Odds ratio for allele 1 versus allele 2.

Table 2. Association of Haplotypes of the *THBS2* Region with Lumbar-Disc Herniation

SNP							Haplotype Frequency		
rs13192849	rs9283850	rs9406328	rs6940420	rs6422748	rs9505891	rs6422747	LDH	Control	p Value[a]
T	T	T	C	C	C	C	0.489	0.409	0.00017
G	T	C	C	G	G	T	0.130	0.181	0.0010
T	T	C	T	G	G	T	0.139	0.156	0.25
T	C	T	C	G	C	T	0.115	0.106	0.50
T	T	C	C	G	C	T	0.076	0.084	0.50
T	T	C	T	G	C	T	0.022	0.020	0.66

LDH, lumbar-disc herniation.
[a] Not corrected for multiple testing.

we considered the presence of a hidden causal SNP to be unlikely.

We analyzed potential confounding factors such as age, BMI, and sex to evaluate for pseudopositive associations with LDH. There was no significant difference in mean age, BMI, or sex distribution among genotypes of the SNP IVS10-8C → T, indicating that the SNP IVS10-8C → T in *THBS2* is an independent risk factor of LDH. By using genomic control methods,[20] we also assessed the stratification of the Japanese A population by genotyping 24 randomly selected SNPs. The significance of stratification (p) and the inflation factor (λ) were 0.98 and 1.00 (95^{th} percentile upper bound 1.53), respectively. Because the maximum factor (the highest χ^2 statistic/threshold χ^2 statistic) in this study is 1.56, it is unlikely that population stratification is the cause of our case-control association result.

To confirm the association, we genotyped the SNP IVS10-8C → T by using an independent Japanese case-control population consisting of 322 cases and 332 controls (Japanese B population). Significant association was replicated in this population (Table 3). Again, we saw no significant difference in mean age, BMI, and sex distribution among genotypes of the SNP IVS10-8C → T. In total, the SNP IVS10-8C → T was genotyped in 847 individuals with LDH and 896 control individuals, and our results clearly demonstrated a strong association with LDH in the Japanese population ($\chi^2 = 21.79$, $p = 0.0000028$; comparison of allele frequency, Table 3). The association was significant after Bonferroni's correction (corrected $p = 0.000045$).

To investigate potential effects of the SNP IVS10-8C → T on *THBS2* transcription, we performed RT-PCR with CS-OKB and OUMS-27 cells. The genotypes of the SNP IVS10-8C → T in these cell lines were C/C and T/T, respectively. Both cell lines contained a 418 bp PCR product in addition to the expected 592 bp product (Figure 2A). Sequence analysis revealed that the 418 bp product lacked exon 11 but retained the original reading frame. Further, we observed a greater amount of the 418 bp product in OUMS-27 than in CS-OKB, suggesting potential allelic differences in the rate of exon 11 skipping.

We followed up this initial observation in vivo by performing RT-PCR and allele-specific real-time PCR in 29 normal human fibroblast cell samples. Fourteen of the samples had the genotype T/T at the SNP IVS10-8C → T locus, 11 had T/C, and the remaining four had C/C. Our analysis revealed significant association between the genotype of the SNP IVS10-8C → T and the rate of exon 11 skipping in vivo (Figures 2B and 2C). The rate of exon 11 skipping for the T/T genotype was approximately 3.8-fold higher than that of C/C, confirming allelic differences in *THBS2* transcription.

Because exon 11 of *THBS2* encodes one of three THBS type 1 repeat (TSR) domains that interact with MMP2 and MMP9,[11] we examined the effect of exon 11 skipping on the interaction between THBS2 and MMP2 or MMP9. We performed immunoprecipitation experiments with S-tagged, recombinant partial human THBS2 proteins produced in *E. coli*. Both the wild-type TSR domains (WT-THBS2) and the exon-11-skipped TSR domains (Skip-THBS2) that lack the third TSR domain coprecipitated with MMP2 and MMP9

Table 3. Association of rs9406328, IVS10-8C → T, in *THBS2* with Lumbar-Disc Herniation

	LDH					Control						
	Genotype					Genotype					Test for Allele Frequency	
Population	TT	TC	CC	Sum	Allele T Frequency	TT	TC	CC	Sum	Allele T Frequency	p Value[a]	Odds Ratio (95% CI)
Japanese A	203	243	79	525	0.618	164	271	129	564	0.531	0.000040	1.43 (1.20–1.70)
Japanese B	106	173	43	322	0.598	92	170	70	332	0.533	0.018	1.30 (1.05–1.62)
Combined[b]	309	416	122	847	0.610	256	441	199	896	0.532	0.0000028	1.38 (1.21–1.58)

LDH, lumbar-disc herniation; CI, confidence interval.
[a] Not corrected for multiple testing.
[b] The sum of the two Japanese populations.

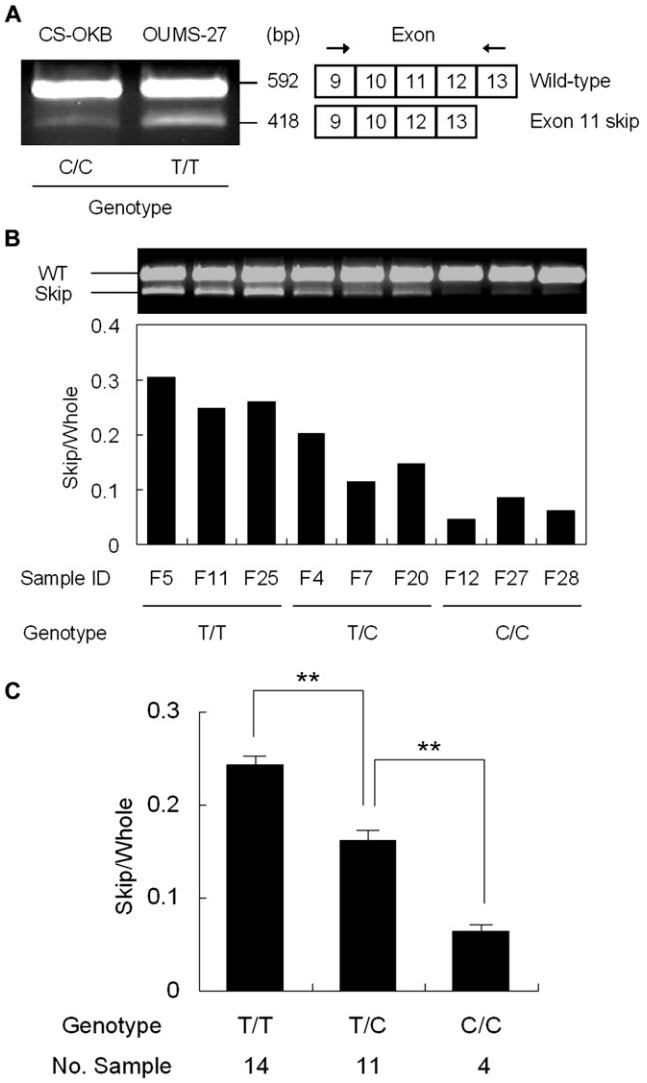

Figure 2. Effects of the LDH-Associated SNP, IVS10-8C → T, on *THBS2* Exon 11 Splicing In Vivo

(A) Left: RT-PCR products from CS-OKB and OUMS-27 cells representing the wild-type and exon-11-skipped transcripts. Right: patterns of exon skipping. Arrows indicate the positions of PCR primers.

(B) Rates of exon 11 skipping for each genotype, detected by RT-PCR (top) and allele-specific real-time PCR (bottom). Of the 29 normal human fibroblast cell samples examined (F1-F29), three representatives for each genotype are shown.

(C) Allelic differences in the rate of exon 11 skipping in 29 normal human fibroblast cells, detected by allele-specific real-time PCR. Data represent the mean ± SEM. ** indicates $p < 0.01$ (Student's t test).

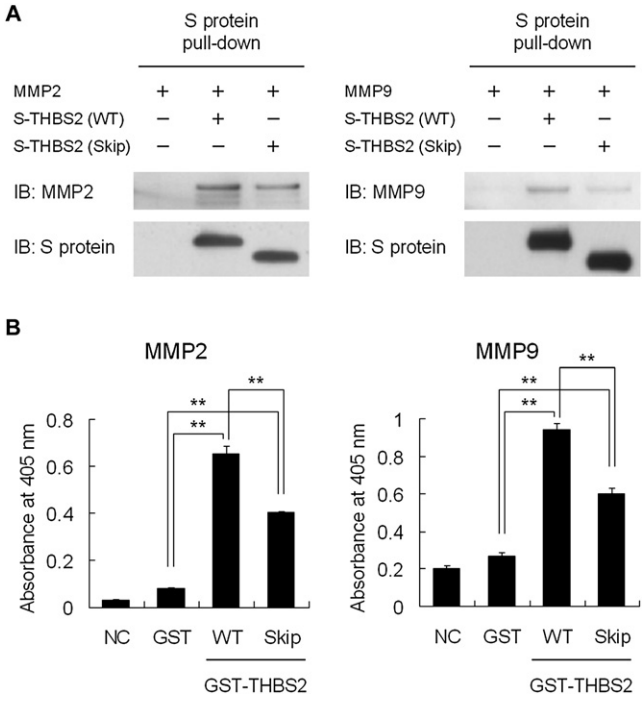

Figure 3. Effects of Exon 11 Skipping on the Interaction of THBS2 with MMP2 and MMP9 In Vitro

(A) S protein pull-down assay. S-tagged recombinant human THBS2 was incubated with purified human MMP2 (left) or MMP9 (right). MMP2 and MMP9 were detected with antibodies, and S-tagged THBS2 was detected with S protein HRP conjugate.

(B) Solid-phase binding assay. Microplate wells coated with GST or GST-tagged recombinant human THBS2 were incubated with or without purified human MMP2 (left) or MMP9 (right). Binding of GST-tagged THBS2 to MMP2 or MMP9 was quantified by colorimetric assay at $A_{405\ nm}$. Data represent the mean ± SEM in quadruplicate assays. ** indicates $p < 0.01$ (Student's t test). NC indicates noncoated.

(Figure 3A). Solid-phase binding assays confirmed that WT-THBS2 bound to MMP2 or MMP9 more strongly than did Skip-THBS2 (Figure 3B).

Binding of THBS2 with MMP2 and MMP9 implies a causal link of MMPs with LDH. Therefore, we evaluated whether *MMP2* and *MMP9* also are associated with LDH. We genotyped 15 tag SNPs in *MMP2* and *MMP9*, selected with the same criteria as for the *THBS* genes, in the Japanese A population (525 individuals with LDH and 564 controls). One missense SNP (Q279R, rs17576) in *MMP9* was strongly associated with LDH (Table 4). *MMP2* tag SNPs did not show any significant association. We resequenced in and around all exons of *MMP9* and its promoter sequence (approximately 1 kb) in 24 LDH individuals and identified a total of nine sequence variations. We genotyped the landmark SNP (rs17576) and other two SNPs that were in LD with the landmark SNP in 847 LDH cases and 896 controls (Japanese A and Japanese B populations). We found the most significant association in the landmark SNP (combined $p = 0.00049$; comparison for allele frequency, Table 4), which was significant even after Bonferroni's correction (corrected $p = 0.0083$).

The functional relationship between *THBS2* and *MMP9* raises the possibility of a combinatorial effect of these genes in LDH. We examined this possibility by combining genotype data on a pair of loci in 847 cases and in 896 controls. Significant combinatorial association was observed for *THBS2* (rs9406328) and *MMP9* (rs17576) with LDH. The genotype that was homozygous with respect to

Table 4. Association of rs17576, Q279R, in *MMP9* with Lumbar-Disc Herniation

	LDH					Control						
	Genotype					Genotype					Test for Allele Frequency	
Population	GG	GA	AA	Sum	Allele G Frequency	GG	GA	AA	Sum	Allele G Frequency	p Value[a]	Odds Ratio (95% CI)
Japanese A	265	203	57	525	0.698	234	250	80	564	0.637	0.0023	1.32 (1.10–1.58)
Japanese B	154	133	35	322	0.685	142	140	50	332	0.639	0.077	1.23 (0.98–1.55)
Combined[b]	419	336	92	847	0.693	376	390	130	896	0.637	0.00049	1.29 (1.12–1.48)

LDH, lumbar-disc herniation; CI, confidence interval.
[a] Not corrected for multiple testing.
[b] The sum of the two Japanese populations.

susceptible alleles of both SNPs showed the highest odds ratio (3.03; 95% confidence interval = 1.58-5.77, Table 5).

Discussion

Our genetic and functional data indicate that THBS2 is involved in LDH via the regulation of MMPs in the intervertebral disc ECM. MMP2 and MMP9 activities are mainly regulated by endocytosis, a general clearance mechanism for ECM. Endocytic clearance of MMPs is mediated by the scavenger receptor, low-density lipoprotein receptor-related protein (LRP).[25,26] THBS2 promotes LRP-mediated endocytosis of MMP2 and its subsequent lysosomal degradation.[25] Thus, THBS2 may play a major role in intervertebral disc ECM homeostasis as a modulator of MMP2 and MMP9 endocytosis.

MMP activity is also regulated at different levels with activation of the latent enzyme and inhibition by tissue inhibitor of metalloproteinase (TIMP). MMP2 and MMP9 mostly exist in tissues as latent enzymes that make complexes to their specific inhibitors, TIMP2 (MIM 188825) and TIMP1 (MIM 305370), respectively.[27,28] These MMP inhibitors, as well as other MMP regulators, could also be a candidate for LDH-susceptibility genes, although the clearance of MMP2-TIMP2 complex is THBS2-independent process.[29]

Our findings suggest that the third TSR in THBS2 plays an important role in its interaction with MMPs and in LDH pathogenesis overall. TSRs are small cystein-knot modules of approximately 60 amino acids, containing six conserved cystein residues as well as conserved tryptophan, serine, and arginine residues.[30] The TSR domain of THBS2 consists of three repeats and interacts with MMP2 and MMP9.[11] These three repeats share conserved cystein and tryptophan residues but differ in other amino acid positions, suggesting that each repeat functions differently. Several short motifs within TSRs have been reported to support protein-protein interactions.[31,32]

The susceptibility SNP in *MMP9* (Q279R) resides within the highly conserved gelatinase-specific fibronectin type II domains, which presumably enhance substrate binding.[33,34] This SNP has previously been reported to associate with metastasis of lung cancer[35] and trachoma;[36] however, its function is unknown. We can hypothesize that this nonconservative amino acid change affects enzyme activity by modifying its substrate binding capacity. Further characterization of the interaction between THBS2 and MMPs, as well as the identification of other genes functioning within this metabolic pathway, should reveal promising targets for treatment and novel therapeutic strategies for LDH.

Acknowledgments

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Table 5. Combinatorial Effect between *THBS2* and *MMP9*

	Genotype of *THBS2* (rs9406328)								
	LDH (n = 847)			Control (n = 896)			Odds Ratio (95% CI)		
Genotype of *MMP9* (rs17576)	TT	TC	CC	TT	TC	CC	TT	TC	CC
GG	154	213	52	105	193	78	3.03 (1.58–5.77)	2.28 (1.21–4.27)	1.38 (0.69–2.75)
GA	123	159	54	116	186	88	2.19 (1.14–4.18)	1.76 (0.94–3.32)	1.27 (0.64–2.51)
AA	32	44	16	35	62	33	1.89 (0.88–4.06)	1.46 (0.72–2.98)	1

LDH, lumbar-disc herniation; CI, confidence interval.

Web Resources

The URLs for data presented herein are as follows:

Applied Biosystems, http://www.appliedbiosystems.com/index.cfm

International HapMap Project, http://hapmap.org/

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/

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