A functional polymorphism in COL11A1 is associated with susceptibility to lumbar disc herniation


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INTRODUCTION
Lumbar disc herniation (LDH), one of the most common musculoskeletal diseases, causes sciatica and low back pain, which affect the activities of daily living of the patient at the same time, resulting in enormous economic burden to the society. Many risk factors of lumbar disc degeneration have been reported; however, its exact etiology and pathogenesis remain unknown. Extracellular matrix (ECM) molecules play important roles in the maintenance of the integrity and functions of the intervertebral disc. Through extensive case-control association studies of the candidate genes encoding ECM proteins in the intervertebral disc, we identified a single nucleotide polymorphism (SNP) in a gene “CILP” encoding cartilage intermediate layer protein that has a strong association with the occurrence of LDH. Objective of current study is to report another potent susceptible gene for LDH.

METHODS
All subjects were Japanese who visited participating hospitals and were diagnosed as LDH after thorough medical examinations. All patients had unilateral pain radiating from the back along the femoral or sciatic nerve to the corresponding dermatome of the affected nerve root with duration over 3 months. They underwent radiographic examination including routine X-ray and MRI and were found to have positive findings indicating LDH.

We obtained informed consent from all subjects. This study was approved by the ethical committees of RIKEN and participating hospitals.

Chi-square tests were used to compare cases with controls on allelic and genotypic frequencies; the odds ratio and 95% confidence intervals were calculated. A permutation test with 10,000 permutations was used to adjust the significance in the initial analysis of the association between the suspected SNP and LDH. Bonferroni correction for the number of comparisons was applied. MRI data, real-time PCR data and mRNA stability data were examined using Student’s t-test.

We processed and embedded intervertebral disc samples in paraffin by the AMeX method. We visualized the immunoreactive products using a DAB reagent and counterstained them with hematoxylin.

A large case-control association study of various SNPs in 823 cases of LDH and 841 healthy controls using the candidate gene approach were conducted. We selected sequence variations of SNPs from the International HapMap Project and the JSNP databases. For the first screening, we recruited 188 cases with LDD and 179 controls and genotyped tag SNPs in the candidate genes and identified three SNPs, showing p values of less than 0.05. The case group included 58 patients who had no herniation (disc degeneration only) and 130 patients with LDH. We identified additional sequence variations in a potent gene by direct sequencing of 230-kb region with DNA from 24 case individuals. Next, we genotyped these SNPs using independent 359 LDH cases and 286 controls. We estimated haplotype frequencies using the expectation-maximization algorithm and pair-wise linkage disequilibrium (LD) index. We finally identified a SNP that had the most significant association with LDH. To confirm the association, we examined another 359 LDH cases and 286 controls for the SNP and also identified the gene that include the SNP and confirmed the expression of the gene in the intervertebral disc by quantitative real-time PCR. We quantified the allelic difference by allele specific PCR and also undertook RNA stability assay to clarify the mechanisms of undertranscription.

RESULTS
A SNP (c.4603C>T; rs1676486) in COL11A1 that encodes one of chains of collagen type XI, an ECM which is highly expressed in the intervertebral disc, showed a significant association with the occurrence of LDH (P=0.000003). We examined whether confounding effects such as age and gender affect the associations with LDH and found no relationship between the genotype and these factors. The association was positive in both sexes. COL11A1 expression was decreased in the intervertebral disc of LDH patients compared to that of the control subjects, and the expression level of COL11A1 was inversely correlated with the degree of disc degeneration judged by MRI. We further analyzed the expression and localization of type XI collagen in intervertebral disc by immunohistochemistry. Normal discs had a highly uniform ECM structure with intense immunostaining of type XI collagen in the nucleus pulposus cells and ECM. In degenerative discs, however, we observed weak immunostaining of type XI collagen around the nucleus pulposus cells. These findings implicate a decrease of type XI collagen in the pathogenesis of LDH.

To clarify the functional impact of c.4603C>T, the mRNA expression level of each allele was compared by direct sequencing of the genomic DNA and cDNA from three LDH patients heterozygous for the allele. The expression level of the susceptibility c.4603T allele was lower than that of the c.4603C allele. We quantified the allelic difference by real-time PCR and confirmed the decreased expression of the susceptibility allele (T) in vivo.

To investigate the cause of decreased expression, we examined the stability of COL11A1 mRNA. The transcript containing the susceptible allele degraded faster than that without the susceptible allele.

DISCUSSION
We identified that a SNP in COL11A1 was one of disease susceptible genes of LDH. The unstable COL11A1 transcript degraded faster resulting in altered ECM function and might have lead to the occurrence of LDH. The 4856-4865 nucleotides in COL11A1 mRNA closely match the consensus for the mRNA stability motifs. The sequence variation might affect the mRNA stability motifs and disrupt a cis-element critical for mRNA stability. A few cis-elements have been implicated in mRNA stabilization. Alternatively, the sequence variation might induce a conformational change in the mRNA that would decrease mRNA stability or increase the sensitivity to RNase. Type XI collagen regulates the diameter of cartilage collagen fibrils. Its N-terminal non-collagenous region limits the appositional lateral growth of the fibril by blocking further accretion of type II collagen. Chondrodysplasia (cho) is an autosomal recessive disorder in mouse mutation of COL11A1. The collagen fibrils of cho mice are much thicker than normal. Mutations in type XI collagen cause various types of chondrodysplasias in human, which are collectively termed type XI collagenopathies, and all are complicated by abnormalities of the spine, including narrowing of the intervertebral disc. In particular, patients with Stickler syndrome have spondylar abnormalities and Schmorl’s node. These human mutations are in vivo evidence that, type XI collagen has a critical role in the organization of the supra-molecular architecture of cartilage collagen. Our results would lead to the understanding of the pathomechanism of LDH, which may lead to the development of novel therapeutic strategies for LDH.

Figure

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