Introduction
Lumbar disc disease (LDD) is one of the most common musculoskeletal disorders accompanied with intervertebral disc degeneration. Recent studies have identified possible susceptibility genes that are associated with LDD, such as COL9A2, COL9A3, MMP3, VDR, AGC1 and COL11A1. However, the etiology and pathomechanism of LDD are for the most part unclear. We have recently reported that CILP, encoding cartilage intermediate layer protein, shows a highly significant association with LDD (1). We also demonstrated that the CILP protein inhibits TGF-β-induced transcriptional activation of cartilage matrix genes in nucleus pulposus (NP) cells in vitro by binding to TGF-β1 and down phosphorylation of Smads. To further extend the study and to demonstrate the functional significance of CILP in vivo, we established transgenic mice that show differential expression of CILP in the intervertebral disc tissues and analyzed whether CILP is responsible for enhanced disc degeneration.

Materials and Methods
CILP vector constructs and generation of transgenic mice with differential expression in the intervertebral disc tissues.

Expression plasmids of the full-length mouse CILP cDNA were constructed using previously described Col1a2 promoter/IVS1 (2) that enables differential expression in the intervertebral disc tissues; 453mCILPInt induces CILP expression specifically in the NP of the intervertebral disc, whereas 742mCILP does not (Figure 1). DNA sequence encoding the influenza virus hemagglutinin (HA) epitope was fused in-frame with the 3’-end of the cDNA. Transgenic (Tg) mice were produced by microinjecting each of the inserts into pronuclei of fertilized eggs of C57BL/6 mice. Founder mice were identified by PCR analysis of genomic DNA extracted from the tail.

Analysis of CILP expression
Transgene expression in each mouse line was analyzed by RT-PCR. For CILP protein analysis, whole mouse tissue (13.5 d.p.c.) was homogenized and total proteins were collected using T-PER protein extraction kit (Pierce). Affinity chromatography was performed using HA-agarose (Sigma). The fractions were confirmed by silver staining. For CILP protein analysis, whole mouse tissues (13.5 d.p.c.) were homogenized and total proteins were collected using T-PER protein extraction kit (Pierce). Affinity chromatography was performed using HA-agarose (Sigma). The fractions were confirmed by silver staining.

Results and Discussion
Differential Expression of the transgene in the NP of the 453mCILPInt line and 742mCILP line was confirmed (Figure 1). Analysis of HA tagged CILP protein from 453mCILPInt mouse showed presence of full length and processed form of the protein as expected. (Figure 2).

Mice from each line developed normally without any apparent skeletal abnormality. We initially examined the mice until 5-month of age and soft X-ray analysis demonstrated no morphological differences in the spine or other skeletal components between the mouse lines. To further examine the spine in detail, MRI assessment was performed. MRI analysis of the lumbar intervertebral discs (L1/2- L5/6) indicated that the 453mCILPInt mouse showed significantly lower signal intensity of the NP where CILP was overexpressed (Figure 3a), suggesting an early deterioration of the intervertebral disc matrix after CILP expression.

Histological assessment further confirmed the presence of mild but enhanced lumbar disc degeneration. 453mCILPInt mouse tended to show shrinkage of NP, focal loss of the lamellar structure of the annulus with partial disruption of the end-plate. Image analysis of the histological section of the discs displayed a significant decrease of the staining intensity and NP area in the 453mCILPInt mouse compared to those in 742mCILP, again suggesting loss of proteoglycan and enhanced degeneration in the NP of the mouse overexpressing CILP (Figure 4a, b). Thus, current study indicated for the first time that CILP, a matrix protein encoded by a susceptibility gene for the LDD, was indeed responsible for the enhanced degeneration of the intervertebral disc.

Conclusion
Overexpression of CILP protein in the nucleus pulposus promotes disc degeneration and, therefore, CILP seems to be directly involved in the pathomechanism of the LDD.

References