INTRODUCTION:
Intervertebral disc (IVD) degeneration starts to occur in the second decade of life and is often first diagnosed by MRI of patients with clinical symptoms. The loss of intensity of NP in the T2 image is the typical finding of IVD degeneration; this reflects the decrease of water content that is typically associated with a loss of proteoglycan (PG), mainly aggrecan. [1]. It is also recognized that the rate of progression of disc degeneration differs at different levels in the lumbar spine; typically L4/5 is the most susceptible level. Although the mechanism of PG degradation in the IVD is not well established, two classes of proteinases, matrix metalloproteinases (MMPs) and aggrecanases (a disintegrin and metalloproteinase with thrombospondin motifs, ADAMTS) [6, 8] are believed to be involved in the degradation of aggrecan core protein. The two major sites of proteolytic cleavage by these enzymes are found within the interglobular (IGD) domain generating new C- and N- terminal aggrecan fragments [5]. Antibodies, known as neo-epitope antibodies, generated against these new C- and N-terminal amino acid sequences have helped immensely in studying aggrecan catabolism [2, 3]. Because these enzymes are secreted as pro-forms and are inhibited by proteinase inhibitors, the utilization of antibodies against the proteinases and the neo-epitopes they create shed light on the mechanisms responsible for matrix degradation. In the IVD tissue, most investigations of the involvement of MMPs and ADAMTSs [4, 7, 8] utilized mostly surgical specimens with attendant inflammatory cell populations. However, inter-subject variability makes the comparison among different grades of degeneration difficult. In this study, we have used human cadavers exhibiting different degrees of MRR, with grades of degeneration at different levels of the spine.

The purpose of this study was to examine a possible role for ADAMTS-4 in disc generation by measuring ADAMTS-4 protein and neoepitopes generated by aggrecanases at early (Grade 2) and later (Grade 4) stages of disc degeneration.

MATERIALS AND METHODS:
Isolation of the NP and AF: Human IVDs (5 donors, age 62-71, average 66) were dissected from cadaveric human spines, obtained from the regional organ bank within 24 hours of death, MRI scans were obtained. The degree of disc degeneration was graded using the Thompson scale [10]. To avoid inter-subject variability, a pair of samples from discs exhibiting grade 2 (early degeneration stage) and grade 4 (late degeneration stage) changes were collected from the same donor, frozen immediately in liquid nitrogen and stored in -80°C until further processed.

Protein and PG extraction: The frozen AF and NP tissues (30-50 mg wet weight) were pulverized in a cryopress (Microtech Nichion, Japan) and lyophilized and resuspended in appropriate buffer for protein or PG extraction with chondroitinase ABC, keratanase I and II [9] and separated on 4-10% SDS-PAGE (Camberx, MD). Immunoblotting was performed with antibodies BC-3 (Abcam, Inc, MA; against a neo-epitope ARGSV) and JSCNIT (against neo-epitope NITEGE) for both NP and AF tissue.

RESULTS:
Immunoblot analysis for ADAMTS-4 protein: In both AF and NP (figure 1, A and D) active ADAMTS-4 protein levels (68kd) were significantly elevated in grade 4 tissues when compared to the enzyme levels in grade 2. The pro-form of ADAMTS-4 (98Kd) was also seen in AF and NP. The inter-subject variability was large but the differences within the same donor were consistent.

Immunoblot analysis for aggrecan fragments generated by aggrecanases: In AF tissue (Figure 1, B and C), the ARGSV sequence, detected with BC-3 was observed in both grades 2 and 4 of disc degeneration. Also, the NITEGE sequence, detected with JSCNIT, was found at a higher level in grade 4 than in grade 2 discs.

In the case of NP tissue (Figure 1, E, and F), both neo N-terminal (ARGSV) and C-terminal (NITEGE) aggrecan fragments were observed at higher levels at the later (grade 4) than earlier (grade 2) stage of disc degeneration.

DISCUSSION: We report here that active ADAMTS-4, detected by immunoblotting, is present at a higher level in human AF and NP at late (grade 4) rather than in early (grade 2) stages of disc degeneration within the same individual. In the NP, aggrecan fragments (NITEGE and ARGSV), resulting from cleavage of aggrecan by aggrecanases, were also observed at a higher level in the later stage of IVD degeneration. In the AF, NITEGE fragments were found at higher levels in grade 4, whereas, levels of ARGSV fragments were not markedly different at early and late stages of disc degeneration. These results are consistent with a role for ADAMTS4 in this process and suggest that aggrecanases act in a different way in NP and AF tissues. However, because the retention time of fragments and enzymes in the IVD is not known, further research on mechanisms involved in enzyme activation and inhibition studies are essential before one can elucidate the specific contribution of ADAMTS-4 and other aggrecanases to IVD degeneration.

REFERENCES:

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