INTRODUCTION: Intervertebral disc (IVD) degeneration has been identified in approximately 40% of low back pain diagnoses [1]; however, the understanding of the mechanism of its pathogenesis is currently limited. It is believed that degeneration occurs during normal aging in conjunction with genetic predisposition. Other factors, such as mechanical stress, may influence the process of disc degeneration through an imbalance of cellular metabolism [2]. Previous studies showed the increased expression of cytokines [3, 4] and matrix-degrading enzymes [5, 6] and changes in growth factor expression [7] at the protein level using surgical or donor samples. However, differences among the donors and/or patients make data interpretation difficult. Therefore, the analysis of disc tissues from two distinct levels within one spine showing different degrees of disc generation may shed light on the cellular status at each degeneration stage by a pair-wise comparison [6].

The purpose of this study was to compare the expression of a panel of genes in paired samples with early and advanced disc degeneration from the same donor and to elucidate the role of each of the genes. The target genes included matrix molecules (aggrecan, collagen type I and II, and decorin), matrix-degrading enzymes (matrix metalloproteinase-3 (MMP-3), MMP-2 and ADAMTS4) and those that are regulating cellular homeostasis of disc cells, such as cytokinins (interleukin-1β (IL-1β), IL-6, and tumor necrosis factor-α (TNF-α)) and the bone morphogenetic protein families (BMP-2, -4, and -7).

MATERIALS AND METHODS: Donors and Sample Preparation: Over 30 cadaveric donor spines were obtained from a regional organ bank within 24-48 hours of death. The gross morphology of each disc was graded by the Thompson grading scheme after MRI T2 weight imaging. A total of 10 donor spines (with an average age of 53 yrs (7-male, and 3-female), specifically exhibiting the same donor and to elucidate the role of each of the genes. The target genes included matrix molecules (aggrecan, collagen type I and II, and decorin), matrix-degrading enzymes (matrix metalloproteinase-3 (MMP-3), MMP-2 and ADAMTS4) and those that are regulating cellular homeostasis of disc cells, such as cytokinins (interleukin-1β (IL-1β), IL-6, and tumor necrosis factor-α (TNF-α)) and the bone morphogenetic protein families (BMP-2, -4, and -7).

Quantitative PCR (q-PCR): Total RNA was isolated from annulus fibrosus (AF) and nucleus pulposus (NP) tissues (RNAeasy, Qiagen, CA) and q-PCR performed using the gene-specific primers for matrix components-aggrecan, collagen types I and II and decorin; cytokines-IL-1β, IL-6, TNF-α; matrix-degrading enzymes-MMP-2, -3, ADAMTS4; and growth factors- BMP-2, -4, -7. Standards were made by cloning the PCR products into the pDrive vector using a PCR cloning kit (Qiagen, CA). The results are expressed as molecules/ml and normalized by copy number of 18s rRNA as the internal control.

Statistical Analysis: Data are presented as mean ± SEM. The effect of disc grade (early and advanced) was analyzed by the Mann-Whitney U test (p=0.05).

RESULTS: Matrix components (Fig. 1): Expression levels of aggrecan were significantly lower in the advanced group of both tissues (p<0.01). Contrary to our expectation, the expression of aggrecan was higher in the AF than in the NP (p<0.01). Collagen type I and II expression showed similar trends, except in the advanced group of the NP. Both types of collagen expressions were higher in the NP than the AF (Fig. 1).

Decorin expression was increased as discs degenerated both in the AF and NP (Cytokine Expression (Fig. 2, top)): In the AF, the expression of IL-1β was higher in the advanced group than in the early group; this pattern was reversed in the NP. Interestingly, TNF-α and IL-6 showed a reverse pattern of gene expression with degeneration grade. Expression of Matrix-degrading enzymes (Fig. 2, middle): The expressions of MMP3 and ADAMTS4 (TS4) were elevated significantly in the advanced group in the AF (p<0.01), whereas the expression was significantly higher in the early group in the NP (p<0.01). MMP-2 was significantly upregulated in the advanced group in both the AF and NP (p<0.01).

Expression of BMPs (Fig. 2, bottom): In the AF, the expression of BMP-2 and BMP-4 was significantly higher in the early group than the advanced group (p<0.01); BMP-7 showed the opposite result. In the NP, BMP-2 and BMP-7 expression was significantly higher in the advanced group (p<0.01), while the results for BMP-4 expression were reversed.

DISCUSSION: Although a number of cytokines have been implicated, in particular IL-1 and TNF-α, as contributors to disc degeneration, the expression pattern of these genes in the different stages of disc degeneration is not well understood. Our data on a comprehensive analysis of four matrix genes and nine genes that contribute to cellular metabolism indicated that the levels of expression differ in the different stages of disc degeneration, and also in the AF and NP. For the matrix gene analysis, the expression level of collagen type I was higher in the NP; that may suggest the phenotypical change of NP cells in the advanced stage of disc degeneration. The increase in decorin expression supported the previous finding [8]. It was interesting that the high expression of IL-1β was observed at the early stage of disc degeneration and decreased as degeneration advanced, while TNF-α expression increased in the advanced stage. This sequence of expression of cytokines may suggest that the proper application of cytokine inhibitors at specific stages of degeneration is required.

Because the sampling of tissue can only be performed 24-48 hours after death of the donor, our concern was that mRNA expression status may vary significantly among donors. However, the differences in mRNA expression levels of biomolecules among individual donors after normalization with 18s rRNA were relatively consistent. Importantly, our pair-wise comparison was advantageous in analyzing the gene expression pattern between two different stages of disc degeneration within one donor. Further studies comparing protein content with gene expression and matrix fragmentation pattern may further advance our knowledge of human disc degeneration.