Increased collagen accumulation and enrichment of TGFβ pathways in the mouse caudal intervertebral disc following growth rod instrumentation

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INTRODUCTION: Early-onset scoliosis can negatively impact quality of life and, if not treated, may shorten life expectancy; treatment often involves growth rod instrumentation to correct spinal curvature in skeletally immature patients (1). The most common surgical intervention is the use of distraction-based growing rods, which allows the spine to be lengthened by the surgeon during followup. While efficacious initially, the spine may prematurely stiffen to such an extent that lengthening is no longer possible, resulting in a state known as autofusion. Defined as the spine stiffening post-rod insertion, autofusion occurs in as many as 90% of patients within five years and can lead to limited thoracic height, diminished lung capacity, impaired pulmonary function, and reduced quality of life (2). Yet, the biological mechanisms of how the spine respond to growth rod loads remain unknown. Thus, the overall objective of this study is to examine the matrix and transcriptomic changes in the IVD of the juvenile spine subjected to growth rod like loads.

METHODS: 6-week old female C57BL/6 mice were used for this study (n=8/group). We utilized a murine system that models the growth rod instrumentation and the associated distraction forces. Human females are more likely to develop early onset and adolescent scoliosis requiring surgical intervention, and female mice were used to align with clinical observations. In the experimental group metal pins were placed in two vertebrae and used to anchor 3D printed washers. Two screws then linked the two washers and guided overlaying springs to distract the Co7/8-Co12/13 caudal spinal segments (Fig 1A). The segments within the springs were 'distracted,' while the adjacent segments in the same animal were 'non-distracted.' The total distraction force was 2x body weight of the mouse, and this instrumentation was maintained for 20 weeks. Sham animals were instrumented with metal pins / washer construct, but without the spring or the screw. Structural changes, measured by the Disc Height Index (DHI), of the spinal segment were assessed though longitudinal x-ray and (normalized by non-distracted segments). After sacrifice, contrast enhanced microCT, dynamic cyclic compression, assays for sulfated glycosaminoglycan (sGAG) and collagen (n = 4), and bulk RNA sequencing (n=3) were compared between the distracted and non-distracted IVDs. One way ANOVA was used to compare Sham/Spring animals, and paired t-tests were used to compare non-distracted/distracted IVDs.

RESULTS: For the first two weeks DHI of the growth rod instrumented group was higher than the sham group (Fig 1B). The forces placed on the distracted segments transitioned from tension to compression between 4-6 weeks as the spine grew as the spring lengths remained constant (3). When the growth exceeded the original length of the springs, the distracted segments experiences compression, and this is evidenced by the lower DHI from 7-weeks and onwards. The whole disc volume of distracted discs was lower than non-distracted discs ($p = 0.0002$). This is accompanied by lower annulus fibrosis volumes ($p = 0.001$), and nucleus pulposus volumes ($p = 0.0187$). The sGAG content per disc was higher in the non-distracted discs ($p = 0.0350$). The collagen content in the IVD trended lower in the non-distracted discs ($p = 0.0547$) (Fig1C). RNA sequencing identified upregulation of genes involved in TGF beta signaling, mechanical stimulus response, and inflammation including Acta1, Ttn, Atp1a2, Tpm1, Map3k20, Csrp3, Lrrc32 and Mef2c (Fig1D). The Gene Ontology analyses revealed the upregulation of key pathways including TGFβ signaling; inflammatory responses; mechanical responsiveness; and collagen formation (Fig1E).

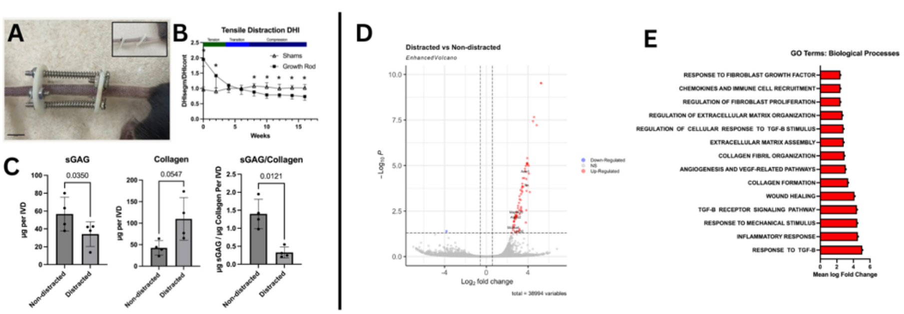

Figure 1: (A) Image of instrumentation for growth rod instrumented and sham animals (B). Disc Height Index of both experimental conditions taken biweekly from the time of instrumentation, and it showed the transition from tensile distraction to compression likely occurs within the 3–7 week period. (C) Quantification of sulfated glycosaminoglycans, and hydroxyproline in the distracted and non-distracted IVDs of the growth rod instrumented group. The distracted IVDs had less GAGs, more collagen, and diminished ratio of GAGs/collagen. (D) Volcano plot highlighting upregulated genes in distracted IVDs relative to non-distracted IVDs. (E). Gene ontology analyses reveals the significantly enriched pathways in the distracted IVDs.

DISCUSSION: This study aimed to characterize changes in disc morphology and connective tissue composition after a regimen of tensile distraction and compression mimicking growth rod treatment for scoliosis. Previously, we have found that sustained tension applied to the IVD did not appear to adversely affect the IVD structure, representing an ideal scenario of growth rod treatment where the spine can be placed under prolonged tension with weekly adjustments. In contrast, this study applies an initial tension that transitions to compression. This loading regimen may be more relevant to the spinal stiffening observed clinically in cases of autofusion. The diminished volumes of the distracted IVDs indicate that prolonged compression can constrain growth, despite the greater DHI during the tensile regime of the instrumentation period. Additionally, the proteoglycan content was decreased and collagen content of distracted discs increased after the entire period of growth rod instrumentation indicating a reduction in IVD hydration and potential stimulation of collagen deposition. Gene ontology analyses from unbiased bulk RNA sequencing indicated enrichment of key pathways including TGFb signaling, inflammation, mechanical response, and collagen formation. Since TGFβ has been shown to be a potent driver of fibrosis, it is likely that the collagen accumulation in the distracted IVD is orchestrated by TGFβ signaling as well as other inflammatory pathways. Increases in collagen content have been shown to occur in degenerative discs, potentially as an attempt to repair disrupted tissue. Further research is needed to establish a potential incidence of stiffness in the juvenile spine after growth rod distraction treatment arising from mechanisms of fibrosis.

SIGNIFICANCE/CLINICAL RELEVANCE: This research demonstrates that a murine model of growth rod distraction may lead to the development of fibrosis and collagen dysregulation in the intervertebral disc.

REFERENCES: 1. Menapace et al. *Spine Deform*. 2024. 2. Cahill et al *Spine* 2010. 3. Salari et al *J Bone Joint Surg Am*. 2025.

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