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Short communication

Lubricin deficiency in the murine lumbar intervertebral disc results in elevated torsional apparent modulus

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ABSTRACT

The purpose of this study was to investigate the mechanical consequences of proteoglycan 4 (Prg4) deficiency on intervertebral disc mechanics using a Prg4 knockout mouse model. Prg4, also called lubricin, was first identified as the boundary lubricant in synovial fluid but has subsequently been localized within a number of musculoskeletal tissues in areas subjected to shear and tensile stresses, including the intervertebral disc. The function of lubricin in the intervertebral disc has not been determined. Lumbar level 1–2 vertebral body-disc-vertebral body motion segments were isolated from Prg4 null mice and wild type (WT) litter mate controls. Disc dimensions were measured and motion segments were tested in axial loading and torsion. Torque measurements and disc dimensions were used to calculate the torsional apparent modulus for discs from Prg4 null and WT discs. Discs from Prg4 null mice had a significantly smaller mean transverse disc area ($p=0.0057$), with a significantly larger proportion of this area occupied by the nucleus pulposus ($p<0.0001$), compared to WT specimens. Apparent torsional moduli were found to be elevated in Prg4 null lumbar discs compared to WT controls at 10–10° ($p=0.0048$) and 10–30° ($p=0.0127$) rotation. This study suggests a functional role for Prg4 in the murine intervertebral disc. The absence of Prg4 was associated with an increased apparent torsional modulus and the structural consequences of Prg4 deficiency in the intervertebral disc, with expansion of the area of the nucleus pulposus relative to the transverse disc area in Prg4 null specimens.

1. Introduction

Synovial joints and intervertebral discs are uniquely adapted for the demands of their specific articulations (Pattappa et al., 2012). An interesting similarity between synovial joint articular cartilage and intervertebral discs is the selective localization of the mucinous glycoprotein lubricin (Shine et al., 2009; Shine and Spector, 2008). Lubricin, also called superficial zone protein (SZP) or proteoglycan 4 (Prg4), was first identified as the boundary lubricant in synovial fluid (Rhee et al., 2005). In synovial joints, lubricin is produced by superficial zone chondrocytes and synovial cells; it is present in synovial fluid and deposited as a protective layer over the articular cartilage surface and synovium (Rhee et al., 2005). Lubricin is also present within the superficial zone of articular cartilage (Ateshian and Hung, 2005). It has been proposed that lubricin facilitates microscale lubrication between collagen fibrils when the surface is subjected to compression and shear. This hypothesis is supported by the selective localization of lubricin in other areas of elevated shear and tensile stresses: the collagen bundles throughout the entire knee meniscus, the tendon fascicles in digital flexor tendons, and the rotator cuff (Funakoshi et al., 2010; Sun et al., 2006; Zhang et al., 2011).

Lubricin has also been identified in the lumbar intervertebral discs of goats (Shine and Spector, 2008) and humans (Shine et al., 2009). Notably, while lubricin immunostaining in caprine discs identified lubricin primarily in the outer annulus fibrosus (Shine and Spector, 2008), lubricin was identified in the cells, matrix, and tissue surfaces of the annulus and nucleus pulposus in human intervertebral discs (Shine et al., 2009). The consequences of

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lubricin deficiency for the functional integrity of the intervertebral disc, and the potential role of insufficient lubricin in degenerative disc disease, have not been investigated.

Camptodactyly–arthropathy–coxa vara–pericarditis (CACP) syndrome is an autosomal recessive syndrome resulting from loss of function mutations of Prg4, the gene coding for lubricin. Patients affected with CACP have normal-appearing joints at birth but develop early joint degeneration (Bahabri et al., 1998). In a longitudinal case series of patients affected by CACP, the development of scoliotic and kyphotic deformities were noted in some individuals in adolescence and adulthood (Faivre et al., 2000). Potential mechanisms by which lubricin deficiency might impact intervertebral disc mechanics include direct effects on tissue mechanical properties, accumulated damage in the setting of inadequate natural lubrication, and indirect effects on the disc from altered motion and loading in the setting of widespread, early onset degenerative spondylosis.

A Prg4 knockout mouse was developed to study the mechanisms by which lubricin deficiency results in the phenotype of CACP syndrome (Rhee et al., 2005). Prior studies using the Prg4 knockout mouse have focused on the consequences of lubricin deficiency in articular joints. The objective of this study was to investigate the mechanical consequences of lubricin deficiency on intervertebral disc mechanics using the Prg4 knockout mouse model. Our hypothesis was that the apparent torsional modulus is elevated in the intervertebral discs of young adult Prg4 null mice compared to wild-type (WT) littermates.

2. Materials and methods

Lumbar disc specimens were obtained from an active breeding colony of $Prg4^{-/-}$ mice maintained on the BL6 background strain. For mechanical testing, 26 male mice, 10 weeks of age, formed the two groups, $Prg4^{-/-}$ (KO) and $PRG4^{+/+}$ (WT) with $n=13$ specimens per group. Following harvest, each specimen was sealed in an airtight container and frozen at −20 °C until the day of testing. All animals were euthanized according to an Institutional Animal Care and Use Committee (IACUC)-approved protocol.

On the day of testing, each specimen was thawed for two hours at room temperature. The lumbar spine was dissected free (Fig. 1), and the lumbar L1–L2 disc and its adjacent vertebral bodies were identified. The posterior elements including the facet joints and surrounding soft tissues were dissected off of the sample to isolate a vertebral body-disc-vertebral body motion segment.

Mechanical testing was a destructive process. In order to estimate the dimensions of the nucleus pulposus relative to total disc dimensions, matched L1–L2 discs were obtained from another five Prg4 null and five WT 10-week-old male specimens. Disc height, transverse disc major axis, and transverse disc minor axis were measured using a digital caliper (Mitutoyo Corporation, Kawasaki, Japan). Discs were sectioned axially at midheight so that the nucleus pulposus transverse major and minor axes could be measured. These values were averaged for each group and the averaged values were used to estimate the dimensions of the nucleus pulposus relative to the total disc area in the tested specimens.

2.1. Mechanical testing

Following specimen preparation, the vertebral body-disc-vertebral body superior and inferior end plates were secured in the testing frame using cyanoacrylate glue (Super Glue, Pacer Technology, Rancho Cucamonga, CA). Specimens were kept moist by regular applications of normal saline. Mechanical testing was performed using an ELF 3200 material testing system (Bose, Framingham, MA). A 1.5 N m torsion load cell and 22 N axial load cell were used in series to apply a constant compressive force of 0.35 N (approximately 1.5 times mouse bodyweight) across the testing segment while the torque-rotation data were recorded. The torsional actuator was located at the base of the testing frame and rotated the inferior vertebra relative to the fixed superior vertebra under rotational angle control (Fig. 2). Prior to data collection, the motion segment was loaded with 0.35 N of compression and rotated from 5° to −5° for 34 cycles at 0.07 Hz to establish a reproducible torque-rotation response. A rotation from 10° to −10° was then exerted on the disc. Following a sequence of three rotations from 10° to −10°, a single rotation from 0° to 30° was performed while torque and angular rotation were recorded. Torsional modulus calculations were performed using the torque versus angular rotation data from the first rotation and the final rotation from 0° to 30° (Supplement Fig. 1).

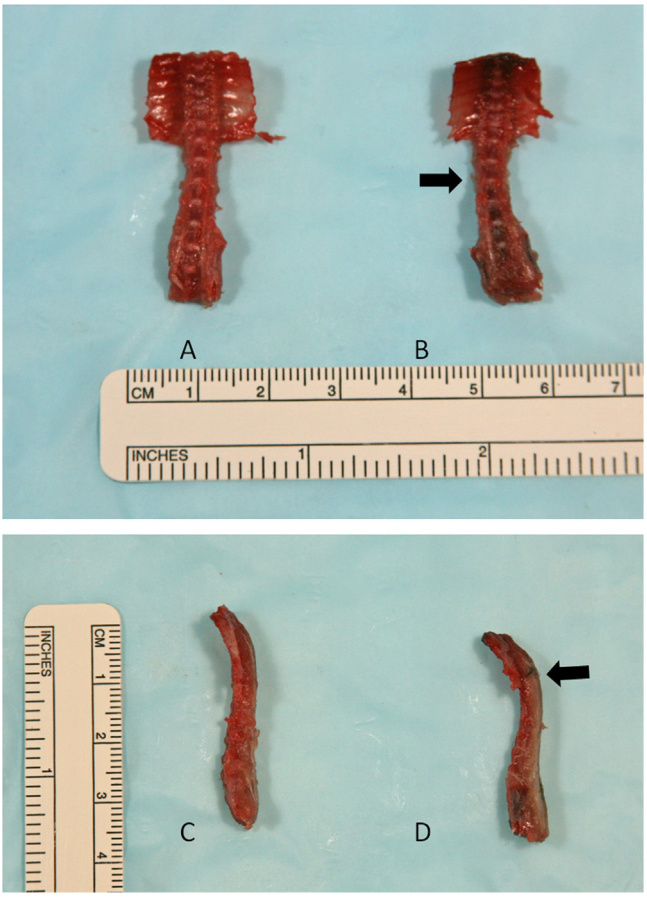

Fig. 1. Gross dissection of the thoracolumbar spine for 10-week-old male mice, WT (A, C) and $PRG4^{-/-}$ (B, D). Note the scoliosis (B, arrow) and kyphosis (D, arrow) of the $PRG4^{-/-}$ specimen.

Torsional apparent modulus was calculated according to the method of Elliott and Sarver (2004) from moment–angular deflection (M–Θ) curves that were constructed for the first 10° to −10° and the 10–30° data sets. Torsional stiffness (K, N mm/deg) was calculated by linear regression using the moment–angular deflection curves. Apparent torsional modulus (G^A, MPa) was calculated from torsional stiffness (K), disc height (h), and the polar moment of inertia of the disc (J, mm^4), where $G^A=Kh/J$ and $J=\pi([W_{AP}W_L^3-W_{AP}^3W_L]-[N_{AP}N_L^3+N_{AP}^3N_L])/64$. W_{AP} and W_L represent the anteroposterior and lateral disc diameters, and N_{AP} and N_L represent the diameters of the nucleus pulposus.

In the referenced method for calculating the polar moment of inertia (Elliott and Sarver, 2004), the nucleus pulposus was assumed to be 0.2 of total disc area. In this study, measurements were taken to estimate the dimensions of the nucleus pulposus relative to the area of the disc.

2.2. Statistical analysis

Generalized linear models for Gaussian data were used to compare genotypes on all dependent variables. Classical sandwich estimation was used to adjust for any model misspecification. Alpha was set to 0.05 per dependent variable.

3. Results

For specimens sectioned to calculate the relative volume of the nucleus pulposus, significant differences were observed between the $Prg4^{+/+}$ and $Prg4^{-/-}$ specimens in the dimensions of the disc, nucleus pulposus, and ratio of the area of the nucleus to the total disc area (Table 1). Significant differences were observed between the mechanically tested $Prg4^{+/+}$ and $Prg4^{-/-}$ discs in transverse disc area ($p=0.0057$), transverse disc major axis ($p=0.0045$), and disc height ($p=0.0002$) (Table 2). There was a trend towards difference in disc minor transverse axis dimensions ($p=0.0574$). Polar moment of inertia for the annulus, which predicts an object's