Lubricin/*PRG4* Expression in Equine Flexor Tendons is Greater in Intrasynovial Regions and in Tendon Injury

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INTRODUCTION: Equine superficial digital flexor tendinopathy (SDFT) is a valuable model for human exercise-induced Achilles tendinopathy due to shared structure, function, and pathology.[1,2] Both are energy-storing tendons exposed to high cyclic loads, with limited vascularity, poor intrinsic healing capacity, and a high rate of injury after clinical recovery. Naturally occurring equine SDFT injury demonstrates hallmark features of human Achilles tendinopathy—including collagen disorganization, tenocyte hypercellularity, neovascularization, and matrix degeneration—making it a robust translational model for studying disease mechanisms and therapies.[3] In addition, equine flexor tendons are partially contained within tendon sheaths, and intrasynovial tendon injuries can be especially challenging to treat due to the potential for persistent non-healing tendon tears, tenosynovitis, and adhesions formation. Lubricin/proteoglycan 4 (*PRG4*) has been studied in human, equine, canine and ruminant tendons. Human patients with camptodactyly-arthropathy-coxa vara-pericarditis syndrome caused by a lack of functional *PRG4*/lubricin, lose tendon elasticity and may develop digit contracture.[4] In equine tendon, lubricin staining is reported to be highest in the interfascicular matrix of the SDFT; however, *PRG4* expression and immunostaining has not been evaluated in injury or between intra- and extrasynovial regions within the flexor tendons.[5] Therefore, the primary objective of this study was to describe *PRG4* gene expression, synovial fluid lubricin concentrations, and lubricin immunostaining in healthy equine digital flexor tendons and tenosynovial structures. A secondary objective was to compare synovial fluid lubricin concentrations and tissue distribution in healthy and injured tendons and tendon sheaths.

METHODS: Tissues were collected *postmortem* from six adult (4 to 6-year-old) female horses euthanized for reasons unrelated to this study and without evidence of tendon injury (convenience sample). Superficial and deep digital flexor tendons (DDFT), manica flexoriae, and synovial membrane samples were obtained from 5 proximo-distal locations**,** flash frozen and stored at -80°C for RNA isolation or fixed in 10% formalin for histology and immunohistochemistry (IHC). Synovial fluid was obtained from 16 healthy horses, in addition to 11 horses diagnosed with tendinopathy or tendon sheath lesions ranging in age from 4 to 24-years-old, including 3 females, 1 intact male, and 7 castrated males. Tendon and synovial membrane tissue samples were also obtained from a subset of 3 horses within the injury cohort that were euthanized due to the severity of their injuries. This study was performed with IACUC approval and, where appropriate, owner consent. Hematoxylin and eosin (H&E) staining was performed to assess histologic architecture, and lubricin immunostaining (mAb 9G3) was scored on a 0-3 scale. Synovial membrane samples were graded using the OARSI criteria, and tendon samples were scored using a modified Bonar scale by a boarded veterinary pathologist.[6,7] *PRG4* gene expression was quantified by qRT- PCR, and synovial fluid lubricin was quantified using a custom sandwich ELISA. Paired histology and gene expression data for the healthy cohort were evaluated using Wilcoxon matched pairs signed rank tests with Friedman post hoc tests. A mixed linear model was used to compare synovial fluid lubricin concentrations.

RESULTS: *PRG4* expression was detected in healthy tendon and tenosynovial tissues, with 5-fold greater expression levels in intrasynovial than extrasynovial tendon locations (p=0.009) and greater expression in the manica flexoriae compared to the SDFT or DDFT (p=0.02, **Fig. 1A,B).** Synovial membrane *PRG4* expression and synovial fluid lubricin concentrations were similar between healthy tendon sheath locations and healthy middle carpal joints (MCJ); however, synovial fluid lubricin concentrations were greater in injured versus healthy tendon sheaths (839.4 µg/mL ± 84.8 vs. 184.7 µg/mL ± 40.1, p=0.007, **Fig. 1C)**. Lubricin staining was detected in all tendons, with the most intense staining in peripheral as compared to central regions and intrasynovial compared to extrasynovial locations. Injured tendons had more intense lubricin staining than healthy tendons (IHC median score: 10 vs. 3), with strong staining detected in intra-fascicular collagen fibers at the site of a chronic 'core' SDFT lesion **(Fig. 2)**. Strong lubricin staining was also evident adjacent to and within the fibrillated tendon fibers of a linear deep digital flexor tendon (DDFT) tear and in one horse with mature fibrous adhesion tissue present between synovial membrane and intrasynovial tendon structures.

DISCUSSION: Lubricin was present in healthy tendon sheath synovial fluid at similar concentrations to those reported in healthy equine synovial joints, suggesting that lubricin may have similar roles in interfacial tendon lubrication.[8] Consistent with this hypothesis, lubricin immunostaining was most intense in the peripheral and interfascicular regions of non-injured tendons, with more intense staining in intrathecal as compared to extrathecal locations. Lubricin staining was more intense at sites of injury, including tendon surface fibrillation, adhesions, intra-tendinous 'core' lesions, and linear tears. Lubricin localization to cellular areas within these lesions suggests that *PRG4* may be produced by the surrounding tenocytes or immune cells. Lubricin is hypothesized to enhance interfascicular tendon gliding and promote healthy tendon function; thus, increased lubricin immunostaining within and adjacent to tendon lesions may represent a mechanism to restore tissue homeostasis and tendon function.

SIGNIFICANCE: This study motivates further investigation into lubricin expression and staining patterns in tendon injury, especially with respect to different injury types, locations and chronicity and as a function of age and sex. Mechanistic roles of lubricin/*PRG4* are understudied in tendon, especially its role in interfascicular regions and tendon 'core' lesions post-injury. Lubricin may have value not only as a biomarker for tendon and tendon sheath injury, but also as a potential treatment option to reduce tendon adhesions, limit tendon fibrosis, and improve tendon elasticity and interfascicular gliding.

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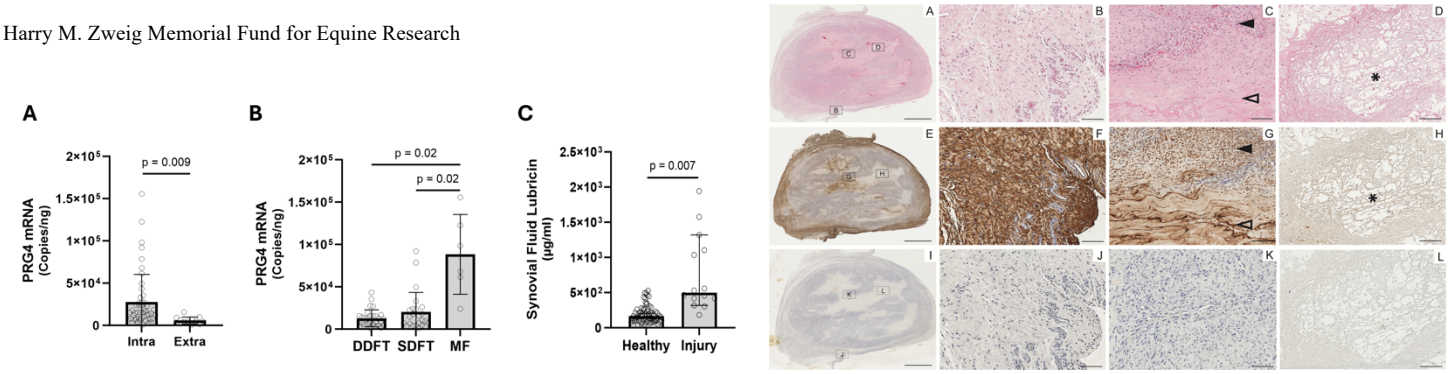

Fig. 1. *PRG4* expression was greater in intrasynovial than extrasynovial locations **(A)** and within the manica flexoriae (MF) as compared to the flexor tendons **(B)**. Lubricin concentrations were greater in tendon sheaths with injury than healthy tendon sheaths **(C). Fig. 2**. H&E **(A-D)** and lubricin IHC **(E-H)** images obtained from an injured SDFT with strong lubricin staining detected in intra-fascicular collagen fibers. Panels **(I-L)** show the absence of antigen-independent lubricin staining.