# Evaluation of Tendon and Ligament Microstructure in a Canine Model of Mucopolysaccharidosis I

Keerthana Iyer[1], Yian Khai Lau[1], Chet S. Friday[1], Michael W. Hast[1], George R. Dodge[1,2], Rahul Gawri[1,3], Margret L. Casal[1], Lachlan J. Smith[1]
[1]University of Pennsylvania, Philadelphia, USA; [2]MechanoTherapeutics LLC, Philadelphia, USA; [3]McGill University, Montreal, Canada
keerthana.iyer@pennmedicine.upenn.edu

**Disclosures:** Keerthana Iyer (N), Yian Khai Lau (N), Chet S. Friday (N), George R. Dodge (4-MechanoTherapeutics LLC), Michael W. Hast (N), Margret L. Casal (N), Rahul Gawri (8- JOR Spine, 9-ORS Spine Section), Lachlan J. Smith (8-JOR Spine, Connective Tissue Research, 9-ORS Spine Section, National MPS Society)

**INTRODUCTION:** Mucopolysaccharidosis (MPS) I is an autosomal recessive disorder caused by deficiency of lysosomal enzyme α-L-iduronidase (IDUA). This enzyme is required for the degradation of the glycosaminoglycans (GAGs) dermatan and heparan sulfate [1]. Abnormal accumulation of GAGs in cells leads to disease manifestation in multiple organ systems, which includes the musculoskeletal, central nervous, and cardiopulmonary systems. Amongst the musculoskeletal manifestations, abnormalities of the shoulders, elbows, hips, knees, ankles and small joints of the hands and feet are prevalent, negatively impacting patient mobility and independence [2]. Current systemic treatments for MPS I show limited efficacy for improving joint disease [3]. The anterior cruciate ligament (ACL) and Achilles tendon play critical roles in the stability and biomechanical function of the knee and ankle joints, respectively. However, few studies have examined the underlying pathological changes to these tissues in MPS I, and their contributions to progressive joint disease are poorly understood. Previously, using the naturally-occurring canine model, we demonstrated that cranial cruciate ligaments (CCLs, equivalent to ACLs in humans) from MPS I animals exhibit significantly lower stiffness and failure properties compared to those from healthy animals, implicating them in progressive joint dysfunction [4]. The goal of the current study was to elucidate the microstructural basis of these functional abnormalities by undertaking comprehensive histological characterizations of both the ACL and the Achilles tendon.

**METHODS:** With institutional IACUC approval, CCLs (n=6 MPS I and n=5 control) and Achilles tendons (n=2 MPS I and n=4 control) were isolated postmortem from the stifle (knee) and ankle joints, respectively, of 12 month-old MPS I and healthy control dogs. Tissues were fixed in 10% buffered formalin for 1 week and processed into paraffin. Sections 8 µm thick were cut from the mid-substance of each sample parallel to the fiber direction. Sections were stained with hematoxylin and eosin for cellularity, Alcian blue for GAGs or picrosirius red for collagen. Semi-quantitative grading was performed for both CCL and Achilles tendon sections using parameters adapted from previously established schemes [5, 6], including fiber fragmentation, fiber arrangement, rounding of cell nuclei, overall cell density, vascularity and GAG content. Each parameter was graded on a scale of 0 to 3, with 0 being normal and 3 being severely abnormal. Overall grade was calculated as the sum of individual parameters. Picrosirius red-stained sections were imaged using a polarizing microscope to determine the degree of collagen fiber disorganization quantified as circular standard deviation, using a custom Matlab program [7]. Statistically significant differences in parameters between MPS I and controls were calculated using Mann-Whitney U tests. Results are reported as median and interquartile range (IQR), with $p<0.05$ considered significant.

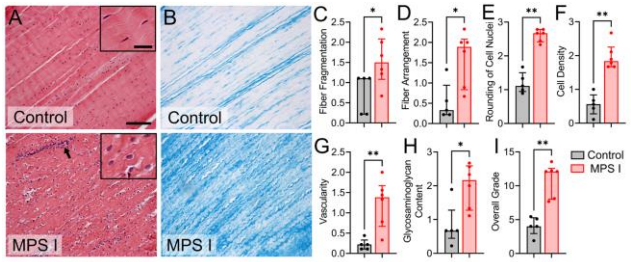


**Figure 1.** Representative histology of control and MPS I CCLs. **A.** Hematoxylin and eosin staining showing altered cellularity (inset), vascularity (arrow) and collagen fiber disorganization and **B.** Alcian blue staining showing elevated GAG content in MPS I CCLs. **C-I.** Semi-quantitative grading. *p<0.05; median/IQR; n=5-6; scale=100um (inset 20µm).

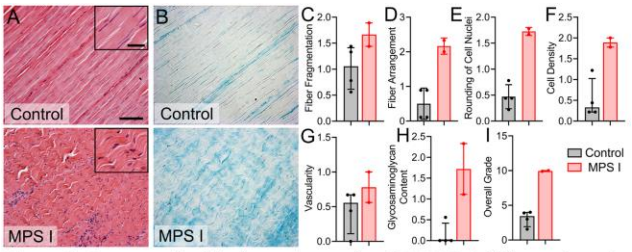


**Figure 2.** Representative histology of control and MPS I Achilles tendons. **A.** Hematoxylin and eosin staining showing altered cellularity (inset), vascularity and collagen fiber disorganization and **B.** Alcian blue staining showing elevated GAG content in MPS I tendons. **C-I.** Semi-quantitative grading. Median/IQR; n=5-6; scale=100µm (inset 20µm).

**RESULTS:** MPS I CCLs subjectively exhibited higher fiber fragmentation and disorganization, rounding of the cell nuclei, hypercellularity, vascularity, and GAG content compared to controls (**Figs 1A and B**). These findings were confirmed through semi-quantitative grading, with all parameters along with overall grade significantly worse for MPS I CCLs compared to controls (**Figs 1C-I**). With respect to the Achilles tendon, while the small sample size did not enable statistical evaluations, findings were similar to those for CCLs, with clear abnormalities with respect to cellularity, GAG content and microstructure (**Fig 2**). Additionally, circular standard deviation, a quantitative measure of collagen fiber disorganization, was higher for both MPS I CCLs ($p<0.05$) and Achilles tendons compared to controls (**Fig 3**).

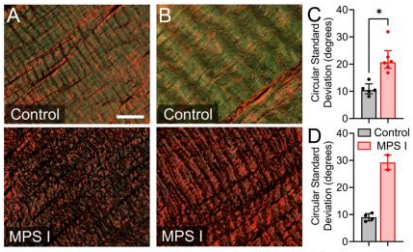


**Figure 3.** Representative polarized light histology of **A.** Control and MPS I CCLs and **B.** Control and MPS I Achilles tendons. **C.** Circular standard deviation. Picrosirius red stain; *p<0.05; median/IQR; n=2-6; scale=100µm.

**DISCUSSION:** There is lack of understanding of the roles of tendons and ligaments in progressive joint disease in MPS I, despite the critical role of these tissues in joint mechanical function. The increased GAG content observed is similar to that observed in other tissues in MPS I, as a result of IDUA deficiency. Notably, abnormal intrafibrillar GAG accumulation is frequently observed in pathologic tendons and ligaments in the general population [8]. Other microscopic abnormalities, including collagen fiber disorganization and hypercellularity, are similar to those seen in tendon overuse injuries in the general population [5, 6], and are a likely basis of the diminished mechanical properties we previously reported [4]. Pathological changes may be driven in part by elevated local inflammation and collagen breakdown, secondary to GAG accumulation [9, 10]. These findings demonstrate that pathological changes to both ligaments and tendons contribute to abnormal joint function in MPS I, and suggest that effective clinical management of joint disease in patients should incorporate treatments targeting these tissues.

**SIGNIFICANCE:** MPS I patients exhibit progressive synovial joint dysfunction resulting in pain and impaired mobility. Our findings implicate pathological changes to both tendons and ligaments in the etiology of joint disease in MPS I and highlight the need for improved treatments targeting these tissues.

**REFERENCES:** [1] Hampe+ Cells 2020; [2] Costi+ Diagnostic 2023; [3] Miller+ J Inherit Metab Dis 2023; [4] Lau+ ORS 2023; [5] Movin+ Acta Orthop 1997; [6] Astrom+ Clin Orthop. Relat. Res. 1995; [7] Freedman+ J Biomech 2013; [8] Han+ Nat Mater 2016; [9] Simonaro+ Am J Path 2008; [10] Natel+ J Inherit Metab Dis 2020.

**ACKNOWLEDGEMENTS:** Funding from the University of Pennsylvania and the NIH (R01AR071975, P40OD010939 and P30AR069619).