Mucopolysaccharidosis I and VII Dogs Exhibit Impaired Anterior Cruciate Ligament Mechanical Properties

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Introduction

The mucopolysaccharidoses (MPS) are a family of inherited lysosomal storage disorders characterized by deficient activities of enzymes that degrade glycosaminoglycans (GAGs) due to mutations in associated genes.[1] MPS I and VII are characterized by deficient α-L-iduronidase and beta-glucuronidase activities, respectively.[2,3] Both subtypes accumulate heparan (HS) and dermatan sulfate (DS) GAGs, while MPS VII additionally accumulates chondroitin sulfate (CS).[2,3] MPS I and VII patients commonly exhibit progressive skeletal abnormalities that are associated with chronic pain, impaired mobility, and overall decreased quality of life.[2,3] Synovial joint (e.g., hip, knee, hands and shoulder) disease manifestations are prevalent in both subtypes, which can be traced in part to a combination of developmental abnormalities and chronic inflammation, which accelerates soft tissue degeneration.[4,5] However, understanding of the underlying pathological mechanisms is limited, hampering the development of effective treatments. In particular, there is a poor understanding of how component joint tissues contribute to progressive mechanical dysfunction of the overall joint. The anterior cruciate ligament (ACL) performs a critical role in maintaining the rotational stability of the knee joint.[6] Therefore, the objective of this study was to establish whether ACL tensile mechanical properties are altered in MPS I and VII using the naturally-occurring canine disease models.

Methods

Animals and Sample Collection

The naturally-occurring canine models of MPS I and VII exhibit progressive synovial joint dysfunction similar to human patients, making them clinically-relevant platforms for studying joint disease pathophysiology. With IACUC approval, MPS I (n = 5) and heterozygous control (n = 4) dogs were euthanized at 12 months-of-age, and MPS VII (n = 5) and heterozygous control (n = 6) dogs were euthanized at ± months-of-age. Prior to euthanasia, all animals received physical examinations from a veterinarian. Left stifle (knee) joints were excised postmortem, all soft tissue between the distal femur and proximal tibia except the ACL was carefully removed, and ACL cross-sectional area was measured using a laser-based device.

Mechanical Testing

The femur and tibia were potted in polymethylmethacrylate, mounted in custom fixtures of a servohydraulic mechanical testing system (Instron 8874; Figure 1), and the ACL tested in uniaxial tension to failure. Briefly, following 10 cycles of preconditioning, samples were subjected to a quasi-static ramp to failure at a strain rate of 0.3%/second. Testing was conducted at room temperature and samples were sprayed with saline to prevent dehydration. The following parameters were calculated for all samples: stiffness, modulus, toughness, failure load, failure stress and failure strain.

Statistical Analyses

All results are reported as median and interquartile range. Significant differences in ACL tensile mechanical properties between MPS I or VII and their respective controls were established using Mann-Whitney tests ($p < 0.05$).

Results

MPS I Dogs

By 12 months-of-age MPS I dogs were able to ambulate unassisted but exhibited a hopping gait while running, with moderate effusions and laxity in stifle joints. With respect to ACL mechanical properties, stiffness, toughness, failure load and failure stress were all significantly lower for MPS I dogs compared to controls (65, 56, 45, and 72% of control, respectively, Figure 2), while modulus and failure strain were not significantly different.

MPS VII Dogs

In contrast to MPS I dogs, by ± months-of-age, MPS VII dogs were no longer able to ambulate, and exhibited severe joint effusions and laxity. With respect to ACL mechanical properties, stiffness, modulus, toughness, failure load and failure stress were all significantly lower for

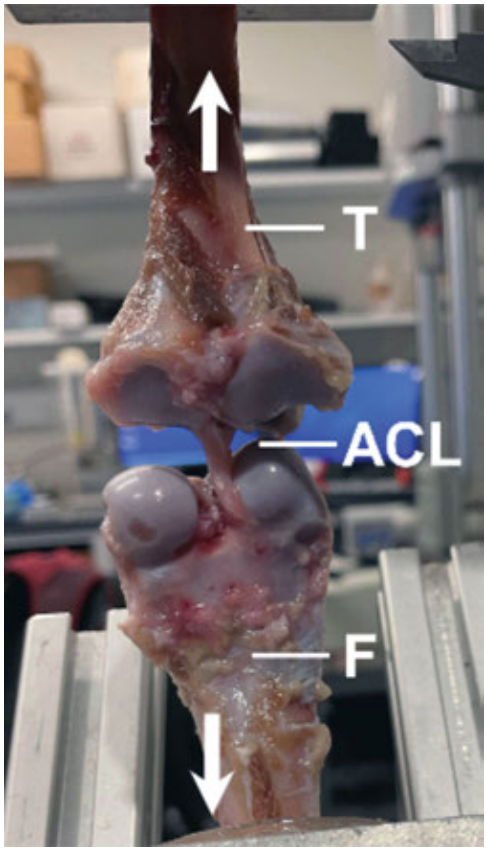

Figure 1. Canjne anterior cruciate ligaments (ACLs) were tested in uniaxial tension to failure (arrows indicate testing direction; F = femur; T = tibia)

MPS VII dogs compared to controls (20, 13, 26, 19 and 16% of control, respectively, Figure 3), while failure strain was not significantly different.

Discussion

In this study we established that impaired mechanical function of the ACL is a key feature of synovial joint disease in both MPS I and VII, implicating pathological changes in this tissue in the overall etiology of joint instability. While we are yet to determine associated changes at the structural and functional levels, they may include extracellular GAG accumulation and resulting abnormal collagen fiber organization, which could reasonably be expected to negatively impact tensile load-bearing capacity. Lower stiffness and modulus may contribute to joint laxity, which was observed clinically in MPS dogs, while diminished failure properties may increase the risk of ACL rupture. Interestingly, mechanical properties of ACLs from MPS VII dogs were significantly worse than those from MPS I dogs, despite the younger age of these animals. Potential explanations may include the fact that MPS VII dogs accumulate CS in addition to HS and DS, which may further exacerbate structural abnormalities. Additionally, prolonged limb disuse in MPS VII animals, which were unable to ambulate at all at the time of euthanasia, may have contributed to diminished ACL mechanical properties. Ongoing studies are focused on establishing the molecular mechanisms of ligament and other soft tissue degeneration in the synovial joints of MPS dogs

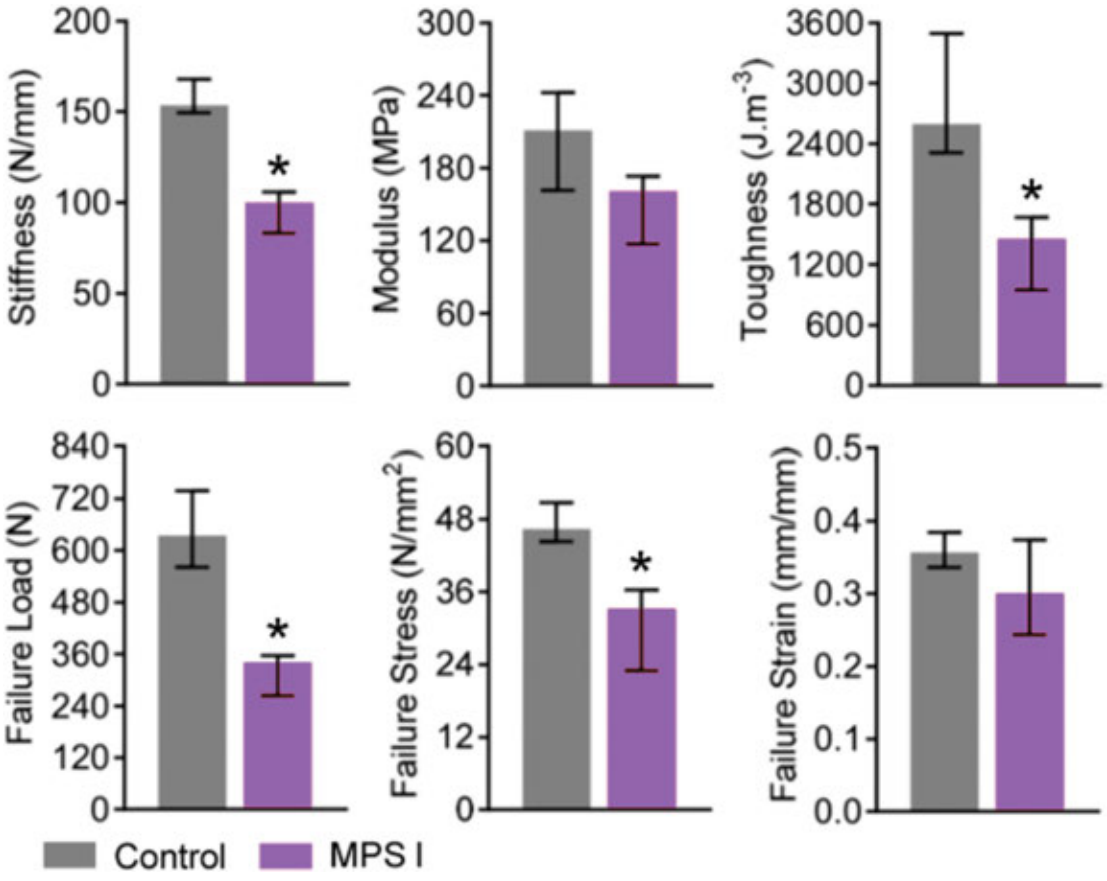

Figure 2. Tensile mechanical properties of anterior cruciate ligaments from control (n=4) and MPS 1 (n = 5) dogs at 12 months-of-age. *$p < 0.05$ vs control.

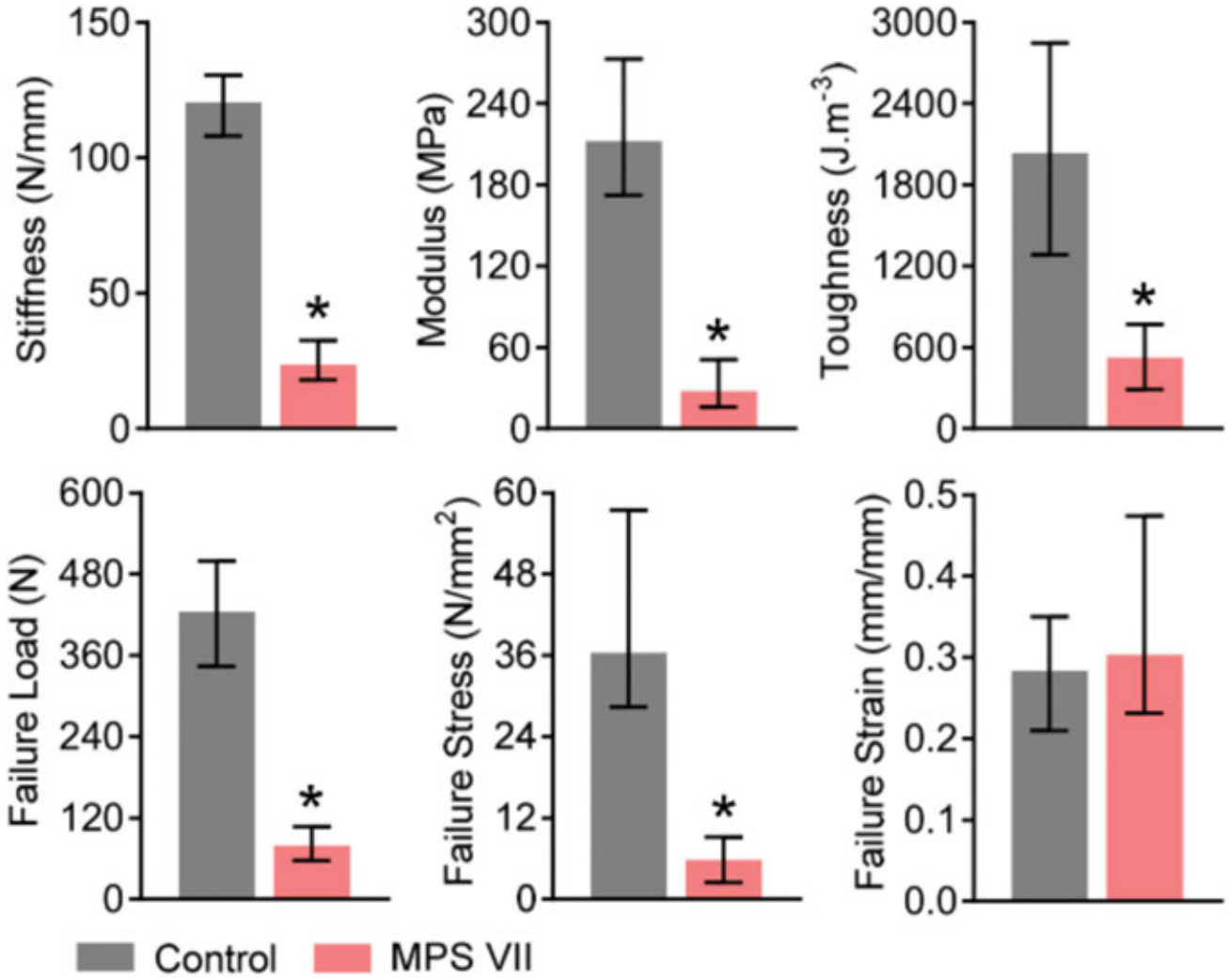

Figure 3. Tensile mechanical properties of anterior cruciate ligaments from control (n=6) and MPS VII (n = =5) dogs at ± months-of-age. *p < 0.05 vs control.

and human patients, with the long-term goal of developing effective, tissue-specific treatment strategies.

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