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Critical Regulatory Role for Collagen V in Establishing the Unique Mechanical Properties of Joint Stabilizing Tendons

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Introduction

Classic (type I) Ehlers-Danlos Syndrome (EDS) is a rare genetic disease associated with heterozygous mutations in collagen V.[1] Patients with classic EDS exhibit connective tissue hyperelasticity, as well as joint laxity and dislocations, indicating a potential role for collagen V in structures that contribute to joint stability.[2,3] Recent studies demonstrated that collagen V plays a crucial role in the anterior cruciate ligament (ACL)[4] which is a primary knee stabilizer, when compared to its role in the flexor digitorum longus (FDL) tendon[5] that does not have a major function in providing joint stability. However, due to the differences in anatomy and structure between these tissues, assessing the differential effects of collagen V in joint stabilizing tissues is difficult. Therefore, the purpose of this study was to determine if the contribution of collagen V to the establishment of mechanical properties in tendons and ligaments is primarily related to the role of the tissue in joint stability. Tendons that are similar in structure, exhibiting a tendon-to-bone insertion site graded in composition and organization, but different in their contributions to joint stability were analyzed. Accordingly, we examined the supraspinatus tendon (SST), which plays a crucial and direct role in joint stability of the shoulder, and the Achilles tendon (ACH), which does not contribute directly to joint stability. We hypothesized that the absence of collagen V would result in decreased mechanical properties in the supraspinatus tendon, but not in the Achilles tendon.

Methods

Sample Preparation Mice from two genotypes, *Col5a1*$^{+/+}$ (Wild Type, n = 5-9) and a conditional knockout targeted to tendon/ligament, *ScxCre+Col5a1*$^{-/-}$ (*Col5a1* KO, n = 8-13) were sacrificed at P60 (IACUC approved).[6] Achilles tendons were carefully dissected from the hind limb and the muscle was removed, leaving the Achilles tendon attached to the calcaneus. The supraspinatus tendon-bone complex was similarly dissected to remove muscle and other surrounding soft tissue. The cross-sectional areas of both tendons were measured using a custom measurement device and stain lines were applied to denote a 2.5mm (supraspinatus) or 5mm (Achilles) gauge length. The humerus was then potted in PMMA and the supraspinatus tendon was secured in custom grips with sandpaper. The Achilles tendon and calcaneus were both secured in custom grips for mechanical testing.

Mechanical Testing The tendons were then mechanically tested with the same loading protocol consisting of ten cycles of preconditioning from 0.02N to 0.04N, a stress relaxation at 5% strain and a constant ramp to failure at 0.1% strain per second. Local strain was measured optically and mechanical parameters were calculated.

Statistics Comparisons were made between wild type and collagen V null tendons using Student's t-tests with significance set at $p < 0.05$.

Results

Achilles Tendon Cross-sectional area and maximum load were reduced in the collagen V null group (Figure 1). In addition, stiffness at the midsubstance and insertion site was also reduced in the null group (Figure 2). However, there was no difference between groups in maximum stress or modulus at either location.

Supraspinatus Tendon Cross-sectional area, maximum load and maximum stress were all significantly reduced in the collagen V null group (Figure 1). In addition, the collagen V null group exhibited severely reduced stiffness and modulus at both the insertion site and midsubstance (Figure 2).

Discussion

Removal of collagen V resulted in severely decreased maximum load and stiffness in both tendons. However, in the Achilles tendons, neither the maximum stress nor modulus demonstrated changes, indicating that these results were due to tissue size rather than a change in tissue material quality. These results were consistent with our previous results in the FDL[5], which like the Achilles tendon does not contribute directly to joint stability. Conversely,

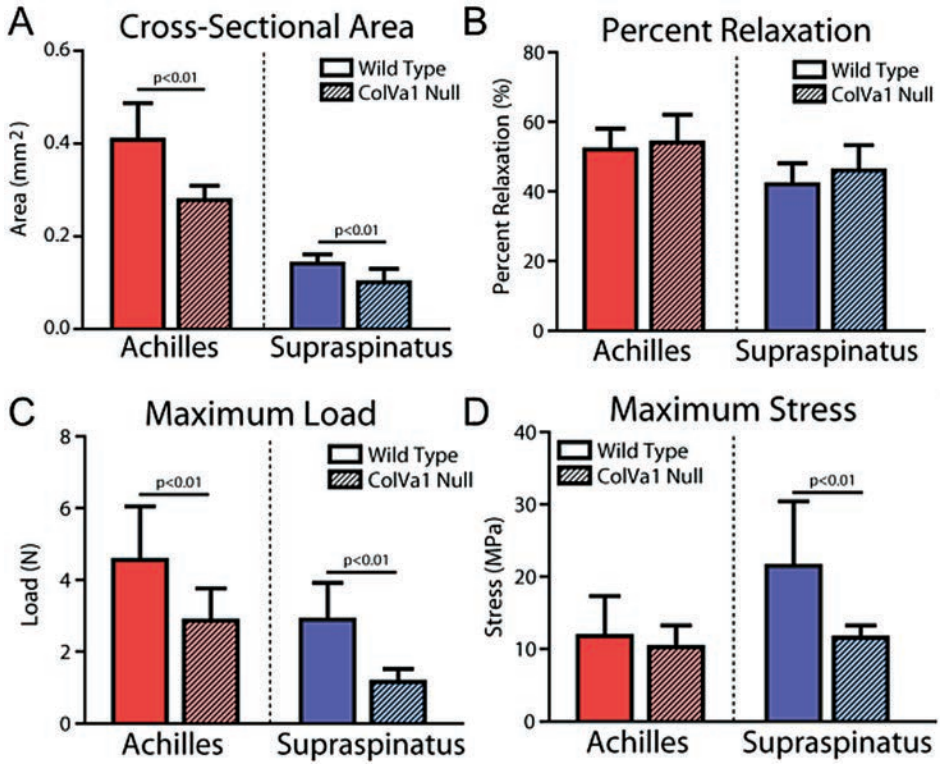

Figure 1. (A) Collagen V Null tendons were significantly smaller in both the Achilles and supraspinatus tendons, but there was no difference in (B) percent relaxation in either tendon. (C) Maximum load was also significantly reduced in the collagen V null group for both tendons. (D) Maximum stress was significantly decreased in the null group in the supraspinatus tendon, but no differences were found in the Achilles tendon.

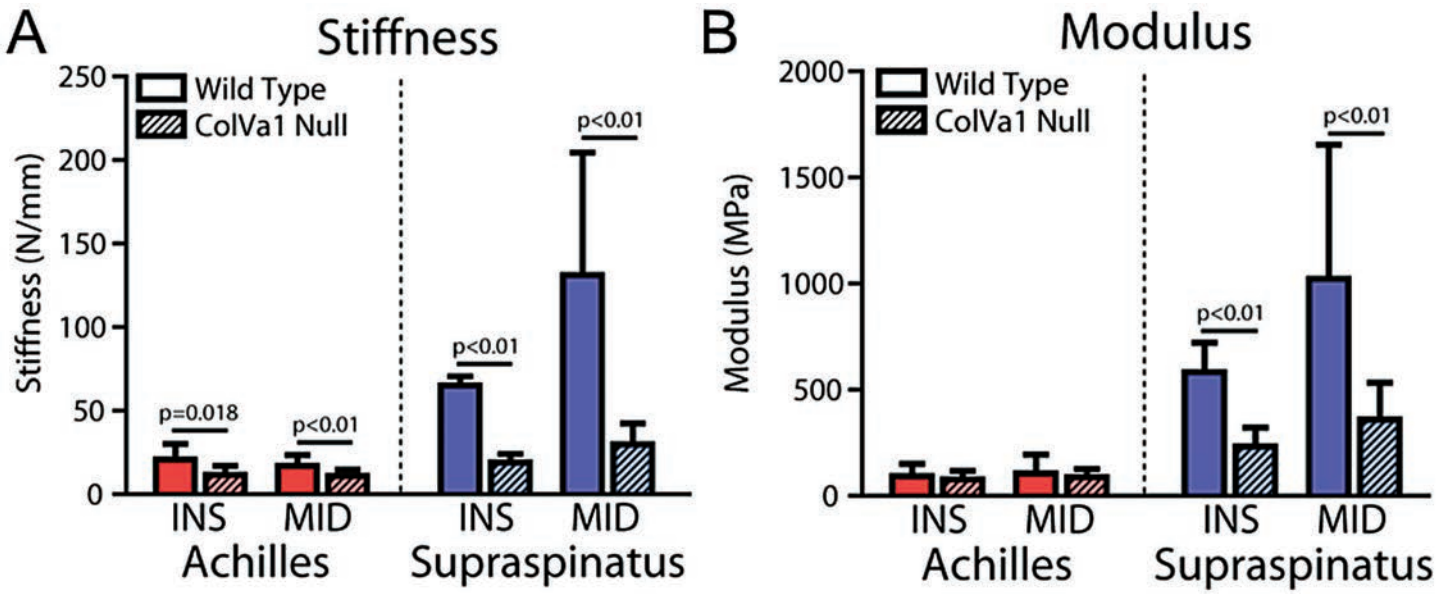

Figure 2. (A) Stiffness was significantly decreased in the collagen V null group in both tendons at both the insertion and midsubstance of the tissue. (B) Modulus was decreased in the null group at both locations only in the supraspinatus tendon. There were no differences in modulus between groups in the Achilles tendons.

the supraspinatus tendon did show significantly and severely reduced modulus and maximum stress. These material and structural mechanical results are consistent with previous work in the ACL.[4] Since both the ACL and the supraspinatus tendon act primarily as joint stabilizers, these results support that collagen V plays a critical role in joint stabilizing tendons and ligaments. Furthermore, the synthesis of results from these four tissues (Figure 3) which span a variety of structural architectures, functional roles, and tissue type indicate that the functional role of joint stability may be a major determinant of the importance of collagen V in that tissue. While this is a significant and interesting finding, this study did not directly measure joint stability or mechanical changes in the other soft tissues that surround joints, which could further support

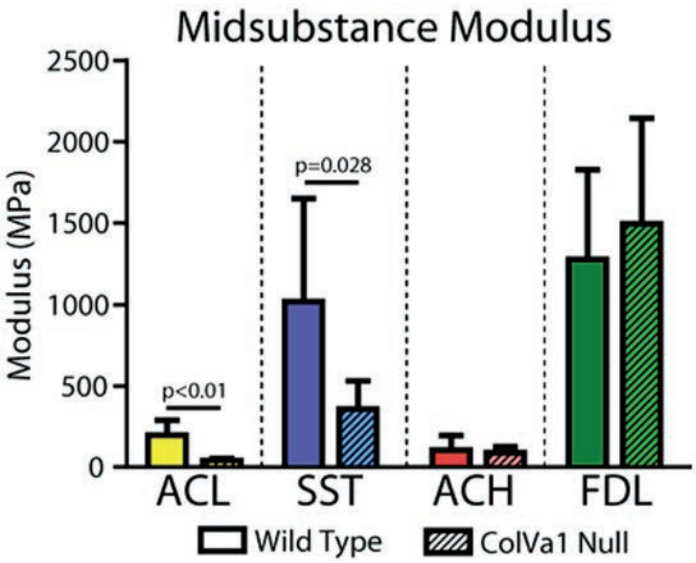

Figure 3. Collagen V plays a significant role in establishing the mechanical properties in joint-stabilizing tissues (ACL and SST), but not in tissues that do not have a direct role in joint stability (ACH and FDL).

this explanation. Additional investigation is also necessary to elucidate other functional alterations in collagen V deficient tendons such as their viscoelastic and fatigue responses.

Significance

Collagen V plays a crucial role in establishing tendon-specific mechanical properties only in tendons and ligaments that contribute to joint stability, suggesting that EDS-related joint instability observed in the clinical population may be directly related to inferior soft tissue mechanical function.

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