Tendon-Targeted Collagen V Deficiency and Knockout Attenuate Mature Supraspinatus Tendon Mechanics

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Disclosures

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Introduction

Collagen V is a critical tendon matrix protein that regulates fibrillogenesis and is expressed throughout development and in mature tendons.[1] Clinical manifestation of collagen V deficiency is the classic form of Ehlers-Danlos syndrome (EDS), a connective tissue disorder with greater than 50% of patients being haploinsufficient for COL5A1, characterized by hyperextensible skin, joint hypermobility and instability, and abnormal wound healing.[2] Recent data from mouse supraspinatus tendon, which experiences a complex, region-dependent (insertion and midsubstance) loading environment within the rotator cuff of the shoulder, demonstrated that deficiency of collagen V during development resulted in severely altered collagen fibril structure, biomechanical properties, and dynamic responses to load.[3] However, the region-specific roles of collagen V tendon-targeted deficiency and knockout on mature supraspinatus tendons remain unknown. The objective of this study is to elucidate the regulatory role of collagen V on supraspinatus tendon whole-tissue and regional mechanics in mature mice using tendon-targeted (Scleraxis-Cre) collagen V heterozygous and knockout mice. Due to the role of collagen V in the regulation of tendon structure during development, we hypothesized that collagen V heterozygous and knockout supraspinatus tendons would have inferior whole-tissue and regional elastic mechanical properties, whole-tissue viscoelastic mechanical properties and reduced regional collagen fiber realignment compared to wild type control tendons.

Methods

Animals

Supraspinatus tendons (n = 10/genotype) from tendon-targeted collagen V heterozygous (TEN-HET) mice (ScxCre;Col5a1f/wt), knockout (TEN-KO) mice (ScxCre;Col5a1f/f), and wild-type (WT) control mice (Cre-littermates) were used (IACUC approved).

Mechanics and Collagen Fiber Realignment

All mice were sacrificed at 150 days old and were subjected to our mechanical testing and collagen fiber realignment protocol: stress relaxations at 3%, 5%, and 7% strain each with subsequent frequency sweeps at 0.1, 1, 5, and 10 Hz, followed by a quasistatic ramp-to-failure.[3] Throughout the ramp-to-failure, dynamic collagen fiber realignment was quantified using cross-polarization imaging, and regional fiber alignment data was interpolated with a polynomial fit as a function of strain from the load-displacement data. Images were acquired during the ramp-to-failure for optical strain tracking of stain lines demarcating the insertion and midsubstance regions of the tendon.

Statistics

Comparisons between genotypes were conducted using one-way ANOVAs followed by Bonferroni post-hoc tests. Significance was set at $p \leq 0.05$ and trends at $p \leq 0.1$.

Results

Whole-tendon cross-sectional area was reduced in the TEN-KO group compared to the TEN-HET and WT groups (Figure 1A). Consistent with our hypothesis, collagen V deficiency and knockout resulted in dose-dependent reductions in elastic mechanical properties (e.g., failure load and linear stiffness (Figure 1B, C)). Viscoelastic differences were also observed. Percent relaxation was increased in TEN-KO tendons compared with TEN-HET and WT tendons at all strain levels (7% strain shown in (Figure 2A). Additionally, collagen V TEN-HET and TEN-KO resulted in dose-dependent reductions in dynamic modulus, while phase shift was increased in TEN-KO tendons relative to TEN-HET and WT across all strain levels and frequencies (7% strain at 1 Hz shown in Figure 2B and 2C). As hypothesized, collagen V TEN-HET

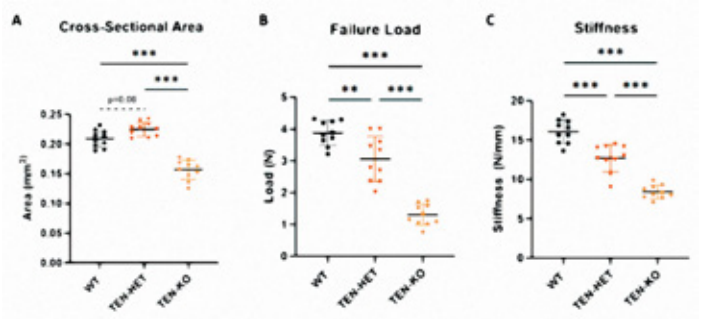

Figure 1. TEN-KO tendons demonstrated reduced cross-sectional area relative to TEN-HET and WT tendons **(A).** Tendon-targeted deficiency and knockout of collagen V resulted in significant reductions in elastic mechanical properties failure load and stiffness in a dose-dependent manner **(B-C).** Data as mean ± standard deviation (--p≤0.1, *p≤0.05, **p≤0.01, ***p≤0.001).

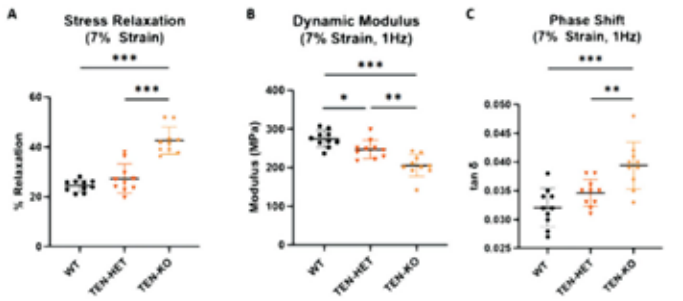

Figure 2. TEN-KO tendons had increased percent relaxation relative to TEN-HET and WT tendons **(A).** Tendon-targeted collagen V deficiency and knockout resulted in significant reductions in dynamic modulus in a dose-dependent manner **(B),** while phase shift was significantly increased in TEN-KO tendons relative to TEN-HET and WT tendons **(C).** Data as mean ± standard deviation (*p≤0.05, **p≤0.01, ***p≤0.001).

and TEN-KO resulted in dose-dependent reductions in insertion modulus, while midsubstance modulus was reduced in TEN-KO tendons relative to TEN-HET and WT tendons (Figure 3A, B). These results are supported by reductions in collagen fiber realignment in TEN-HET and TEN-KO tendons across region, as demonstrated by greater normalized circular variance values for insertion and midsubstance regions from 3-7% strain (Figure 3C-D), encompassing the toe and linear elastic regions of these tendons.

Discussion

This study investigated the role of collagen V on supraspinatus tendon elastic and viscoelastic mechanics using TEN-HET and TEN-KO mice. Consistent with previous data, we demonstrated that tendon-targeted collagen V TEN-HET and TEN-KO 5esulted in reductions in regional and whole-tissue elastic and viscoelastic mechanical properties.[3] Further, reductions in these properties in our collagen V TEN-HET tendons highlight the allele-dependency of collagen V on tendon elastic and viscoelastic mechanical function and collagen fiber realignment. These functional deficits could be attributed to the improper hierarchical assemblies of TEN-HET and TEN-KO tendons resulting in disorganized tendon matrices with an inferior ability to respond to load.[4] This was evidenced by marked

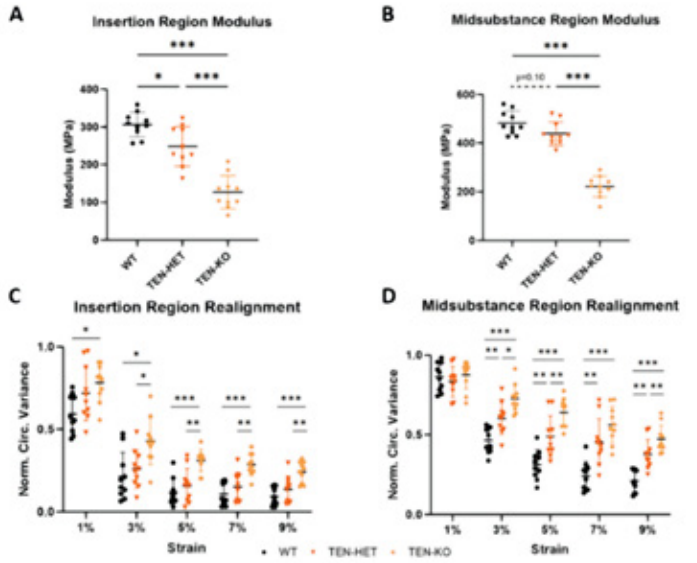

Figure 3. TEN-HET and TEN-KO tendons demonstrated reduced moduli and collagen fiber realignment in the insertion **(A, C)** and midsubstance **(B, D)** regions. Decreased normalized circular variance is indicative of increased collagen fiber realignment. Data as mean ± standard deviation (--p≤0.1, *p≤0.05, **p≤0.01, ***p≤0.001).

reductions in the TEN-HET and TEN-KO tendons' responses to realign resulting in inferior whole-tissue and regional elastic and viscoelastic mechanical properties. Overall, results demonstrate that decreased collagen V expression detrimentally affects supraspinatus whole-tissue and regional elastic and viscoelastic mechanical properties and collagen fiber realignment.

Significance/Clinical Relevance

This study elucidates the critical role of collagen V in regulating supraspinatus tendon function. Future studies will evaluate the structural and compositional mechanisms that contribute to these mechanical results. Understanding the effects of collagen V in tendon can be used to develop potential treatments modalities for classic Ehlers-Danlos syndrome.

Acknowledgement

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References

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