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

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Disclosures: AF Kuntz (5, Integra Lifesciences, Orthofix, Inc., FX Shoulder USA, Inc.; 9, American Shoulder and Elbow Surgeons, American Board of Orthopaedic Surgery, Inc.), no other disclosures

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;*Col5a1*$^{f/wt}$), knockout (TEN-KO) mice (ScxCre;*Col5a1*$^{f/f}$), 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 [3]: 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. 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 (**Fig. 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 (**Figs. 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 **Fig. 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 **Figs. 2B and 2C**). As hypothesized, collagen V TEN-HET 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 (**Figs. 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 (**Figs. 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 [3], we demonstrated that tendon-targeted collagen V TEN-HET and TEN-KO resulted in reductions in regional and whole-tissue elastic and viscoelastic mechanical properties. 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 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: This study was supported by NIH/NIAMS (AR070750) and the Penn Center for Musculoskeletal Disorders (NIH/NIAMS, P30 AR069619).

REFERENCES: [1] Wenstrup et al. J Biol Chem. 2011. [2] Steinmann et al. Conn Tissue, Heritable Disorders. 2002. [3] Connizzo et al., Interface Focus. 2016. [4] Connizzo et al., J Orthop Res. 2016.

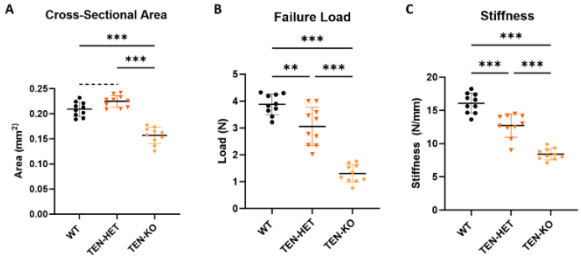

Figure 1. TEN-KO tendons demonstrated reduced cross-sectional area relative to TEN-HET and WT tendons (A). Tendon-targeted deficiency and knockout of collagen V resulted in significant reductions in elastic mechanical properties failure load and stiffness in a dose-dependent manner (B-C). Data as mean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

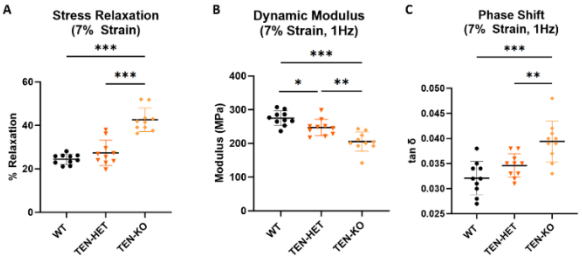

Figure 2. TEN-KO tendons had increased percent relaxation relative to TEN-HET and WT tendons (A). Tendon-targeted collagen V deficiency and knockout resulted in significant reductions in dynamic modulus in a dose-dependent manner (B), while phase shift was significantly increased in TEN-KO tendons relative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

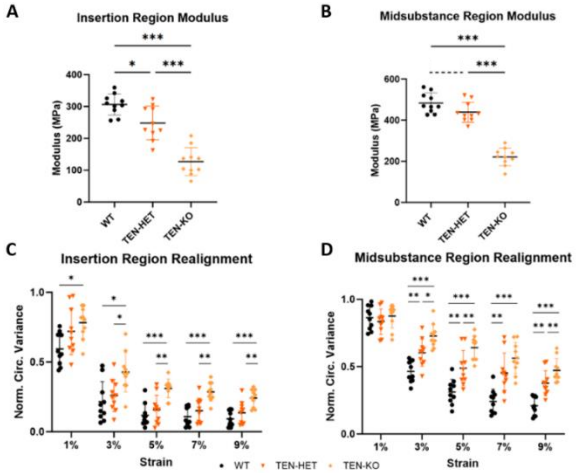

Figure 3. TEN-HET and TEN-KO tendons demonstrated reduced moduli and collagen fiber realignment in the insertion (A, C) and midsubstance (B, D) regions. Decreased normalized circular variance is indicative of increased collagen fiber realignment. Data as mean ± standard deviation (--p≤0.1, *p≤0.05, **p≤0.01,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