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

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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 specific roles of collagen V-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 (SccCre;Col5a1F/f), knockout (TEN-KO) mice (SccCre;Col5a1f/f), and wild-type (WT) control mice (Cre- littersmates) 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 alignment data was interrogated 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<0.05 and trends at p<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-HET and 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.

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