Collagen XII is a Critical Regulator of Supraspinatus Tendon Mechanics and Collagen Fiber Realignment Across Sex

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INTRODUCTION: Collagen XII is a Fibril-Associated Collagen with Interrupted Triple Helices (FACIT) that regulates collagen fibril assembly and is primarily expressed throughout tendon growth and development. Mutations in the *Col12a1* gene result in myopathic Ehlers-Danlos syndrome, a connective tissue disorder in which patients exhibit weakness at birth, absence of deep tendon reflexes and distal joint hypermobility and contracture [1]. Our novel tendon-targeted collagen XII mouse model demonstrated that patellar tendons exhibited reduced elastic, viscoelastic, and dynamic collagen fiber realignment properties across sex [2]. However, the role of collagen XII on the supraspinatus tendon, which experiences a complex, region-specific (insertion and midsubstance) loading environment within the rotator cuff of the shoulder, remains unknown. Therefore, the objectives of this study are to (1) elucidate the regulatory role of collagen XII on supraspinatus tendon whole-tissue and regional mechanics and dynamic response to load in mature mice using tendon-targeted (Scleraxis-Cre) collagen XII deficient and knockout mice and (2) understand whether the role of collagen XII on supraspinatus tendon biomechanical function and dynamic response to load is dependent on sex. We hypothesized that tendon-targeted deficiency and knockout of collagen XII would result in decreased supraspinatus tendon whole-tissue and regional elastic mechanics, whole-tissue viscoelasticity, and regional collagen fiber realignment across sex.

METHODS: Supraspinatus tendons from male and female, day 60 tendon-targeted collagen XII heterozygous (HET) mice (ScxCre;*Col12a1*$^{f/wt}$, n=8-9/group), knockout (KO) mice (ScxCre;*Col12a1*$^{f/f}$, n=6-9/group) and wild-type (WT) control mice (Cre- littermates, n=7-9/group) (IACUC) were subjected to our established mechanical testing protocol and collagen fiber realignment method [3]. Tendons underwent stress relaxation testing at 3, 5, and 7% strain each with subsequent dynamic 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 also used to optically measure strain to calculate regional moduli (insertion and midsubstance). For each sex, 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: Cross-sectional area (CSA) was not different between male tendons, while female KO tendons exhibited a trending decrease in CSA relative to WT (data not shown). Consistent with our hypothesis, linear stiffness was significantly decreased in KO mice across sex and between female HET and KO mice (**Fig. 1A**). Moreover, insertion modulus was significantly reduced in HET and KO tendons across sex whereas midsubstance modulus was significantly reduced in male KO tendons and female HET and KO tendons (**Figs. 1B, C**). Further, genotypic differences were observed in viscoelastic properties across sex. Percent relaxation was significantly increased in KO tendons across sex at all strain levels (5% strain shown in **Fig. 1D**). Additionally, dynamic modulus was significantly decreased in male KO tendons and in female HET and KO tendons, while phase shift was significantly increased in KO tendons across sex across at all strain levels and frequencies (5% strain at 1Hz shown in **Figs. 1E** and **1F**, respectively). These results are supported by reductions in collagen fiber realignment in HET and KO tendons across region and sex, as demonstrated by significantly greater normalized circular variance values for insertion and midsubstance regions from 3-7% strain (**Figs. 2A-D**), encompassing the toe and linear elastic regions of these tendons.

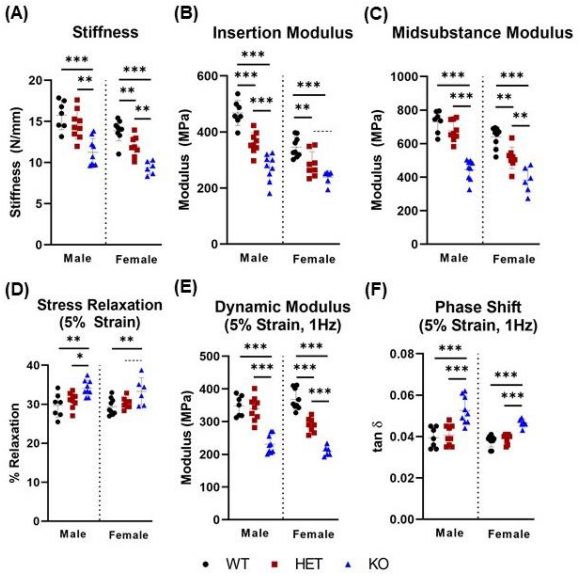

Figure 1. Differences between male and female elastic (A-C) and viscoelastic (D-F) mechanical properties of WT, HET, and KO supraspinatus tendons. Data as mean ± standard deviation (---$p \leq 0.1$ *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$).

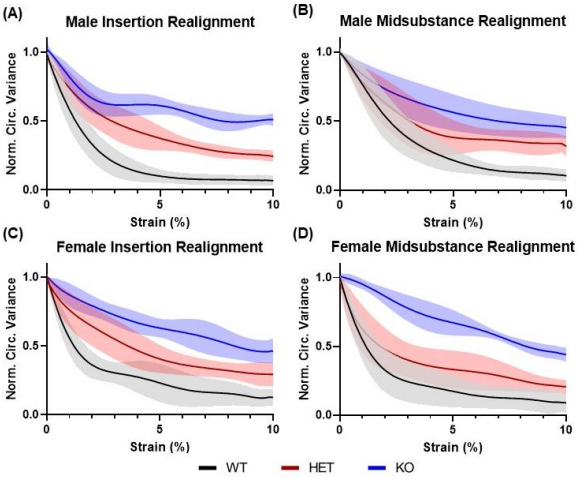

Figure 2. Collagen fiber realignment distribution differences for male (A-B) and female (C-D) WT, HET, and KO supraspinatus tendon insertion and midsubstance regions. Decreased normalized circular variance is indicative of increased collagen fiber realignment.

DISCUSSION: This study investigated the role of collagen XII on supraspinatus tendon elastic and viscoelastic mechanics and dynamic collagen fiber realignment using tendon-targeted male and female ScxCre;*Col12a1*$^{f/wt}$ and ScxCre;*Col12a1*$^{f/f}$ mice. Consistent with previous data [2], we showed that tendon-targeted collagen XII knockout resulted in striking reductions in regional and whole-tissue elastic and viscoelastic mechanical properties and regional collagen fiber realignment. Further, reductions in these properties in our collagen XII deficient HET tendons, highlight the allele-dependency of collagen XII on tendon mechanical function and dynamic collagen fiber realignment. These mechanical deficits could be due to the improper hierarchical assemblies of HET and KO tendons resulting in disorganized tendon matrices with an inferior ability to respond to load. This was evidenced by marked reductions in the HET and KO tendons' responses to realign resulting in inferior mechanical properties, especially whole-tissue stiffness, regional moduli, and dynamic modulus. Although similar differences in elastic and viscoelastic mechanical properties were present across sex in response to collagen XII deficiency and knockout, more genotypic differences were present in female mice. Genetic variations in the *Col12a1* gene have been associated with an increased risk of ACL ruptures in women [4], implicating potential sex-specific effects of collagen XII deficiency and knockout. Overall, our results demonstrate that decreased collagen XII expression detrimentally affects supraspinatus tendon mechanical properties and dynamic collagen fiber realignment across sex.

SIGNIFICANCE/CLINICAL RELEVANCE: This study elucidates the critical role of collagen XII in regulating male and female supraspinatus tendon regional and whole-tissue mechanics and dynamic structural response to load within the complex loading environment of the rotator cuff of the shoulder. Clinically, understanding the effects of collagen XII in tendon across sex can be used to develop and evaluate potential treatments modalities for myopathic Ehlers-Danlos syndrome.

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