Collagen XII Regulates Tendon Dynamic Mechanical Properties and Collagen Fiber Realignment

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Disclosures

None

Introduction

Myopathic Ehlers-Danlos syndrome (mEDS) is a connective tissue disorder caused by mutations in the *Col12a1* gene, which encodes for collagen XII, a fibril-associated collagen with interrupted triple helices (FACIT). Patients with mEDS experience myopathy, joint hypermobility and contractures[1], indicating dysregulation of connective tissue function due to the absence of collagen XII. Our recent data showed that tendons from global collagen XII knockout (*Col12a1*$^{-/-}$) mice exhibited disrupted tendon fiber organization and assembly as well as increased cross-sectional area and stiffness. This suggests that the disruption of tendon structure function in the absence of collagen XII may be caused by a lack of distinct fiber domains resulting in reduced fiber sliding and increased stiffness. However, our previous findings may be confounded by the effects of collagen XII knockdown on other tissues, such as muscle and bone, and the isolated role of collagen XII on tendon mechanical function is still unknown. Therefore, the objectives of this study are to (1) evaluate the specific role of collagen XII in regulating tendon mechanical properties and the dynamic loading response in mature mice using tendon-targeted (scleraxis Cre) collagen XII knockout mice and (2) determine if the role of collagen XII is sex-specific. We hypothesized that a collagen XII knockout would lead to increased tendon stiffness and reduced collagen fiber realignment under loading due to disruptions in tendon matrix assembly in both sexes.

Methods

Patellar tendons from female and male, day 60 tendon-targeted collagen XII knockout (KO) mice (ScxCre;*Col12a1*$^{f/f}$, n = 6-8/group) and control (Cre- littermates, n = 4-6/group) mice (IACUC approved) were mechanically evaluated using viscoelastic and dynamic collagen fiber realignment methods, as described[2]. Tendons underwent a loading protocol of three stress relaxations at 3, 4, and 5% strain each with a dynamic frequency sweep (0.1, 1, 5, 10Hz), followed by a quasi-static ramp to failure. During the ramp, images were continuously acquired through rotating cross polarizers to evaluate dynamic collagen fiber realignment. For each sex, comparisons between genotypes were made using two-tailed, t-tests with significance set at $p \leq 0.05$ and trends at $p \leq 0.1$.

Results

Cross-sectional area was not different between female KO and control mice, while male KO tendons were smaller than control (Figure 1a). Contrary to our hypothesis, linear stiffness was significantly reduced in KO mice for both sexes (Figure 1b), and only female KO tendons exhibited a trending decrease in elastic modulus with no difference between male groups (Figure 1c). Additionally, percent relaxation was significantly reduced in female KO tendons at all strain levels (5% strain shown in Figure 1d). Despite only minor differences in elastic modulus, both sexes demonstrated striking differences in dynamic properties. Compared to their respective controls, dynamic modulus was significantly reduced in KO groups while phase shift was significantly elevated across all strain levels and frequencies (5% strain, 1Hz shown in Figures 1e and f, respectively). This suggests alterations in matrix structure leading to more viscous mechanical behavior during dynamic loading in the KO groups. This finding is further supported by a reduced degree of collagen fiber realignment (Figure 2a) in the female KO group,as shown by increased circular variance at all strain values (Figure 2b), and a reduced rate of fiber realignment in the male KO group (Figure 2c), as shown by increased circular variance at lower strain values (Figure 2d).

Discussion

This study investigated the isolated role of collagen XII on tendon mechanical function using female and male ScxCre;*Col12a1*$^{f/f}$ mice. Interestingly, KO patellar tendons had reduced stiffness, which contrasts the increased stiffness observed in flexor digitorum longus (FDL) tendons of global collagen XII knockout mice. This suggests that the effects of global knockout

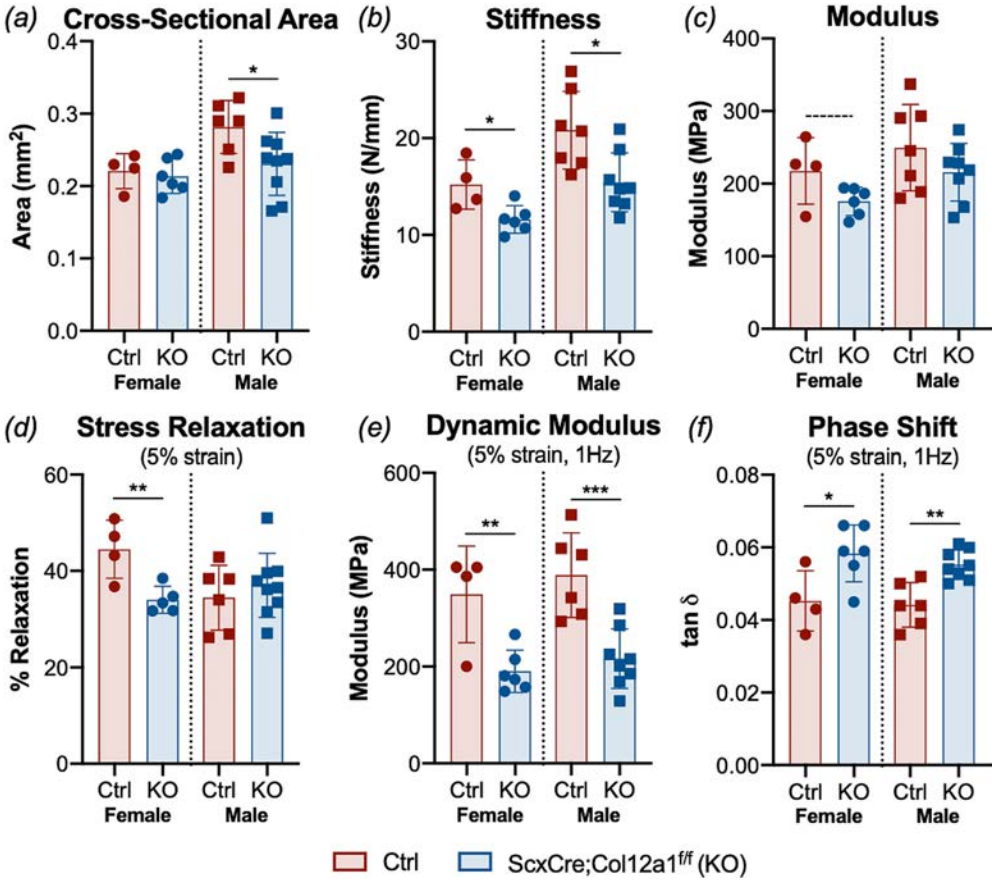

Figure 1. Male KO patellar tendons had **(A)** reduced cross-sectional area, while both sexes exhibited **(B)** reduced tendon stiffness. **(C)** Modulus and **(D)** percent relaxation were reduced in female KO tendons. **(E)** Dynamic modulus was reduced and **(F)** phase shift was elevated in KO groups for both sexes. Data presented as mean ± standard deviation. (---p ≤ .1, *p ≤ .05, **p ≤ .01,***p ≤ .0.001).

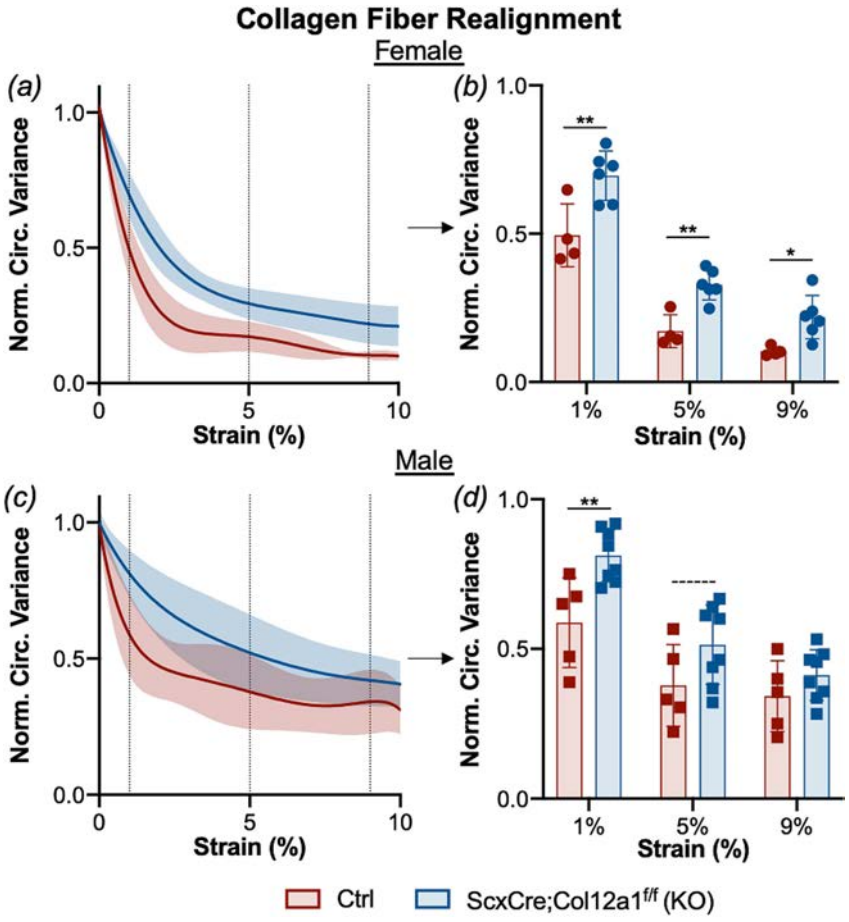

Figure 2. (A) Female KO tendons exhibited reduced collagen fiber realignment with increasing strain, as evidenced by **(B)** increased normalized circular variance at all strain levels compared to control. **(C)** Male KO tendons exhibited a reduced rate of collagen fiber realignment with **(D)** increased normalized circular variance at lower strain values compared to control. Data presented as mean ± standard deviation. (---p ≤ 0.1, *p ≤ 0.05, **p ≤ 0.01).

of collagen XII on muscle[3] and bone[4] may indirectly affect tendon, necessitating the use of this tendon-targeted mouse model. The effects of collagen XII knockout could also be tendon-specific, as mEDS patients present with both distal joint hypermobility and proximal joint contractures. Furthermore, female KO tendons exhibited less stress relaxation at all strain levels, suggesting alterations in the ability to effectively dissipate load. This could be attributed to a disruption in the establishment of proper hierarchical assembly leading to a reduction in fiber and fibril sliding. Matrix disorganization could also explain the striking differences in dynamic properties, as evidenced by reduced dynamic modulus, increased phase shift, and reduced collagen fiber realignment. Interestingly, our preliminary data shows that, in addition to its structural role, collagen XII may also be critical for regulating cellular organization necessary for establishing hierarchical structure and tendon function, and studies are ongoing to investigate these temporal roles of collagen XII throughout development. Finally, though similar trends were observed for both female and male groups in response to collagen XII knockout, there was a more pronounced effect in female mice. *Coll12a1* polymorphisms have been linked to an increased incidence of ACL ruptures in women[5], suggesting a possible sex-specific effect. Our study demonstrates that collagen XII knockout in tendons affects tendon matrix structure and organization, resulting in altered structural, viscoelastic, and dynamic collagen fiber realignment properties.

Significance

This study demonstrates the critical role of collagen XII in regulating tendon dynamic mechanical behavior, highlighting its importance in establishing tendon structure-function and its re-establishment following injury.

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References

1. Punetha *et al.* *Muscle Nerve* 2017
2. Dunkman *et al.* *Matrix Biol.* 2013
3. Zou *et al.* *Human Molecular Genetics* 2014
4. Izu *et al.* *J Cell Biol.* 2011
5. Posthumus *et al.* *Br J Sports Med* 2010.