Collagens V and XI Jointly Regulate Fibril Assembly and Elastic Mechanical Properties during Tendon Maturation

Nat A. Thurlow, Jeremy D. Eekhoff, Ashley K. Fung, Courtney A. Nuss, Stephanie N. Weiss, Nathaniel A. Dyment, Louis J. Soslowsky
McKay Orthopaedic Research Laboratory, University of Pennsylvania, Philadelphia, PA
nthurlow@seas.upenn.edu

Disclosures: None

INTRODUCTION: Tendon hierarchical structure is established during development through the coordinated assembly of matrix proteins, including minor fibril-forming collagens such as collagens V and XI. Collagen V influences collagen fibrillogenesis through nucleating fibril formation and co-assembling with collagens I and II[1], and lack of *Col5a1* expression leads to larger fibrils, reduced fibril density, and smaller tendon cross-sectional area[2]. Collagen XI has a similar role in fibril regulation during development[3] and co-assembles with collagen V to form heterotypic fibrils[1]. The expression of genes for collagen V and XI is similar in developing tendons, but the expression of collagen XI encoding genes is decreased in mature tendons compared to collagen V genes. Moreover, in global knockdown mouse models, haploinsufficiency of both *Col5a1* and *Col11a1* in tandem yielded more irregular fibril shapes and greater heterogeneity of fibril diameters in developing tendons than *Col5a1* haploinsufficiency alone[1]. Together, these findings suggest interactive roles between collagens V and XI during development. However, the structural and functional deficits associated with coordinated knockdown of *Col5a1* and *Col11a1* remain unknown. Since the tendon-specific compound *Col5a1, Col11a1* knockout is postnatally unviable, the objective of this work was to assess the cooperative roles of collagens V and XI during fibril growth and assembly using a tendon-specific (ScxCre) compound *Col5a1* null, *Col11a1* heterozygous mouse model. Based on prior work in tendons lacking *Col5a1* expression, we hypothesized that ScxCre;$Col5a1^{flox/flox}$;$Col11a1^{flox/+}$ (VKO-XIHet) tendons would demonstrate structural changes consistent with aberrant fibril growth.

METHODS: Animals: Male and female postnatal day 30 VKO-XIHet mice (n=10) and ScxCre- littermate controls (Ctrl, n=10) were used (IACUC approved). Transmission Electron Microscopy: Immediately after sacrifice, Achilles tendons (ATs) (n=4/genotype) were isolated, fixed, embedded, sectioned, stained, and imaged as described[4]. Fibril diameters were measured using a custom MATLAB script (n=10 images/sample). Mechanics: AT-calcaneus complexes were harvested, finely dissected, and cross-sectional area was measured using a custom laser device. The free end of the tendon was secured in sandpaper with cyanoacrylate glue, and the calcaneus and sandpaper were gripped in custom fixtures. Tendons were tested in a PBS bath at 37°C using a protocol of preloading to 0.03N, preconditioning for 10 cycles, stress relaxations at 3% and 5% strain, and quasistatic ramp-to-failure at 0.1% strain/sec (Instron 5848). Each stress relaxation was followed by a frequency sweep of 10 cycles at 0.1, 1, 5, and 10 Hz. Statistics: Fibril diameter distributions were compared between genotypes using a Kolmogorov-Smirnov test. Cross-sectional area and mechanical properties were compared across genotypes using a two-sample t-test. Significance was set at $p \leq 0.05$, and all data visualization and statistics were conducted in R (v4.3.1).

RESULTS: VKO-XIHet ATs demonstrated substantial changes in fibril structure and mechanical properties. The collagen fibril distribution in VKO-XIHet tendons was different than Ctrl with a distinct population of larger (>175 nm) fibrils (**Fig 1A**). While fibrils in Ctrl tendons had circular cross-sections, many fibrils in VKO-XIHet tendons had irregularly shaped cross-sections with these irregularities most apparent and severe in the population of larger fibrils (**Fig 1B-C**). Despite larger fibril diameters, overall tendon cross-sectional area was smaller in VKO-XIHet tendons (**Fig 2A**). Maximum load, stiffness, and maximum stress were also lower in VKO-XIHet tendons compared to Ctrl **(Fig 2B-D)**. Viscoelastic properties showed minimal differences between genotypes (data not shown).

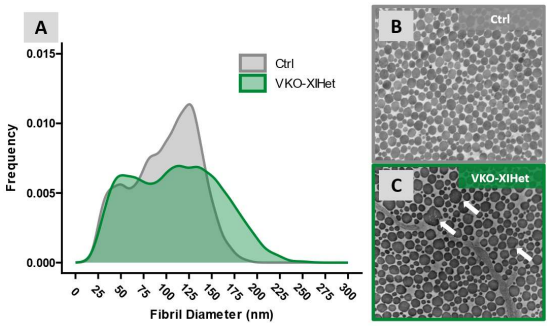

Figure 1: (A) VKO-XIHet fibril distributions demonstrate statistical differences with a population of larger diameter fibrils. Fibril distributions were compared using a Kolmogorov-Smirnov test. (B-C) Fibril boundaries are irregularly shaped in VKO-XIHet tendons (white arrows), especially in larger fibrils.

DISCUSSION: We studied the combined roles of collagens V and XI in establishing structural and mechanical properties of the AT during postnatal growth. Supporting our hypothesis, VKO-XIHet tendons showed fibril-level structural and tissue-level mechanical changes consistent with altered fibril assembly. The shift towards larger diameter fibrils and irregularity of fibril boundaries in VKO-XIHet tendons suggest that these collagen types work in concert to regulate lateral growth of fibrils. This finding is consistent with previous work where the absence of *Col5a1* expression led to larger fibril dimeters[3,5] and irregular fibril boundaries[5]. Additionally, we previously found a 39% decrease in maximum load and a 19% decrease in maximum stress in post-natal day 60 ScxCre;$Col5a1^{flox/flox}$ ATs[2]. In comparison, the post-natal day 30 ScxCre;$Col5a1^{floxflox}$;$Col11a1^{flox/+}$ tendons in this study showed 75% and 45% decreases in the same parameters, respectively. These markedly reduced mechanical properties coupled with increased lateral growth in a sizable portion of fibrils demonstrate that ablation of 1 allele of *Col11a1* in addition to both alleles of *Col5a1* further exacerbates the phenotype during tendon development. Future work will focus on delineating possible compensatory mechanisms between collagens V and XI and understanding interactions at early stages of development.

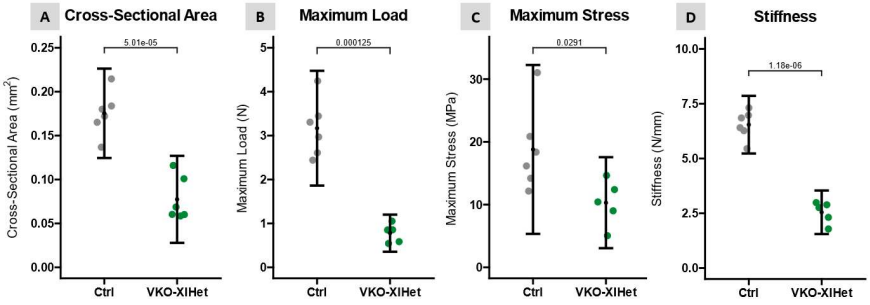

Figure 2: Cross-sectional area (A), maximum load (B), maximum stress (C), and stiffness (D) were significantly decreased in VKO-XIHet tendons. Properties were compared between genotypes using t-tests; p-values are listed above significance bars. Data shown as mean ± SD.

SIGNIFICANCE: Collagens V and XI have known roles in fibrillogenesis and the acquisition of tendon structure during development. Due to their coordinated roles and structural similarities, defining the interactions between collagens V and XI in tendon is essential to understanding mechanisms underlying collagen fibril formation.

REFERENCES: 1. Wenstrup et al., J Biol Chem, 2011. 2. Connizzo et al., J Orthop Res, 2015. 3. Sun et al., Matrix Biol, 2020. 4. Dunkman et al., Matrix Biol, 2014. 5. Connizzo et al., J Orthop Res, 2016.

ACKNOWLEDGEMENTS: This study was supported by NIH/NIAMS (R01AR073231) and Penn Center for Musculoskeletal Disorders (P30AR069619).