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

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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 area2. Collagen XI has a similar role in fibril regulation during development3 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 alone1. 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;Col5a1flox/flox;Col11a1flox/+ (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 described4. 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

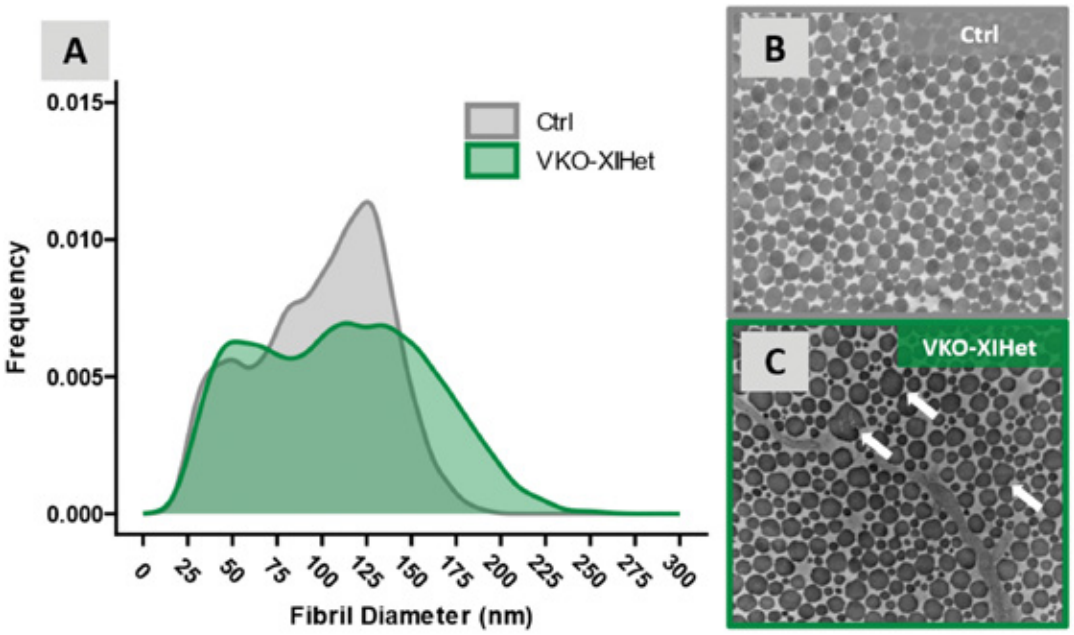

Figure 1. (A) VKO-XIHet fibril distributions demonstrate statistical differences with a population of larger diameter fibrils. Fibril distributions were compared using a Kolmogorov-Smirnov test. **(B-C)** Fibril boundaries are irregularly shaped in VKO-XIHet tendons (white arrows), especially in larger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

VKO-XIHet tendons was different than Ctrl with a distinct population of larger (>175 nm) fibrils (Figure 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 (Figure 1B-C). Despite larger fibril diameters, overall tendon cross-sectional area was smaller in VKO-XIHet tendons (Figure 2A). Maximum load, stiffness, and maximum stress were also lower in VKO-XIHet tendons compared to Ctrl (Figure 2B-D). Viscoelastic properties showed minimal differences between genotypes (data not shown).

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;Col5a1flox/flox ATs2. In comparison, the post-natal day 30 ScxCre;Col5a1floxflox;Col11a1flox/+ tendons in this study showed 75% and 45% decreases in the same parameters, respectively. These markedly reduced mechanical properties coupled with increased lateral

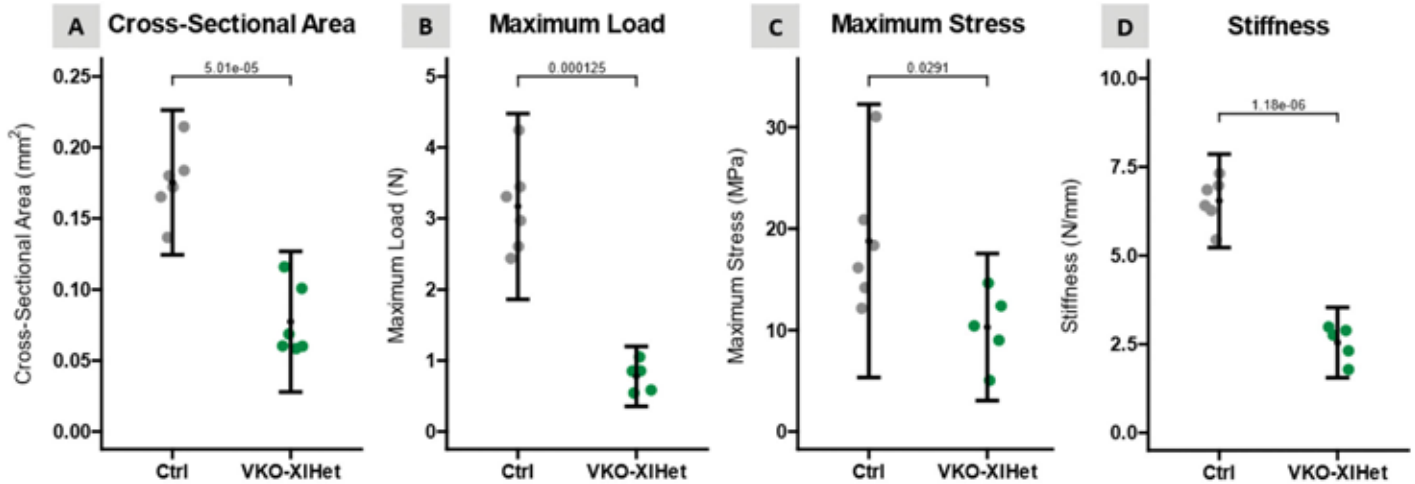

Figure 2. Cross-sectional area **(A)**, maximum load **(B)**, maximum stress **(C)**, and stiffness **(D)** were significantly decreased in VKO-XIHet tendons. Properties were compared between genotypes using t-tests; p-values are listed above significance bars. Data shown as mean ± SD.

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.

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.

Acknowledgements

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