Collagen VI Plays an Important Role in FDL Tendon Mechanics that is Distinct from the Role of Biglycan

Ryan J Leiphart[1]
Snehal S Shetye[1]
Hai Pham[2]
Stephanie N Weiss[2]
Marian F Young[2]
Louis J Soslowsky[1]

1 McKay Orthopedic Research Laboratory
University of Pennsylvania

2 National Institute of Dental and
Craniofacial Research
National Institutes of Health

Introduction

While tendons are largely composed of tension-bearing collagen I fibers, other lower abundance matrix proteins with lesser known functions are also present. For example, collagen VI is a nonfibrillar collagen that enriches the pericellular matrix (PCM), and biglycan is a small, leucine-rich proteoglycan that regulates fibrillogenesis[1,2]. Deficiency in either collagen VI or biglycan is known to impact tendon mechanics[3,4]. While collagen VI and biglycan are known to interact, their interactions in native tendon, and the impact on tendon mechanics, remain unknown[5]. Therefore, the objective of this study was to determine how the roles of collagen VI, biglycan, or interactions involving both molecules affect FDL tendon mechanics. We hypothesized that knockout of collagen VI would reduce FDL tendon mechanical properties more than knockout of biglycan, while blocking interactions by knocking out both molecules would lead to a larger reduction in these properties than the reduction seen in either knockout alone.

Methods

Animals and Dissection

2 month old male wild-type (WT) (n = 16), *Col6a2*$^{-/-}$ (n = 11), *Bgn*$^{-/0}$ (n = 12), and *Col6a2*$^{-/-}$/*Bgn*$^{-/0}$ (n = 13) mice were used in this study (IACUC approved). FDL tendons were dissected from the left hind limb. The tendon sheath was fine dissected off the tendon. Tendon cross- sectional area (CSA) was measured with a custom laser device, and stain lines were applied for optical tracking[6].

Mechanical Testing

The FDL tendon was gripped with sandpaper, leaving a 5mm gauge length. The testing protocol consisted of 10 cycles of preconditioning between 0.01-0.02N at 1Hz, a 5 minute hold, a 5% stress relaxation for 10 minutes, a 1 minute hold, and a ramp to failure at 0.5% strain/s. Stress relaxation, stiffness, max load, modulus, and max stress were computed. Dynamic collagen fiber realignment was measured throughout the ramp-to-failure test using a crossed polarizer setup[7].

Statistics

For mechanical properties, a one-way ANOVA with Bonferroni post-hoc tests was used to compare across genotypes. For fiber alignment data, a two-way ANOVA with Tukey correction for multiple comparisons was used to compare across genotype and strain. Significance was set at $p < 0.05$, and trends were set at $p < 0.10$

Results

WT tendons had larger CSA than tendons from all knockout genotypes (Figure 1A). *Bgn*$^{-/0}$ tendons had larger CSA than *Col6a2*$^{-/-}$ and *Col6a2*$^{-/-}$/*Bgn*$^{-/0}$ tendons. WT tendons were stiffer and had higher max loads than tendons from all knockout genotypes (Figure 1B,C). *Bgn*$^{-/0}$ tendons were stiffer than *Col6a2*$^{-/-}$ and *Col6a2*$^{-/-}$/*Bgn*$^{-/0}$ tendons. *Bgn*$^{-/0}$ and *Col6a2*$^{-/-}$ tendons had higher max loads than *Col6a2*$^{-/-}$/*Bgn*$^{-/0}$ tendons. WT and *Bgn*$^{-/0}$ tendons exhibited a larger percent relaxation than *Col6a2*$^{-/-}$ and *Col6a2*$^{-/-}$/*Bgn*$^{-/0}$ tendons (Figure 2A). No differences in moduli were observed between groups (Figure 2B). *Col6a2*$^{-/-}$ and *Col6a2*$^{-/-}$/*Bgn*$^{-/0}$ tendons had higher max stresses than WT and *Bgn*$^{-/0}$ tendons (Figure 2C). During the

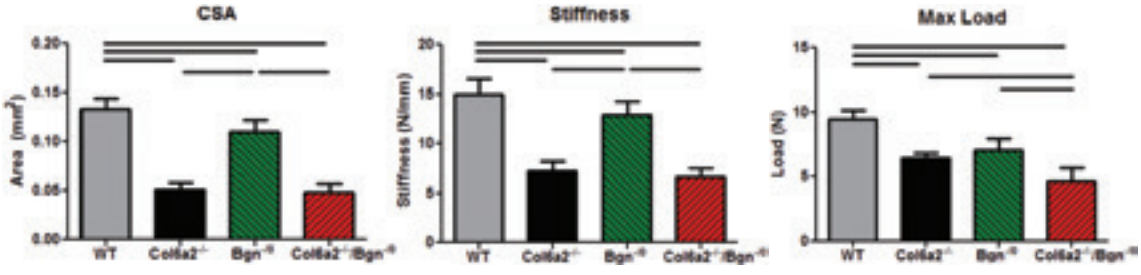

Figure 1. Cross-sectional area and structural-mechanical properties. (A) WT tendons had a larger CSA than all knockout genotypes. **(B)** WT tendons were stiffer than all knockout genotypes. **(C)** WT tendons had a higher max load than all knockout genotypes. Bars indicate $p < 0.05$.

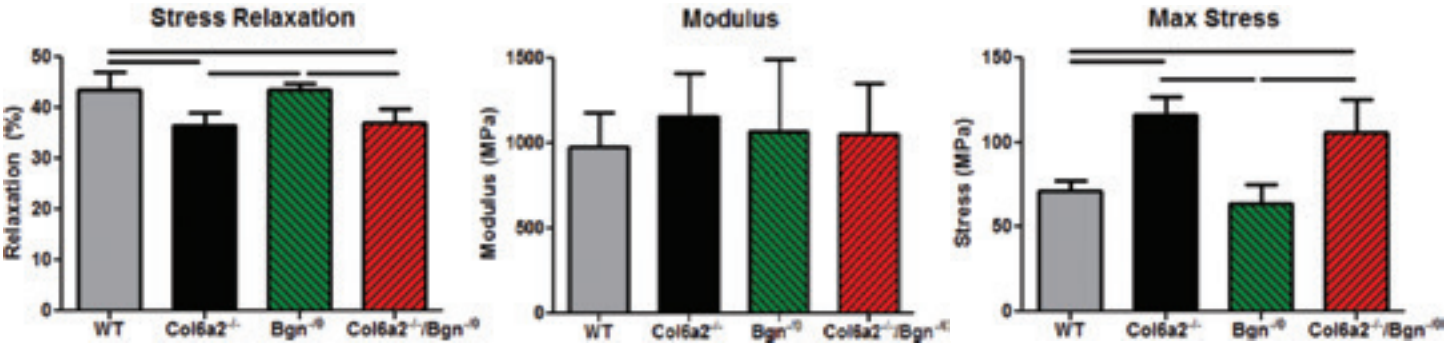

Figure 2. Viscoelastic and material properties. (A) WT and *Bgn*$^{-/0}$ tendons exhibited more stress relaxation than either collagen VI knockout models. **(B)** No differences in moduli were observed between genotypes. **(C)** Both collagen VI knockout tendons had higher max stresses than WT and *Bgn*$^{-/0}$ tendons. Bars indicate $p < 0.05$.

ramp to failure, WT tendons realigned between 3% and 5% strain (Figure 3). Bgn$^{-/0}$ tendons realigned between 5% and 7% strain. *Col6a2*$^{-/-}$ and *Col6a2*$^{-/-}$*/Bgn*$^{-/0}$ tendons realigned between 1% and 3% strain. At 3% and 5% strain, *Col6a2*$^{-/-}$ and *Col6a2*$^{-/-}$*/Bgn*$^{-/0}$ tendons were more aligned, and WT tendons trended towards more alignment, compared to *Bgn*$^{-/0}$ tendons.

Discussion

While biglycan deficiency led to some decreases in FDL structural-mechanical properties (stiffness, max load), collagen VI deficiency led to larger reductions in structural-mechanical and viscoelastic properties. Knockout of biglycan or collagen VI led to smaller, less stiff, and weaker tendons than WT, but *Bgn*$^{-/0}$ tendons were larger and stiffer than *Col6a2*$^{-/-}$ tendons.

Biglycan deficiency led to delayed fiber realignment compared to WT tendons, while collagen VI deficiency led to earlier realignment. *Col6a2*$^{-/-}$ tendons were less viscoelastic than *Bgn*$^{-/0}$ and WT tendons. These results agree with our hypothesis that collagen VI deficiency would reduce tendon mechanical properties more than biglycan deficiency. These mechanical and viscoelastic changes did not correspond to similar differences in material properties (modulus, max stress). There were no differences in moduli between WT, *Bgn*$^{-/0}$, and *Col6a2*$^{-/-}$ tendons, and *Col6a2*$^{-/-}$ tendons had higher max stresses than WT and *Bgn*$^{-/0}$ tendons. Our hypothesis that either knockout would reduce material properties was rejected. The different responses between the structural-mechanical and material properties could be due to smaller CSA in knockout tendons. Contrary to our hypothesis, knocking out both molecules did not amplify the differences seen in the *Col6a2*$^{-/-}$ mice. *Col6a2*$^{-/-}$ and *Col6a2*$^{-/-}$*/Bgn*$^{-/0}$ tendons had similar CSA, stiffness, moduli, max stress, stress relaxation, and fiber realignment. Due to its proximity to tendon cells within the tendon PCM, collagen VI is likely an important regulator of tendon cell behavior. The results of this study suggest that collagen VI regulation is so robust that it dominates any biglycan regulatory effects. This study is limited in that the knockouts are global. Changes in neighboring tissues, such as muscle and bone, may confound the effects of these knockout models on tendon properties specifically. Future studies will aim to elucidate the mechanisms by which collagen VI and biglycan regulate tendon properties.

Another surprising finding in this study is that the biglycan knockout results differ from those of a previous study, which may be due to differences in CSA measurement[4]. The laser device used in the present study is more precise than the previous approach[6]. Overall, this study demonstrates that collagen VI and biglycan play distinct roles in regulating tendon mechanics and that collagen VI has a larger impact on mechanical properties.

This study reveals unique roles of collagen VI and biglycan in tendon mechanics and demonstrates that collagen VI has a larger impact on mechanical properties. These results provide further understanding of the role of lower abundance matrix proteins in tendon function.

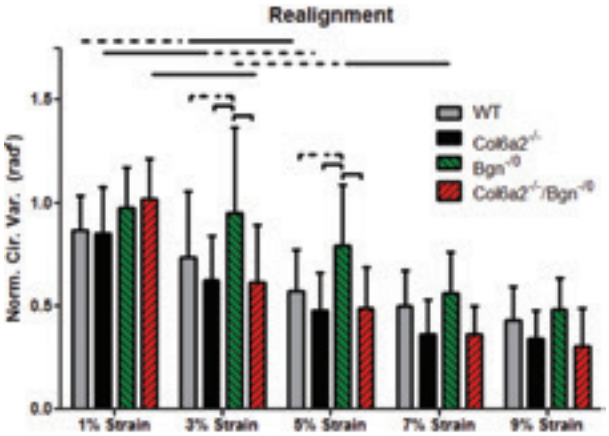

Figure 3. Fiber realignment during ramp to failure. Compared to WT tendons, *Bgn*$^{-/0}$ tendons realigned later. *Col6a2*$^{-/-}$ and *Col6a2*$^{-/-}$*/ Bgn*$^{-/0}$ tendons realigned earlier. A lower circular variance value indicates more alignment. Smooth bars compare adjacent strain values. Notched bars compare between genotypes. Solid bars indicate $p < 0.05$, and dashed bars indicate $p < 0.10$.

References

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