Scleraxis Targeted Deletion of Collagen XI Impairs Tendon Mechanical Function During Postnatal Development

Jordan S. Cohen[1]
Ashley K. Fung[1]
Stephanie N. Weiss[1]
Nathaniel A. Dyment[1]
Louis J. Soslowsky[1]

[1]McKay Orthopaedic Research Lab
University of Pennsylvania

Introduction

Mutations in the *Col11a1* gene are implicated in Type II Stickler Syndrome and result in joint problems including hypermobility and early arthritis.[1] Beyond its role in Stickler Syndrome, collagen XI is highly expressed during tendon postnatal development and interacts with collagen I and II during heterotypic fibril formation.[2] We previously showed that during patellar tendon postnatal development, tendon-targeted (ScxCre) collagen XI deficiency disrupts tendon structure, resulting in tenocyte nuclear disorganization, larger diameter collagen fibrils, and increased tendon length.[3] However, whether these structural findings result in alterations in tendon mechanical function is unknown. Therefore, the objective of this study was to define the role of collagen XI in the acquisition of tendon mechanical function during postnatal development using tendon-targeted collagen XI knockout mice. We hypothesized that tendon-targeted collagen XI knockout would result in inferior tendon mechanical properties.

Methods

Tendon-targeted ScxCre;*Col11a1*$^{flox/flox}$ (KO), ScxCre;*Col11a1*$^{flox/wt}$ (HET), and Cre-littermate control (CTRL) mice were used (IACUC approved). Patellar tendons were harvested at postnatal days (P) 10, 20, and 30 for mechanical testing (n = 12/group). The tibia-patellar tendon-patella complex was dissected, tendon cross-sectional area was measured, and the tibia was potted in polymethylmethacrylate. The patella was gripped with sandpaper (P10 and P20) or clamped directly (P30) using custom fixtures, and tendons were subjected to a protocol consisting of preconditioning, stress relaxations at 3, 4, and 5% strain each followed by a dynamic frequency sweep (0.1-10 Hz), and a ramp to failure at 0.1% strain/s. For each age, genotypes were compared using a one-way ANOVA with Tukey post-hoc tests. Significance was set at $p \leq 0.05$.

Results

Tendon-targeted collagen XI knockout resulted in substantial alterations in patellar tendon mechanical properties. Cross-sectional area (Figure 1A) of KO tendons was greater only at P10, while KO tendons (Figure 1B) were significantly longer than CTRL tendons at all ages, consistent with previous histological findings. Stiffness (Figure 1C) and failure load (Figure 1D) of KO tendons were dramatically reduced compared to CTRL and HET tendons. Material properties of KO tendons showed similar trends, with marked reduction in modulus (Figure 1E), failure stress (Figure 1F), and failure strain (Figure 1G) at all ages compared to CTRL and HET tendons. Dynamic modulus was similarly reduced in KO tendons at all ages (data not shown).

Discussion

Previous work evaluating the role of collagen XI in postnatal development found that tendon-targeted knockout disrupts tendon structure, resulting in tenocyte nuclear disorganization, larger diameter collagen fibrils, and increased tendon length.[3] Results of the present study further elucidate the importance of collagen XI in regulating tendon structure-function during early postnatal development. The mechanical differences in KO mice were particularly striking, with KO patellar tendons demonstrating substantial reductions in modulus of 45%, 78%, and 60% at P10, P20, and P30, respectively. These findings are consistent with previous mechanical findings in mature (day 60) KO tendons[4,5] but the emergence of these stark mechanical differences as early as postnatal day 10 point to a critical and previously undescribed role for collagen XI in the initial establishment of tendon hierarchical structure. In tendon, collagen XI is most highly expressed during embryonic development with minimal expression during adulthood,[2] and therefore, inferior mechanical function in KO tendons may be due to alterations in fibril nucleation and assembly. Additionally, motivation for this study was primarily driven by the observation of significant patellar tendon lengthening and

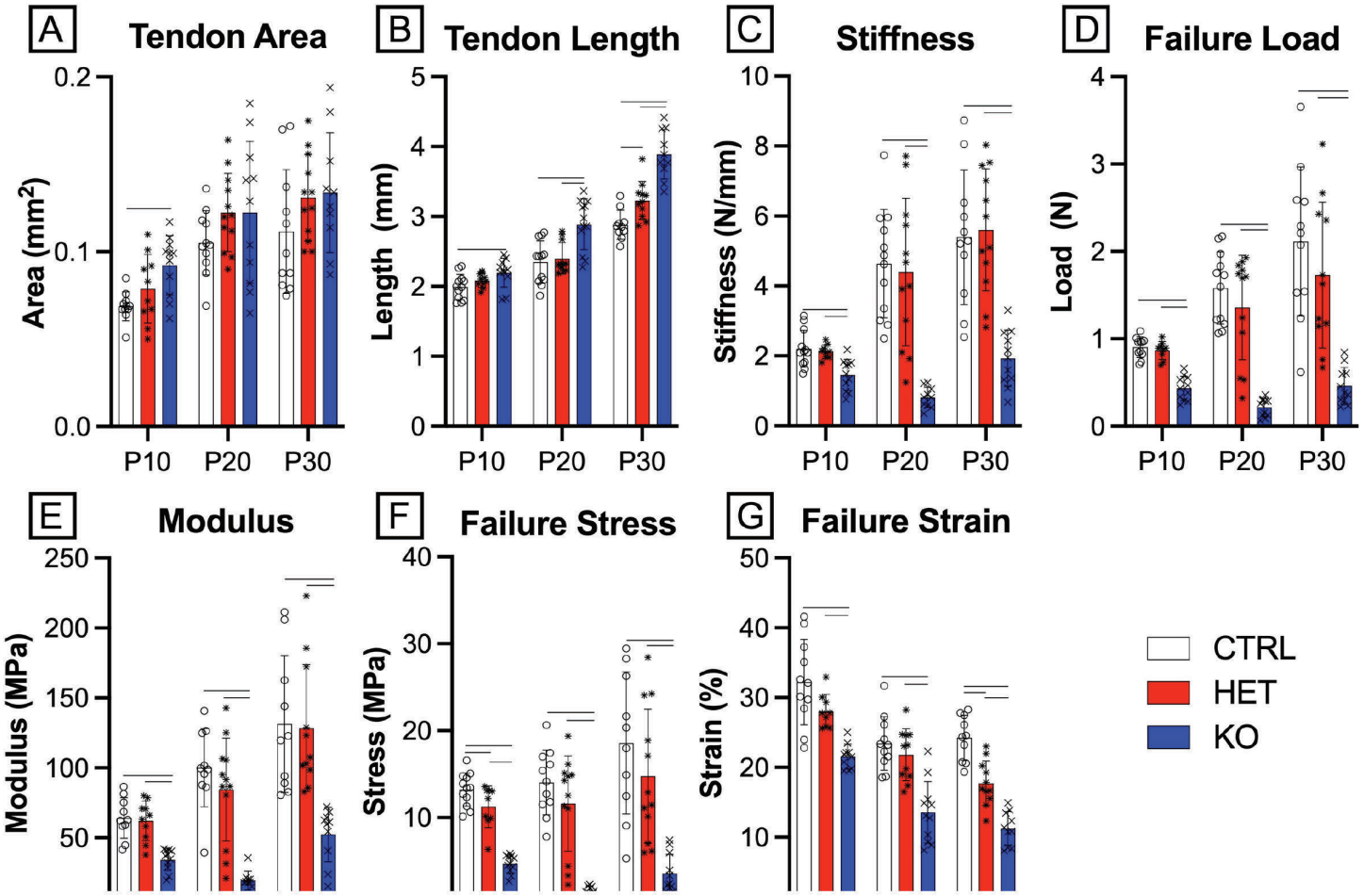

Figure 1. Patellar tendon. **(A)** cross-sectional area was higher in KO tendons at p10; **(B)** tendon length was greater at all ages; **(C)** Stiffness and **(D)** failure load were significantly reduced in KO tendons at all ages compared to CTRL and HET tendons; **(E)** Elastic modulus; **(F)** failure stress; and **(G)** failure strain were significantly reduced in KO mice compared to CTRL and HET tendons at all ages. (—p<0.05)

the presence of granulation tissue in mature tendons. Our findings suggest the absence of collagen XI leads to the deposition of substantially weakened matrix, resulting in injury and elongation following the initiation of ambulation around postnatal day 10. Preliminary gene expression findings at P10 (not shown) support a pathological response with increased expression of genes associated with non-collagenous matrix proteins and remodeling, and studies are ongoing to further elucidate the mechanisms underlying the role of collagen XI in tendon development.

Significance

Collagen XI is critical in the establishment of tendon structure and mechanical function during postnatal tendon development. These findings highlight the need to further define the regulatory role of collagen XI in tendon development and healing, which could lay the foundation for future therapeutic applications.

Acknowledgements

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References

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