Collagen III Haploinsufficiency Alters Fibril Size but Not Mechanical Properties in Uninjured, Young Adult Male Murine Tendons

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Introduction

Clinically, *Col3a1* mutations present as vascular Ehlers-Danlos syndrome (vEDS), a rare but life-threatening condition due to abnormalities in the matrix of the vasculature and hollow viscera. Additional patient morbidity from tendon pathology[1,2] indicates a consequential role for type III collagen (Col3) in tendon. While a role for Col3 in matrix homeostasis has been established in the vasculature,[3] cutaneous skin,[4] articular cartilage,[5] meniscus,[6] and bone,[7] the involvement of Col3 in tendon structure and function is poorly understood. Early investigations focused on understanding the role of Col3 in female murine tendon,[8] but biological sex is known to influence tendon health, Col3 levels,[9] and vEDS presentation,[10] motivating investigation of the role of Col3 in male tendon. Therefore, the objective of this study was to elucidate the role of Col3 in tendon homeostasis using a murine model of male vEDS. We hypothesized that Col3 haploinsufficiency would alter fibrillogenesis and fibril maintenance yielding decreased large diameter fibrils and mechanically inferior tendons.

Methods

Tendons from male wild-type (WT) Balb/cJ and heterozygous *Col3a1*$^{+/-}$ mice at 90 days of age were assessed (IACUC approved). Patella-patellar tendon-tibia complexes were dissected and prepared as described[11] for mechanical testing (n ≥ 9/group). Tendons were assessed with a viscoelastic testing protocol consisting of: 1) preconditioning, 2) stress relaxation at 3% and 6% strain with a subsequent sinusoidal frequency sweep (10 cycles at 0.1, 1, 5, and 10 Hz) at each strain level, 3) return to gauge length, and 4) ramp to failure at a strain rate of 0.1% strain/s. Images were captured during the ramp to failure for elastic modulus measurement. Patellar tendons were fixed, processed, and imaged using transmission electron microscopy as described[12] to measure collagen fibril diameters (n = 3/group). T-tests were used to determine the impact of genotype on mechanical properties. A Kolmogorov-Smirnov test was used to assess the effect on collagen fibril diameter distributions. Significance was set at $p \leq 0.05$.

Results

Compared to WT tendons, *Col3a1*$^{+/-}$ tendons were not significantly different in any quasistatic or viscoelastic mechanical property including stiffness (Figure 1A), elastic modulus (Figure 1B), dynamic modulus (Figure 1C), phase shift (Figure 1D), and percent relaxation (data not shown). In contrast, collagen fibril size distributions were significantly different between genotypes (Figure 2, $p < 0.0001$). WT tendons had a characteristic bimodal fibril diameter distribution (Q1: 70.0 nm, Q2: 116.5 nm, Q3: 154.2 nm). *Col3a1*$^{+/-}$ tendons had a tighter fibril diameter distribution (Q1: 80.6 nm, Q2: 125.0 nm, Q3: 154.0 nm) with a smaller proportion of small diameter fibrils (< 70 nm) and a greater proportion of intermediate and large diameter fibrils (> 110 nm).

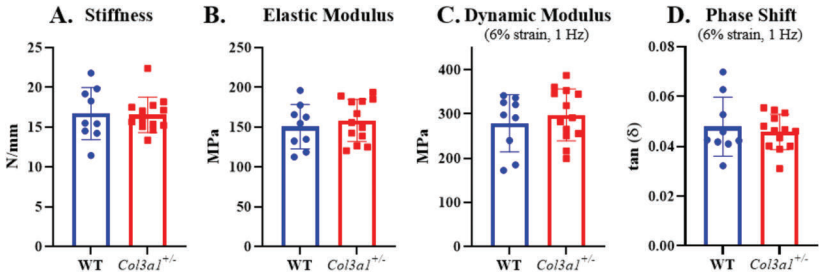

Figure 1. Collagen III haploinsufficiency does not impact male tendon mechanical properties. No differences in stiffness **(A)**; elastic modulus **(B)**; dynamic modulus **(C)**; phase shift **(D)**; or percent relaxation (data not shown) were observed between genotypes. No differences were seen in other strain or frequency levels (data not shown).

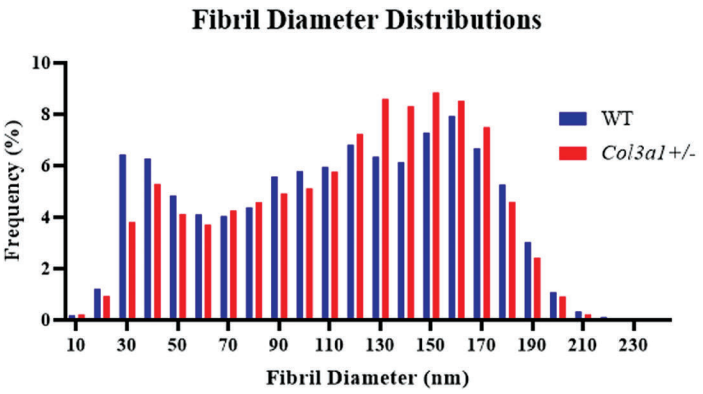

Figure 2. Collagen III haploinsufficiency alters collagen fibril size. Collagen fibril diameter distribution was characteristically bimodal in WT tendons. *Col3a1*$^{+/-}$ tendons had significantly different fibril distribution ($p < 0.0001$) with a lower proportion of smaller fibrils (< 70 nm) and a higher proportion of intermediate and large fibrils (> 110 nm).

Discussion

We studied the role of Col3 in tendon homeostasis using a murine model of male vEDS. In contradiction to our hypothesis, Col3 haploinsufficiency in young adult male mice did not alter uninjured patellar tendon quasistatic or viscoelastic mechanical properties. However, in support of our hypothesis, Col3 haploinsufficiency did significantly alter the fibril diameter distribution. This alteration in matrix structure may have mechanical consequences in settings where fibrillogenesis and maintenance are altered, such as in injury and/or aging. Importantly, genotype-dependent changes to fibril diameter in male mice differ from those observed in female mice. In developing female mice, Col3 haploinsufficiency resulted in a decrease in large diameter fibrils, 8 in contrast to the increase in large diameter fibrils observed in the present study. Interestingly, this indicates a differential influence of biological sex on tendon matrix structure in the context of Col3 haploinsufficiency. Conclusions from this study should be interpreted in the context of a conventional haploinsufficiency mouse model which conflates developmental and regulatory effects. Moreover, dose-dependent effects of Col3 insufficiency cannot be investigated due to perinatal lethality of *Col3a1*$^{-/-}$ mice. Further, in recognition of the critical importance of Col3 in the provisional healing matrix, subsequent investigations will define the effects of Col3 knockdown in injury and advanced aging contexts. Future studies will leverage the power of inducible Col3 knockdown to further delineate the sex-, dose-, and age-dependence of the tendon response to Col3 knockdown in homeostatic and injury environments.

Significance

In addition to direct implications for patients with Col3a1 mutations, insights from this study reveal contributions of Col3 to tendon structure which serve as an important foundation for future investigations of sex-based differences in the regulatory role of Col3.

Acknowledgements

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