

# Collagen Fibril Deformation is Not Observed in Developing Mouse Patellar Tendon Regardless of Collagen XI Expression

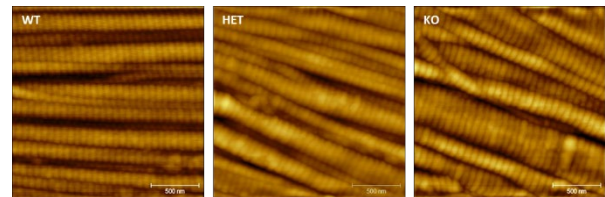
Jaime A. Santillan, Jeremy D. Eekhoff, Stephanie N. Weiss, Louis J. Soslowsky  
McKay Orthopaedic Research Laboratory, University of Pennsylvania, Philadelphia, PA  
[Jaime.santillan@pennmedicine.upenn.edu](mailto:Jaime.santillan@pennmedicine.upenn.edu)

**Disclosures:** Jaime A. Santillan (N), Jeremy D. Eekhoff (N), Stephanie N. Weiss (N), Louis J. Soslowsky (N)

**INTRODUCTION:** The development of functional tendons requires proper collagen assembly in hierarchical structures [1]. As part of this process, collagen XI co-assembles with collagens I and II during heterotypic fibril formation and its disruption leads to abnormal extracellular matrix development [2]. Deficiency of collagen XI disturbs tendon structure, causing nuclear disorganization, increased lateral growth of fibrils, and degradation of mechanical properties [1,3]. However, how nanoscale mechanisms contribute to the weakened mechanical integrity of collagen XI deficient tendon is unknown. Therefore, the goal of this study was to elucidate the role of collagen XI in the fibril deformation mechanisms in developing mouse patellar tendon. We hypothesized that tendon-targeted knockout of collagen XI would result in increased deformation of collagen fibrils.

**METHODS:** Male and female wild-type control (WT), *Scx-Cre;Coll1a1<sup>flax/WT</sup>* heterozygous (HET), and *Scx-Cre;Coll1a1<sup>flax/flax</sup>* knockout (KO) mice at 30 days of age were used (n=7-8/group, IACUC approved). Tibia-patellar tendon-patella complexes were isolated, and the cross-sectional area of all samples was measured using a custom laser-scanning device. The tibia was embedded with polymethyl methacrylate (PMMA) and the patella was gripped with sandpaper and secured in a metal fixture. Tendons were preloaded to 0.025N and subjected to a testing protocol consisting of 10 cycles of preconditioning followed by a 1-minute hold. Based on loading curves obtained from preliminary quasi-static ramp-to-failure data using the abovementioned mouse models [4], tendons were strained at a rate of 0.1% strain/second to the toe region (1% strain for all genotypes) or linear region (8.9%, 6.2%, or 4.4% strain for WT, HET, or KO, respectively) of the stress-strain curves. Then, the samples were flash-frozen at the target strain, precisely cut free from the tibia and patella, and placed in a cryo-embedding medium. Subsequently, tendons were cryo-sectioned at 20µm thickness and fixed in formalin. To observe changes at the nanoscale, a Bioscope Catalyst Atomic Force Microscope was used in tapping mode on tendon sections to obtain topographical images of the sample surfaces. A minimum of five 2.0µm x 2.0µm images at a resolution of 512x512 pixels were acquired within 1mm of the tibial insertion, where tissue strains were expected to be the greatest [5], across 3 sections per sample. The length of the d-band periodicity of collagen fibrils was measured using Fourier analysis in MATLAB. Changes in the average d-period with applied tendon strain were taken as indicative of fibril deformation, and changes in the average variance in the d-period within individual images and across the entire sample were taken as indicative of local and global variation in fibril deformation, respectively [6]. Data was analyzed using two-way ANOVAs with the main effects of genotype and applied strain (i.e., toe vs. linear regions). Significance was set at  $p < 0.05$ .

**RESULTS:** Qualitative analysis of the AFM images revealed the presence of a heterogeneous population of collagen fibrils with larger diameters in the HET and KO groups compared to WT controls (Fig. 1), showing evidence of the poorly regulated lateral growth of fibrils. Despite this difference, the average d-period length was not different across genotypes and did not change across strain levels (Fig. 2A). Similarly, local and global variance in the collagen fibril d-period were not affected by genotype or applied strain (Fig 2B, C).



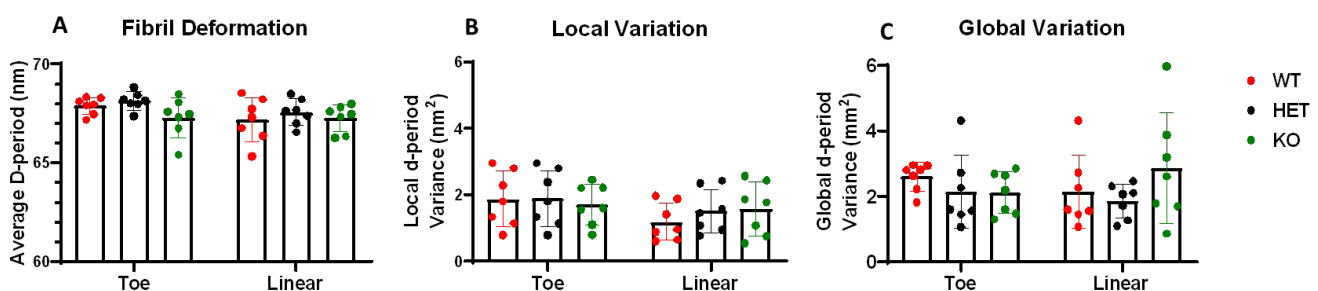
**Figure 1.** Representative AFM images of collagen fibrils of the three different genotype groups (Wild-type, heterozygous, and knockout group, respectively)

**DISCUSSION:** Tensile mechanical testing of tendons was performed to apply macroscale strains and subsequently measure the fibril deformation of collagen fibrils at the nanoscale. We found that tendon-targeted collagen XI knockout disrupted the nanoscale organization and increased the heterogeneity of fibril morphology. This abnormal fibril structure is consistent with prior work that used transmission electron microscopy to observe the collagen fibrillar matrix [1]. Based on previous studies, fibril deformation was expected when different levels of strain were applied to the tendon [6,7]. Contrary to our hypothesis, the fibril d-period did not increase over the applied strain range in the patellar tendon. This result may indicate differences in the nanoscale loading mechanism that occurs within the patellar tendon in comparison to other tendons, which could be due to the patellar tendon's unique structure with two bony attachments. Further work is needed to explain these surprising findings and investigate the role of collagen XI in the deformation mechanism of other tendons.

**SIGNIFICANCE:** Collagen XI regulates collagen fibrillogenesis and is essential during the development of tendons. This study shows that the deficiency or absence of collagen XI causes structural changes in collagen fibrils at the nanoscale and emphasizes its importance in the assembly of tendon hierarchical structure.

**ACKNOWLEDGEMENTS:** This study was supported by NIH/NIAMS (R01AR073231) and the Penn Center for Musculoskeletal Disorders (P30 AR069619)

**REFERENCES:** [1] Sun et al. *Matrix Biol* 2020. [2] Linsenmayer et al. *J Cell Biol* 1993. [3] Holmes et al. *Current Topics in Developmental Biology* 2018. [4] Cohen et al. *ORS* 2022. [5] Gilday et al. *J Biomech* 2013. [6] Connizzo BK et al. *J Biomech* 2014. [7] Rigozzi et al. *Journal of Structural Biology* 2011.



**Figure 2.** Fibril deformation (A), local variation (B), and global variation (C) were unaffected by genotype and applied strain. Data is shown as mean  $\pm$  SD.