Matrix Assembly is Guided by the Coordination of Collagens V and XI during Early Postnatal Tendon Development

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INTRODUCTION: At the nanoscale, minor fibril-forming collagens such as collagen V (Col5) and collagen XI (Col11) assemble with major fibrillar collagens (collagen I and collagen II) to form heterotypic fibrils[1] – the basis of tendon structure and mechanical strength. Col5 and Col11 are highly expressed throughout postnatal tendon development,[2] share similar molecular structures,[1] and contribute to the nucleation and lateral growth of collagen fibrils.[1,2] These similarities indicate that coordination between these collagens may be essential for proper matrix assembly, particularly during the transition between matrix templating and expansion. The objective of this work is to evaluate the cooperative roles of Col5 and Col11 during early postnatal development by comparing tendons with simultaneous tendon-targeted reduction in *Col5a1* and *Col11a1* (VHet-XIHet, VKD-XIHet) to those with *Col5a1* reduction only (VHet, VKD). We hypothesized that processes during development rely more on Col5 and Col11 coordination than Col5 regulation alone; thus, tendons with reduced *Col5a1* and *Col11a1* expression will have inferior mechanical properties and altered fibril diameters, while these effects will be minor in tendons lacking only *Col5a1*.

METHODS: Animals: Patellar tendons (PTs) were collected from 86 male and female postnatal day 10 (p10) mice with tendon-targeted (ScxCre) reductions in *Col5a1* and/or *Col11a1* (VHet: $Col5a1^{f/+}$, VHet-XIHet: $Col5a1^{f/+};Col11a1^{f/+}$, VKD: $Col5a1^{f/f}$, VKD-XIHet: $Col5a1^{f/f};Col11a1^{f/+}$) and ScxCre- littermates (Ctrl, IACUC approved). Mechanics (n≥10/genotype): Patella-PT-tibia complexes were dissected, PT cross-sectional area (CSA) was measured, stain lines were applied for optical tracking, patella was secured in custom grips, and tibia was potted in polymethylmethacrylate. PTs were preloaded to 0.03N and underwent a viscoelastic test: preconditioning, stress relaxations at 2% and 4% strain followed by sinusoidal frequency sweeps (0.1Hz, 1Hz, 5Hz, 10Hz), and a quasistatic ramp to failure (0.1% stain/sec). VKD-XIHets only underwent preconditioning and ramp to failure due to early failure at high strain rates. Fiber alignment was assessed during the ramp to failure using reflectance-mode polarized light imaging and quantified as variance in the angle of polarization (AoP). Transmission Electron Microscopy (n=4/genotype): PTs were dissected, fixed, stained, sectioned, and imaged as described.[3] Fibril diameters were quantified using MATLAB. Tendon Morphology & Cell Properties (n≥4/genotype): Knees were fixed, cryoembedded, sectioned, and stained with Hoechst and toluidine blue to visualize nuclei and tendon morphology. Statistics: Mechanical properties, tendon length, cell density, and average nuclear shape were compared with one-way ANOVA followed by Bonferroni corrected t-tests when indicated ($p \leq 0.05$). Fibril diameters were compared with Kolmogorov-Smirnov tests.

RESULTS: Mechanics: VKD-XIHet PTs had smaller cross-sectional area (Fig 1A) and decreased maximum stress (Fig 1C). While VKD PTs did not show altered CSA, they had decreased maximum stress (Fig 1C) and reduced relaxation at 4% strain (Fig 1D). VHet and VHet-XIHet had moderate decreases in maximum stress (Fig 1C), and VHet-XIHet had reduced relaxation at 4% strain compared to VHet (Fig 1D). There was no change in modulus in any genotype (Fig 1B). All PTs reached similar levels of alignment prior to failure, but VKD and VKD-XIHet PTs spent less time at maximal realignment before failure (Fig 1E). Moreover, at 25% of failure strain VKD and VKD-XIHets had higher variance in AoP, and at 50% of failure strain, the higher variance persisted for VKD PTs (Fig 1F). Fibril Diameter: VHet-XIHet and VKD-XIHet PTs have drastically altered fibril populations with an increase of ~15nm in the median fibril diameter and a doubling of the interquartile range (Fig 2A-C). Conversely, Ctrl, VHet, and VKD have nearly identical fibril distributions. Tendon Morphology and Cell Properties: PT length was unchanged across genotypes (Fig 3A). VKD-XIHets had increased cell density (Fig 3B) and more elongated nuclear shapes (Fig 3C-D). VKD PTs also had higher nuclear aspect ratios compared to their VHet counterparts (Fig 3C-D). VKD-XIHet PTs insert more distally on the tibial tuberosity (red arrow) and joints have regions of hypercellular tissue surrounding the collateral ligaments (black arrow, Fig 3D).

DISCUSSION: During postnatal tendon development, matrix is deposited and expanded to achieve an organized hierarchical structure. We show that this process is regulated through synergism between Col5 and Col11. VKD-XIHet PTs have reduced failure properties, a lack of mechanical integrity at maximal alignment, dysregulated fibril growth, increased cell density, and altered nuclear phenotype, while tendons in VKD mice show deficient failure properties and only minimal changes in fibril morphology or cell properties. These findings align with established roles of Col5 and Col11 in fibril nucleation and control of lateral growth as well as a proposed role in cell-matrix binding and suggest that regulation by Col5, Col11, or their coordination may be specific to different developmental phases. Prior work at p30 showed dramatic decreases in elastic and failure properties with increases in fibril diameter in VKD-XIHet compared to VKD,[4] highlighting that Col5/Col11 coordination may be particularly important between p10 and p30 when matrix assembly switches from the deposition of new fibrils to fibril growth.[5] There may also be temporal roles for Col5 and Col11 in isolation. Current findings contradict the canonical role of Col5 in tendon by indicating that the role of Col5 is less influential during early development; however, by maturity, loss of Col5 alone resulted in fibril dysregulation and inferior mechanics,[6] highlighting a potential role in matrix maturation. Conversely, loss of Col11 alone led to altered fibril morphology, substantially reduced mechanics, and increased non-collagenous matrix expression as early as p10[7] suggesting a role in early matrix templating. Future studies will evaluate gene expression in these mouse models and investigate the temporal and coordinated nature of Col5 and Col11 in matrix assembly during development.

SIGNIFICANCE: Col5a1 (collagen V), Col11al (collagen XI), and their cooperation are critical regulatory elements that highlight the control of matrix assembly and cell organization during development necessary for optimal tendon function.

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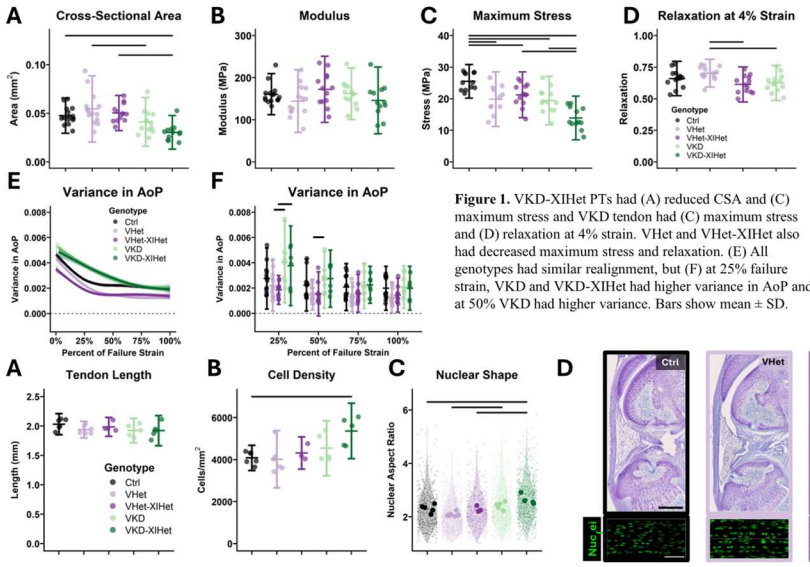

Figure 1. VKD-XIHet PTs had (A) reduced CSA and (C) maximum stress and VKD tendon had (C) maximum stress and (D) relaxation at 4% strain. VHet and VHet-XIHet also had decreased maximum stress and relaxation. (E) All genotypes had similar realignment, but (F) at 25% failure strain, VKD and VKD-XIHet had higher variance in AoP and at 50% VKD had higher variance. Bars show mean ± SD.

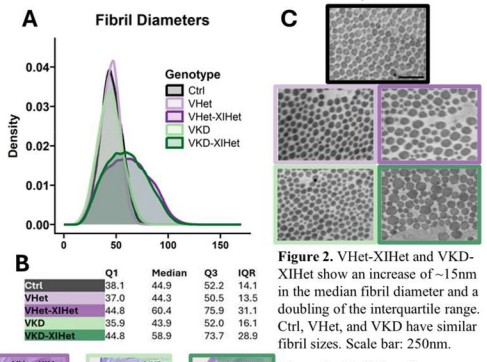

Figure 2. VHet-XIHet and VKD-XIHet show an increase of ~15nm in the median fibril diameter and a doubling of the interquartile range. Ctrl, VHet, and VKD have similar fibril sizes. Scale bar: 250nm.

Figure 3. (A) PT length was unchanged across genotypes. (B) Cell density was increased in VKD-XIHet and (C, D) nuclear aspect ratio was higher in VKD and VKD-XIHet. (D) VKD-XIHet PTs insert more distally on the tibial tuberosity (red arrow) and have hypercellular tissue in the joint space (black arrow). Scale bars: 500μm (tendon), 50μm (nuclei). Bars show mean ± SD.