Structure and Composition in Tendons with Altered Collagen V Expression are Location-Dependent

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Introduction

Classic (type I) Ehlers-Danlos Syndrome (EDS) is a rare genetic disease associated with mutations in collagen V.[1] Patients with classic EDS exhibit connective tissue hyperelasticity and laxity. While collagen V is a quantitatively minor component of collagen fibrils in tendons, it plays a critical role during fibrillogenesis.[2] Recent studies have found that the regulatory role of collagen V in establishing the mechanical properties of tendons is tissue-dependent, with major alterations occurring in the supraspinatus tendon.[3] However, the role of collagen V in determining the regional structure and composition of this particular tendon is still unknown. Therefore, the objective of this study was to investigate structural and compositional changes associated with decreased expression of collagen V at both the insertion site and midsubstance of the supraspinatus tendon. We hypothesized that the fibril structure would be altered (i.e., increased diameter, reduced density) in both locations with decreasing collagen V expression, but that no changes would be present in the cell morphology or extracellular matrix composition at either location.

Methods

Male mice of three genotypes, *Col5a1*$^{+/+}$ (WT), *Col5a1*$^{+/-}$ (HET), and a tendon/ligament-targeted conditional knockout, *ScxCre+Col5a1*$^{-/-}$ (NULL) were sacrificed at P120 (IACUC approved). *Electron Microscopy:* Samples for TEM (n=10/group) were prepared as described.[4] Ten non-overlapping cross-sectioned digital images were obtained from the central areas of each specimen. Diameters were measured along the minor axis of cross sections. Fibril density was obtained as the fibril number per unit area. A measure of fibril roundness, fibril irregularity factor (FIF), was defined as the ratio of the radius as determined from a circle with the fibril's perimeter to the radius as determined from a circle with the fibril's area, where increasing values would define an increasing number of folds along the surface of the fibril. *Histology:* Histological samples (n = 8/group) were harvested from the shoulder and processed for paraffin-embedding. Coronal sections (7 μm) were stained with H&E. Each sample was then evaluated for cellularity and cell shape (tendon proper only).[5] *Biochemistry:* Biochemistry samples (n = 20/group) were prepared for analysis (tendon + surrounding sheaths) as described.[6] DNA content and glycosaminoglycan (GAG) content were quantified using the PicoGreen and dimethylmethylene blue assays, respectively. The remaining digest was hydrolyzed, resuspended, and used to quantify total collagen (COL) using the hydroxyproline assay and pyridinoline crosslinks (PYD) using the MicroVue PYD ELISA kit. GAG and COL content were normalized to DNA content and PYD was normalized to total COL. *Statistics:* Statistical comparisons were made using one-way ANOVAs with post-hoc Bonferroni tests. Statistical significance was set at $p < 0.05$ and a trend at $p < 0.10$.

Results

At the midsubstance, fibril diameter was significantly increased and fibril density was decreased in the heterozygous and null groups (Figure 1). The distribution of fibril diameters broadened from wild type to null to include fewer small diameter fibrils and increased large diameter fibrils (not shown). At the insertion site, fibril diameter was increased in the heterozygous group and fibril density was slightly decreased in the null group (Figure 1). The insertion site fibril diameter distribution displayed an increased number of small and large diameter fibrils in the experimental groups (not shown). In addition, irregular fibril shapes were present at the insertion site of the tendon in the null tendons only (Figure 1C). Cell density was slightly decreased in the null group at the insertion site compared to wild type (Figure 2A). Cell shape was not different between the groups in either region (Figure 2B). There also appeared to be pockets of hypercellular non-fibrillar tissue in between fibers or fascicles in the null group that were not present in the other groups (not shown). At the midsubstance, there were no differences between groups in DNA, GAG, or COL content (Figure 3). At the insertion site, DNA was increased while GAG and COL content were decreased in the null group (Figure 3A-C). Additionally, PYD crosslinks were increased in the heterozygous group at the insertion site and in the null group at both locations (Figure 3D).

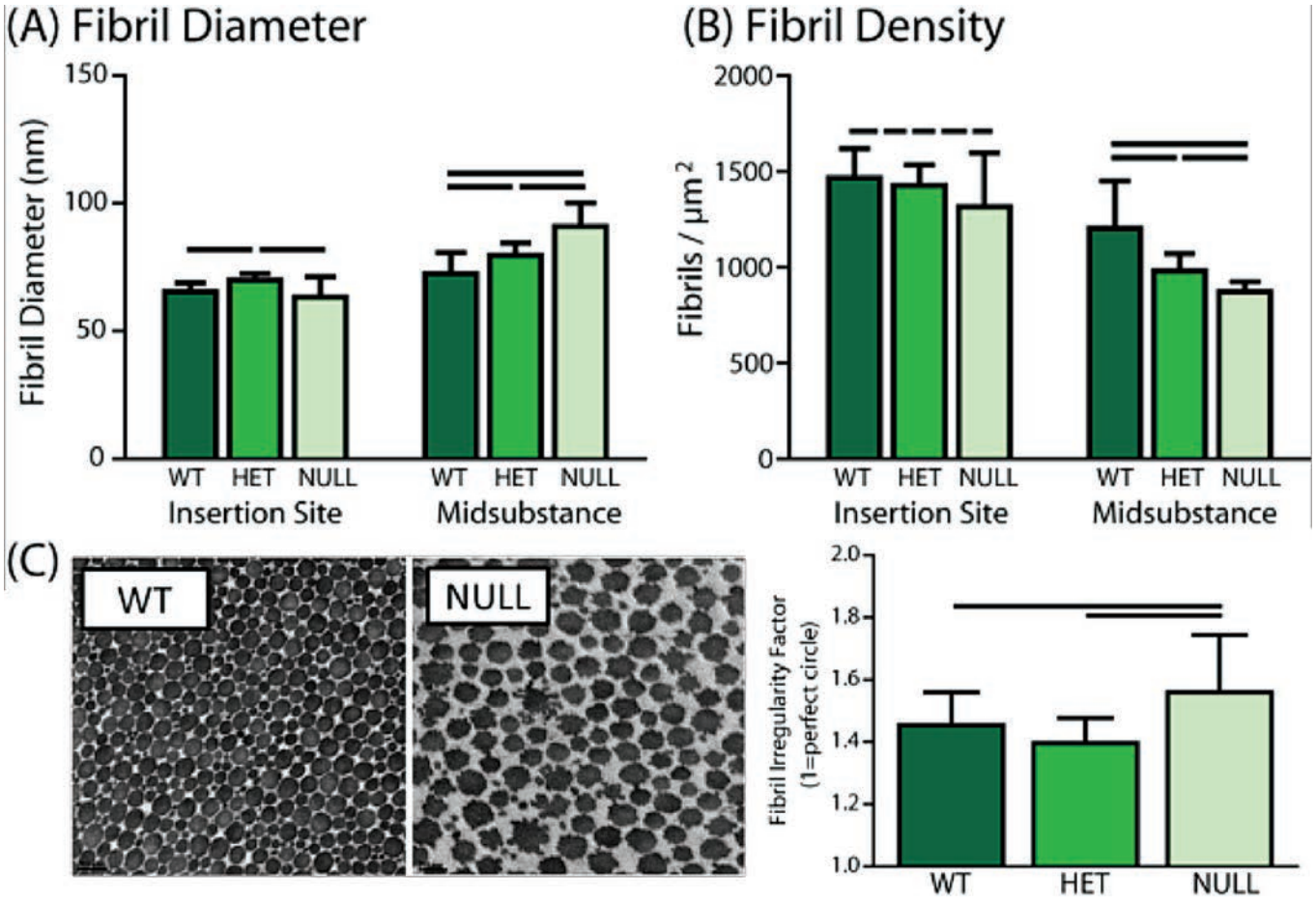

Figure 1. (A) Fibril diameter was increased in the heterozygous groups at the insertion site and midsubstance and in the null group at the midsubstance (significant). (B) Fibril number was decreased in the experimental groups at the midsubstance (significant). (C) Irregular fibril morphology was present in the null tendon insertion site both qualitatively and quantitatively.

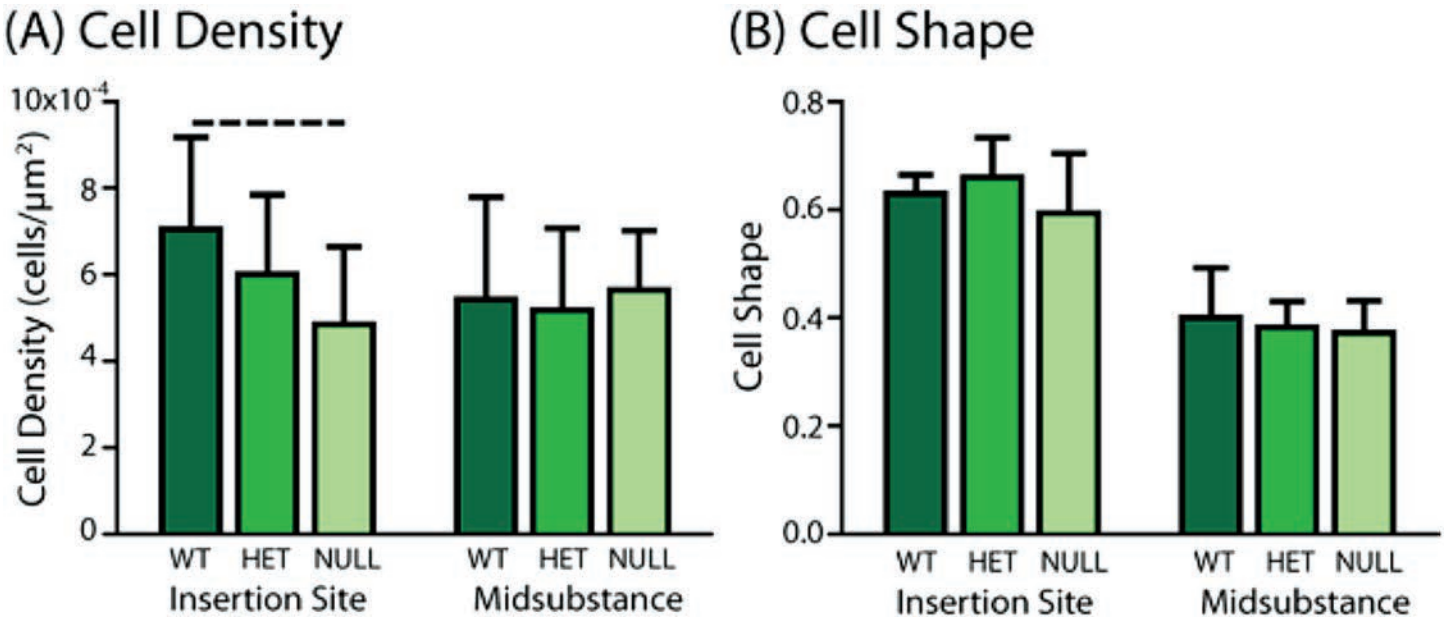

Figure 2. (A) Cell density was decreased in the null group at the insertion site only (trend, $p < 0.1$). (B) Cell shape was not different between groups in either region (significant, $p < 0.05$). Data presented as mean ± SD.

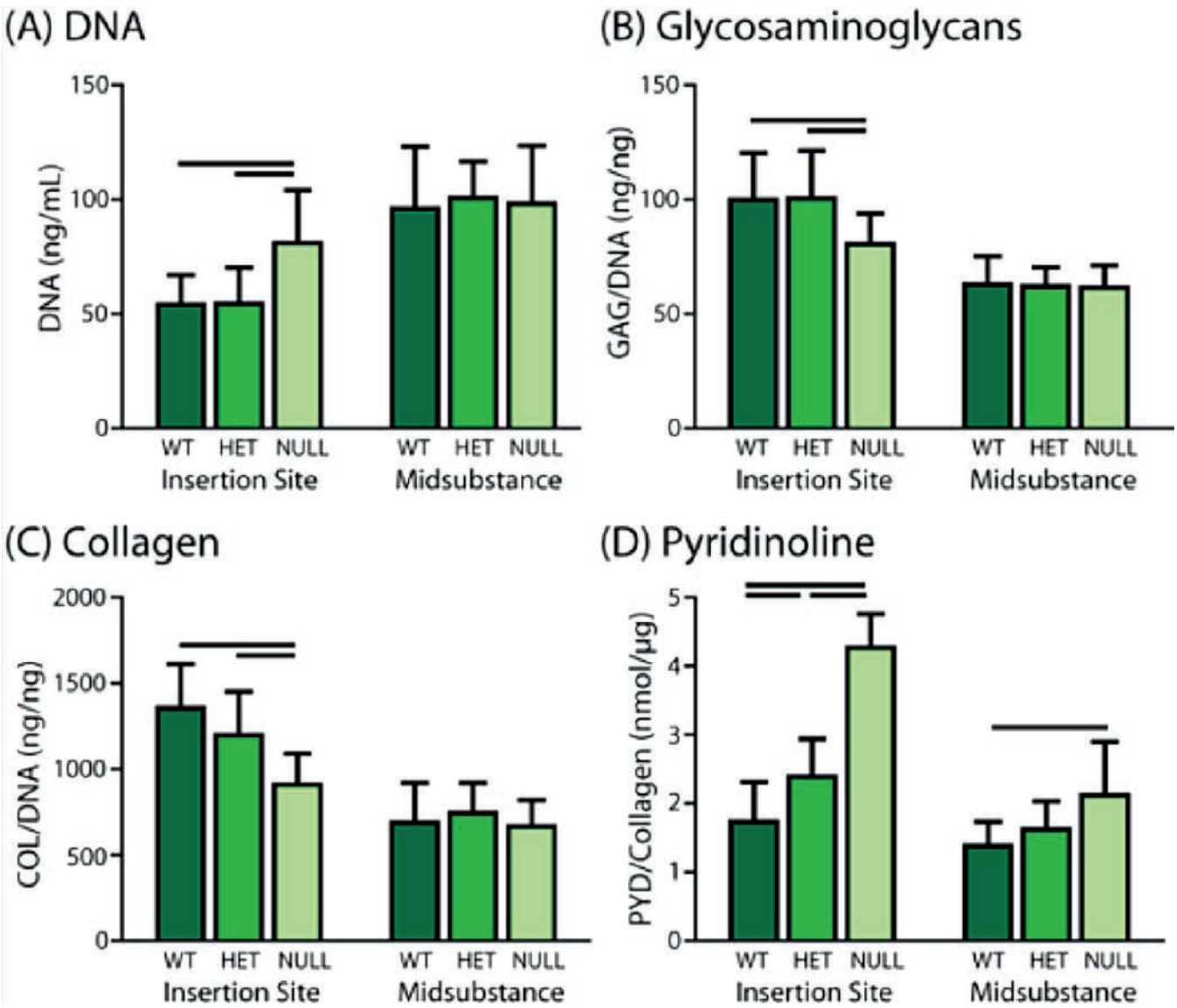

Figure 3. (A) DNA content was increased in the null group at this insertion site (significant). (B) GAG content and (C) COL content was decreased at the insertion site (significant). (D) PYD density was increased in the null group at both regions and in the heterozygous group at the insertion site (significant).

Discussion

Fewer, but larger, collagen fibrils were found with reduced collagen V expression in both the heterozygous and null groups at the midsubstance, consistent with previous literature.[7,8] These changes were present with no other alterations in cell density, cell morphology, collagen content or GAG content, as expected. However, these structural changes were not mimicked at the insertion site. At the insertion site, slightly reduced fibril density was quantified that was not accompanied by an increase in fibril diameter. Instead, increases in both small and large diameter fibrils were present in the null group, demonstrating differences in the distribution of fibril diameters (data not shown). Furthermore, many null tendons had irregularly shaped fibrils, suggesting the packing of collagen molecules inside the fibril may have been disrupted. This finding is currently being investigated further. Although cell density was slightly decreased at the insertion site in the tendon proper, DNA content of the tendon and associated sheaths was significantly increased due to the increased amount of hypercellular tissue between collagen fibers in the null tendons. The insertion site also had decreased collagen and GAG content, alluding to alterations in the viscoelasticity of the tissue shown recently.[9] Finally, pyridinoline, a mature collagen crosslink, was significantly increased with the reduction of collagen V expression in both regions, suggesting an alteration in collagen fibril processing that has not been previously described. This result could explain alterations in the shape of collagen fibrils and will be the subject of further study.

Significance

These studies define a crucial location-dependent role for collagen V in developing supraspinatus tendon structure and composition.

Acknowledgements

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