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Collagen V Null Mice Have Decreased ACL Mechanical Properties and Altered Fibril Morphology

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Introduction

Classic (type I) Ehlers-Danlos Syndrome (EDS) is a rare genetic disease associated with mutations in collagen V.[1] The most common mutations result in a single null COL5A1 allele and haploinsufficiency. Patients with classic EDS exhibit connective tissue hyperelasticity and laxity, suggesting a key role for collagen V in soft tissue function. While collagen V is a quantitatively minor (~2%) component of collagen fibrils in tendons and ligaments, modulation of its expression has dramatic phenotypic effects, indicating critical regulatory roles.[2] In addition, collagen V has been linked to injury,[3] performance deficiencies,[4] Achilles tendinopathy,[5] and anterior cruciate ligament (ACL) rupture.[6] The ACL is the most commonly injured ligament of the knee, with approximately 80,000 surgical repairs per year, resulting in an estimated cost of over one billion dollars.[7] Understanding the structure-function relationships in the ACL could provide valuable insight into mechanisms of matrix maintenance, as well as joint laxity, injury and repair. Therefore, the purpose of this study was to investigate the regulatory roles of collagen V on the mechanical properties and fibril morphology of the mouse ACL using collagen V haploinsufficient and null mouse models. We hypothesized that mechanical properties would be reduced, fibril diameter would be increased, and fibril density would be decreased compared to wild type ligaments when collagen V is reduced or absent in ACLs.

Methods

Mice from two genotypes, Col5a1+/+ (Wild Type, n=19) and a tendon/ligament-specific conditional knockout, ScxCre+Col5a1−/− (Col5a1 KO, n=10) were sacrificed at P60 (IACUC approved).[8] Hind limbs were detached and dissected free of soft tissue leaving only the knee joint intact. Surrounding ligaments, menisci, and soft tissue were carefully removed to expose the ACL and then surrounding soft tissue was carefully trimmed, leaving only the femur-ACL-tibia complex intact. Under a microscope, the ACL was imaged in the coronal and sagittal planes for area measurement. Verhoeff's stain was applied to both insertions of the ACL for optical strain tracking. The femur and tibia were affixed at 60 degrees of flexion in custom fixtures using PMMA (Figure 1). The ACLs were mechanically tested with a standard protocol consisting of preconditioning, stress relaxation and a constant ramp to failure. Cross-sectional area was calculated assuming an ellipsoidal shape from microscope images. Local strain was measured optically and mechanical parameters were calculated using custom software. ACLs from 4 additional mice at P30 of each genotype were analyzed for fibril morphology at 80 kV using transmission electron microscopy.[9] Images were captured at 60,000X and transferred to RM Biometrics-Bioquant software. Fibril diameter analyses were performed using a region of interest (ROI) from images across the central portion of the ACLs. Fibril diameters were determined along the minor axis of the fibril profile. Fibril density was defined as the total number of fibrils within the ROI. Statistics. Comparisons were made using Student's t-tests with significance set at $p<0.05$ and a trend defined at $p<0.10$.

Results

Cross-sectional area was not different between the control and the knockout ACLs (Figure 2A). Maximum load, stiffness, modulus (significant, Figure 2B-D), maximum stress (trend, not shown), and percent relaxation (trend, not shown) were decreased in the knockout group. In addition, the

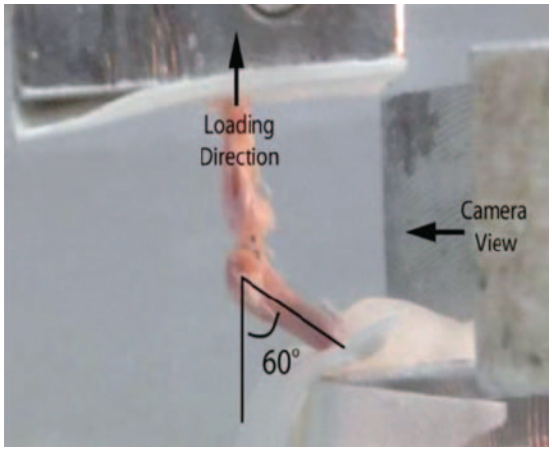

Figure 1. Mechanical testing setup of the tibia-ACL-femur complex.

Paper No: 0110
2014 Annual Meeting
Orthopaedic Research Society
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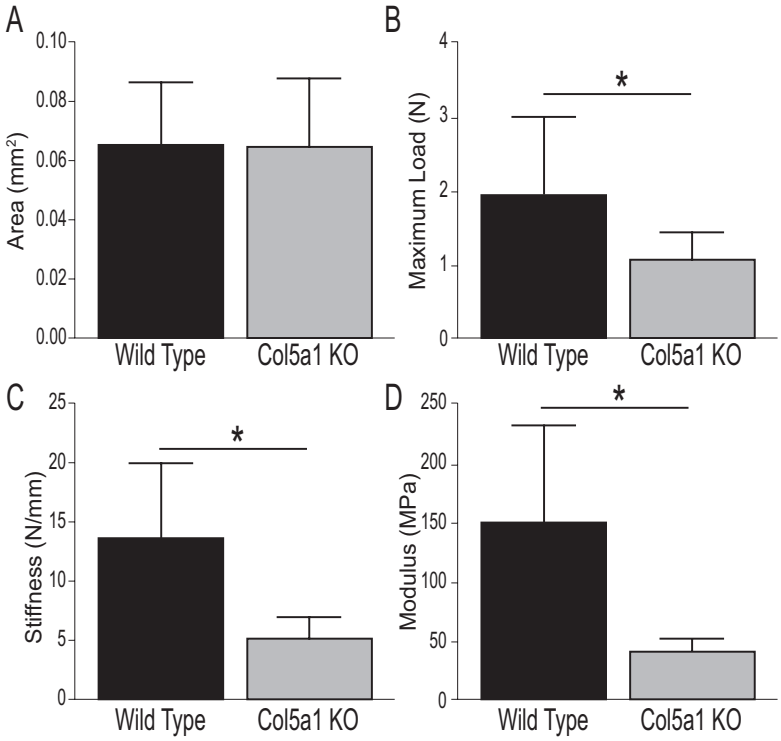

Figure 2. (A) There were no differences in cross-sectional area between groups. (B) Maximum load was decreased when comparing the knockouts to the wild type. (E) Stiffness and (F) modulus were both also decreased in the knockout group when compared to the wild type group. Results are presented as mean ± standard deviation with *p<0.05.

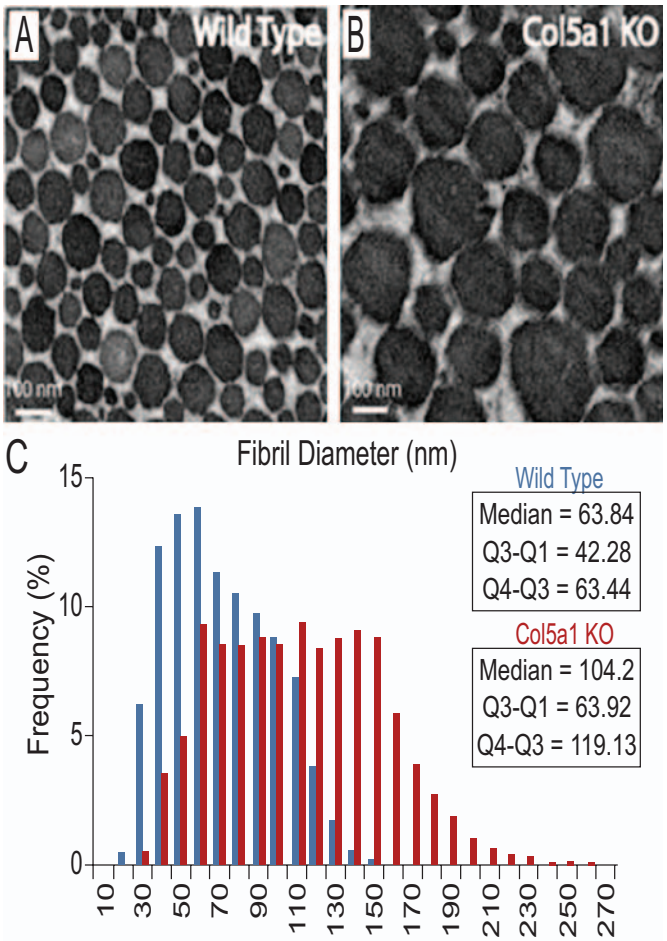

Figure 3. Representative images of fibril morphology in (A) wild type and (B) knockout ACLs confirm quantitative measurements. (C) Fibril diameter distributions shifted towards a broader profile with more larger diameter fibrils. There was a significant increase in fibril diameter in the knockout group when compared to the control group.

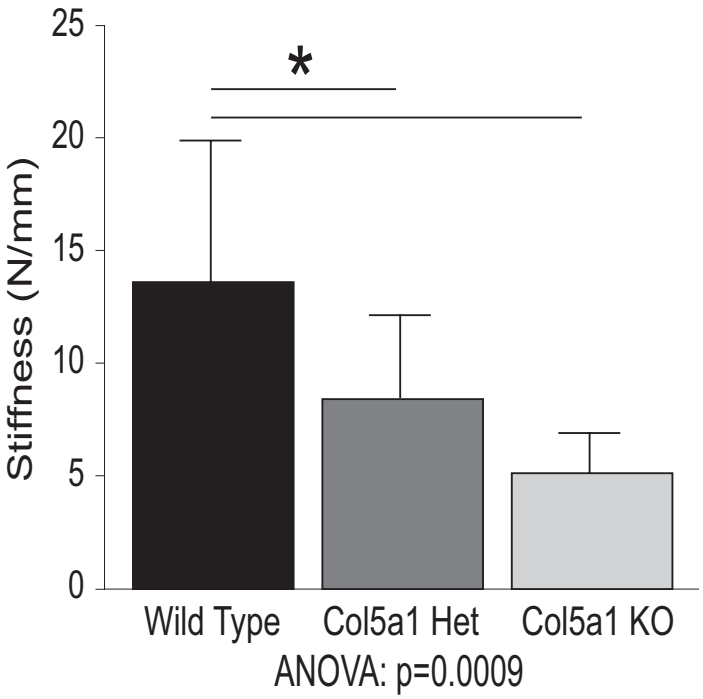

Figure 4. Stiffness was decreased in heterozygous and knockout ACLs. Other mechanical parameters had similar trends in the data.

distribution of fibril diameters was shifted towards increased larger diameter fibrils and a broader profile (Figure 3) in the knockout group. Fibril diameters were significantly larger and fibril density was significantly decreased in the knockout ACLs (not shown).

Discussion

Despite being a quantitatively minor component of the extracellular matrix, removal of collagen V resulted in severely decreased mechanical properties and altered fibril morphology, confirming that collagen V plays a critical role in the ACL. While these mechanical and structural results are consistent with previous work in the FDL,[10] the mechanical changes in the ACL were much more dramatic than the changes in the FDL,[10] suggesting that collagen V may play a larger role in the ACL. Since the ACL acts primarily as a joint stabilizer during knee movement, collagen V could play a key role in tissue laxity or elasticity. In the mouse model of EDS (Col5a1 heterozygous mouse), ACL mechanical evaluation showed that reduction of collagen V decreases ACL stiffness (and other mechanical parameters) in a dose-dependent manner (Figure 4), suggesting that some EDS-related joint laxity may be attributed to the functional deficiencies in the stabilizing soft tissues. Further investigation is necessary to elucidate other functional alterations in collagen V deficient tendons such as their viscoelastic and fatigue responses. In addition, investigation into the role of collagen V during injury, healing, and aging could aid in determining specific mechanisms by which collagen V regulates fibrillar structure and function.

Significance

This study demonstrated that collagen V plays a crucial role in the structure and function of the ACL. More broadly, this study confirms altered structure and function of joint stabilizing components that suggest further research in collagen V deficient tendons and ligaments, such as in EDS, is necessary.

Acknowledgments

This study was supported by NIH/NIAMS (T32-AR007132, AR044745), NIH/NEI EY05129, NSF GRFP and the Penn Center for Musculoskeletal Disorders (NIH, P30 AR050950).

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