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Achilles Tendons from Decorin and Biglycan Deficient Mice Demonstrate Inferior Mechanical and Structural Properties Predicted by an Image-based Empirical Damage Model

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Introduction

The Achilles tendon is among the most commonly injured musculoskeletal structures affecting as many as 2.5 million people per year in North America.[1] Major class I small leucine rich proteoglycans (SLRPs), specifically decorin and biglycan, play an important regulatory role during collagen fibrillogenesis, ultimately affecting tendon mechanical integrity,[2] yet the impact of these SLRPs on the Achilles tendon has not been described. Therefore, the objective of this study was to define the relationship between Class I SLRPs and Achilles structure and function, while also exploring the use of an empirical damage model to predict how these altered structural and regulatory molecules impact the dynamic mechanical behavior of the Achilles tendon. We hypothesized that the absence of decorin and biglycan would lead to altered mechanical and structural properties, and that these altered parameters used in an adapted empirical damage model could be used to successfully predict dynamic mechanical behavior.

Methods

Experimental Design: Achilles tendons (N = 102) from WT (C57Bl/6), decorin-null (*Dcn*$^{-/-}$) and biglycan-null (*Bgn*$^{-/-}$) female mice were harvested at maturity (P = 150days), middle (P = 300 days), and old (P = 570 days) age (n = 9-11 for each group) (IACUC approved).[3-4] All tendons were harvested and then randomized to blind a single dissector at the time of fine dissection and subsequent testing and analysis.

Tendon Mechanical Testing: Tendons were secured proximally using a sandpaper grip while maintaining the calcaneal insertion distally. This construct was loaded into custom fixtures on an Instron 5848Testing system. Samples, with a 5mm gauge length, underwent a dynamic mechanical testing protocol consisting of: 1) preloading to 0.05N, 2) preconditioning, 3) stress relaxations to equilibrium stress at 4%, 6% and 8% strain, each followed by a sinusoidal frequency sweep progressing from 0.1 Hz through 1, 5 and 10 Hz, and 4) a ramp to failure at 0.1%/sec.

Tendon Imaging: Alignment maps of tendons were collected using a polarized light image capture system.[5] Briefly, this system consisted of two rotating polarizing plates and software-synchronized image capture during mechanical testing. Alignment maps were used to quantify collagen organization based on the birefringence signal phase and magnitude. From these measures, the apparent birefringence and circular standard deviation were determined throughout loading.

Damage modeling: A previously described empirical damage model[6] was adapted to evaluate differences in parameters altered through genetic variation. Predictions of dynamic modulus were made using evaluation based on equilibrium stress at 6% strain and the calculated birefringence, again at 6% strain. For these calculations, model fit parameters were taken at moderate strain and middle age.

Statistics: Power analysis was conducted prior to experimentation and based on data variance in previous studies to ensure sufficient power. One-way ANOVAs were used to evaluate significant differences in mechanical properties between genotypes. Significant relationships were subsequently evaluated using post-hoc Student's t-tests with Bonferroni corrections (α=0.05; with significance set at $p < 0.0167$). Damage model fits between predicted and measured dynamic modulus were calculated and reported as Pearson's coefficients of determination (R^2 values).

Results

When compared to Achilles tendons from WT mice, mechanical properties were significantly inferior in the *Dcn*$^{-/-}$ and *Bgn*$^{-/-}$ mice. Ultimate load was significantly higher for WT tendons compared to SLRP null tendons for middle (P = 300) and old (P = 570) age. WT tendons at maturity (P = 150) had significantly higher ultimate loads when compared to *Bgn*$^{-/-}$ tendons, but not when compared to *Dcn*$^{-/-}$ tendons (Figure 1a). The dynamic modulus was similarly reduced for the mature and middle aged

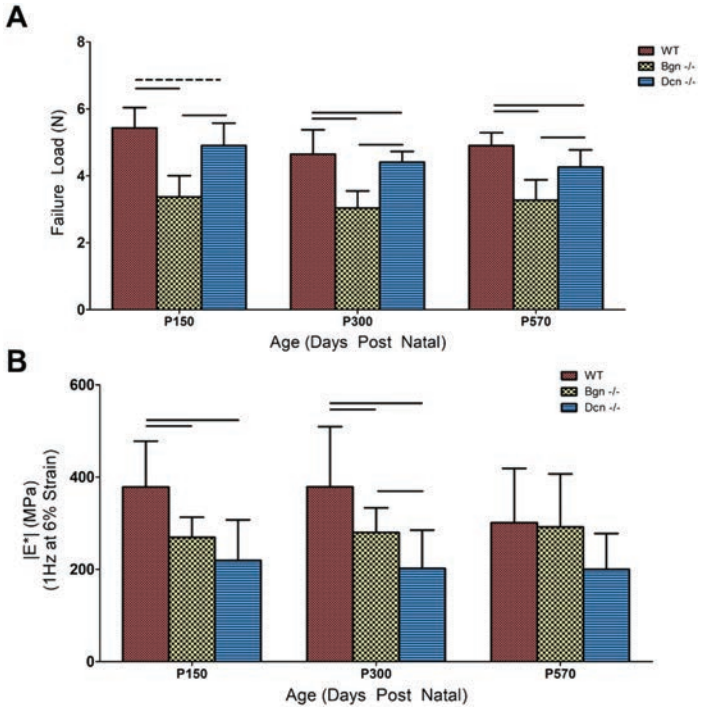

Figure 1. (A) Ultimate load of WT (red), *Bgn*-/- (yellow), and *Dcn*-/- (blue) tendons at maturity (p = 1 50) middle (p = 300) and old (p = 570) age, demonstrating a significant reduction in load for *Bgn*-/- and *Dcn*-/- ($p < 0.0167$; solid bar) Achilles tendons except in *dcn*-/- tendons at maturity, which maintained a consistent trend ($p < 0.05$; dashed bar) (B) Dynamic modulus for WT, *Bgn*-/- and *Dcn*-/- Achilles tendons at maturity, middle and old age, demonstrating a significant reduction ($p < 0.0167$) in dynamic modulus at maturity and middle age which was not observed in old age.

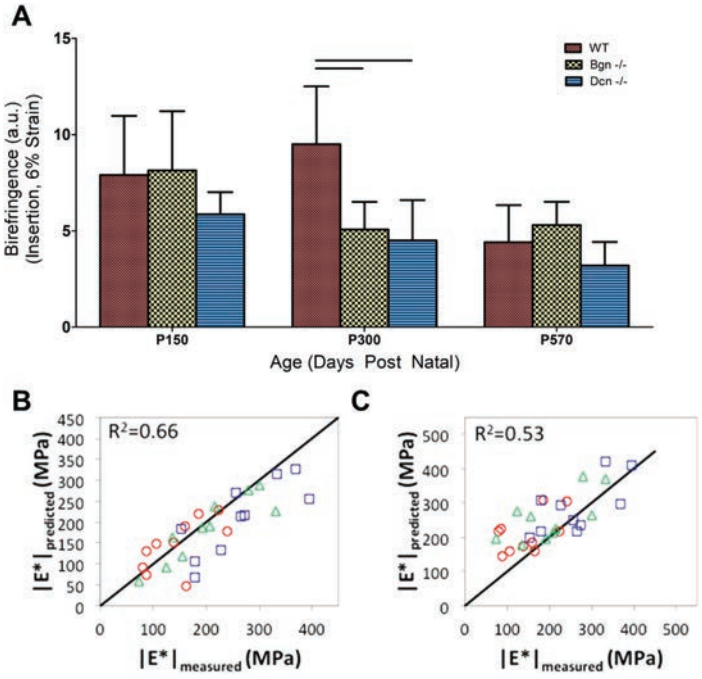

Figure 2. (A) Birefringence in WT (red), *Bgn*-/- (yellow) and *Dcn*-/- (blue) mouse Achilles tendons for mature (P = 150) middle (P = 300) and old (P = 570) age, demonstrating a significant reduction in collagen organization in middle age, but not at maturity or in old age. (B) Model predicted dynamic modulus vs. measured dynamic modulus based on mechanical based parameters and demonstrating moderate correlation ($R^2 = 0.66$) (C) Model predicted dynamic modulus vs. measured dynamic modulus based on image based parameters and, again, demonstrating moderate correlation ($R^2 = 0.53$). Red circles: 4% strain, green triangles 6% strain, blue squares 8% strain.

tendons; however this difference diminished with age (Figure 1b). Age-dependent differences in collagen birefringence were also detected (Figure 2a). When incorporated into an empirical damage model, both image-based and mechanical-based inputs resulted in predicted values of dynamic modulus that were moderately correlated to the experimental values observed (Figure 2b,c).

Discussion

Decorin and biglycan have a varied effect on tendons throughout the body.[2,5,7] In the Achilles tendon, their absence results in inferior mechanical properties. This is consistent with studies exploring the effect of SLRPs in the flexor carpi ulnaris[7] and the flexor digitorum longus.[2] However, similar studies of the patellar tendon demonstrated that the absence of decorin caused an increase in modulus, and neither the absence of decorin or biglycan had any effect on tail tendon fascicles.[2] The age dependent decrease in collagen fiber organization is consistent with previous work that found increased fiber heterogeneity resulting from knockout of SLRPs.[8] The ultimate load was reduced in the SLRP null tendons, suggesting direct implications for risk of Achilles tendon rupture. In light of the prevalence of Achilles pathology, exploring such structure-function relationships is a critical step in understanding how the Achilles tendon might fail, how to prevent failure, and how to better treat Achilles injuries. Additionally, this work extends the use of damage models as applied to predicting tendon dynamic mechanical properties in two ways; extending their use to circumstances involving genetic variance, and demonstrating that they can be used with image-based inputs. Importantly, meaningful differences were detectable using image-based evaluation of collagen organization, a parameter that has previously been measured with high frequency ultrasound,[9] thereby providing a potential opportunity for clinical translation of our basic research findings.

Clinical Significance

By developing a deeper insight into how SLRPs impact tendon and how understanding differences may help predict altered tendon function, we can better monitor, prevent and rehabilitate tendon injuries. This may be particularly clinically relevant in the context of image-based evaluation of structure as it can be used as a predictive tool for function.

Acknowledgements

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