

Proteomics reveal region-specific and inter-individual variability in human Achilles tendons: A pilot study

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INTRODUCTION: Tendons that predominantly serve an energy storing function (e.g., human Achilles tendon) are highly prone to injuries. As in many other diseases, proteomic analysis could enhance our understanding of pathogenesis and facilitate development of new treatments. Prior studies in the patella tendon^{1,2} have highlighted region specific variations in protein composition and material properties, suggesting there might be similar differences in the Achilles tendon. However, exploration in the Achilles is lacking. Understanding these region-specific differences could guide future studies and improve our understanding of what predisposes certain areas of the tendon to degeneration or injury. This pilot study aims to characterize variability of the human Achilles tendon proteome both among individuals and within distinct tendon regions. We hypothesize that proteomic analysis will reveal region specific variations in protein expression distinguishing the core and periphery regions, while individual diversity in protein expression will highlight the effect of factors such as genetics and age on tendon composition.

METHODS: Achilles tendon samples were collected from individuals >18 yrs of age (n=3; 2M,1F), undergoing below knee amputation. Exclusion criteria included diagnosed peripheral artery disease, certain infectious diseases, or inability to obtain informed consent (e.g., altered mental status from trauma or pre-surgical medication). This study was approved by the Washington University School of Medicine in St. Louis Institutional Review Board (#201505110), and informed consent was obtained from all participants prior to enrollment. Samples were transported in PBS-soaked gauze on ice and frozen at -80°C. For proteomic analysis, 1-mm-thick cross-sections from the midportion of Achilles were cut into smaller 1-mm cubes representing the left (LP) and right (RP) periphery and core (C) regions (Fig1B). The samples were cryopulverized, solubilized using urea buffer with sonication, and digested following established protocols^{3,4} with the addition of hyaluronidase prior to digestion⁵. 1µg of protein was labeled with TMT-10 isobaric mass tags according to kit instructions and all samples were combined and injected on a Thermo Fisher Scientific Q-Exactive PlusTM mass spectrometer. The LC-MS data were processed using Proteome Discoverer and Mascot software was used to match MS2 spectra against a RefSeq database of human proteins (41,734 entries). The processing, quality assurance and analysis of isobaric-labeled peptide LC-MS data were carried out using proteoQ software, and R program for statistical computing. Cluster analysis and principal components analysis were used for data interpretation.

RESULTS: The age and body mass index of the participants were 43 yrs, 32 kg/m² (subject 1); 63yrs, 43 kg/m² (subject 2); and 64yrs, 25 kg/m² (subject 3). A total of 1,651 proteins were identified in the specimens obtained from Achilles tendons. Cluster analysis predominantly differentiated samples based on individuals, followed by tendon regions. Robust clustering was observed among the tendon regions, indicating higher similarity in protein expression between samples from tendon periphery compared to those from the tendon core (Fig 1A), which can be clearly visualized when plotted as principal components, with samples primarily distinguished by tendon followed by tendon region, affirming the effectiveness of our proteomic data and its efficacy in differentiating between distinct tendons and tendon regions (Fig 1C).

DISCUSSION: The outcomes of this study offer insights into the proteomic dynamics of tendons, particularly within the Achilles tendon. The identified clustering among different tendon regions highlights region-specific adaptations, which may reflect previously reported differences in collagen turnover and mechanical demands in the tendon core and periphery^{1,2}. The variability in protein expression across individuals emphasizes the influence of factors such as genetics, tendon loading, and participant activity level, including systemic co-morbidities that may have contributed to the amputation. These results underscore the importance of considering individual variability in designing treatment strategies for tendon disorders. Despite limitations in sample size and participant heterogeneity (no participant had a macroscopic tendon injury), this study offers preliminary insights into inter-tendon and intra-tendon differences that may be important to consider in delivering tailored treatment and in experimental design of future studies.

SIGNIFICANCE/CLINICAL RELEVANCE: This study's significance lies in uncovering region-specific adaptations and inter-individual proteomic variability within the Achilles tendon, enhancing our understanding of tendon biology and functionality. These insights hold potential for targeted interventions to improve tendon health and performance, while acknowledging the need for personalized treatment strategies that account for individual proteomic diversity.

REFERENCES: ¹Haroldsson.BT, et.al, 2005, ² Zhang.C, et.al, 2020, ³Mertins.P, et.al, 2014; ⁴Kramer.MH, et.al, 2022; ⁵Anderson.JR, et.al, 2020

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Figure1-1A: Heatmap showing hierarchical agglomerative clustering of samples based on protein abundances.**1B:** Schematic of sample regions. **1C:** Principal component analysis (PCA) showing the first two principal components (PC1 and PC2). Numbers around the circles indicate the sample numbers.

