INTRODUCTION
Tendinopathies leading to rupture are a major source of musculoskeletal disability, affecting the entire spectrum of society. Histopathologic characteristics of tendinopathy include cellular proliferation, collagen fiber disorganization, and increased content of glycosaminoglycans (GAG) and large proteoglycans (i.e., versican, aggrecan) [1,2]. Since altered proteoglycan (PG) gene expression has been extensively reported in human tendon disease [3,4,5], it is likely that an imbalance in PG homeostasis impairs the reparative capacity of tendon extracellular matrix. Patellar tendinopathy is a common disorder characterized by pain at the inferior patellar pole after activity, with histologic alterations commonly seen in the posterior aspect of the proximal tendon. While several recent biopsy studies have begun to describe pathologic changes in the proximal patellar tendon [5,6,7], there remains an incomplete understanding of normal, regional (e.g., patellar vs tibial) histologic and compositional properties of this structure in humans. Therefore, the objective of the present study is to characterize histologic and biochemical features of uninjured human patellar tendons with a focus on large PGs relevant to tendinopathy.

METHODS
Six entire patellar tendons (three male, three female, ages 26, 29, 38, 42, 65, & 80 yrs) were procured within 48 h post-mortem from the Gift of Hope (Elmhurst, IL, USA). None of the knees exhibited overt signs of arthritic disease, fractures, or evidence of prior surgery. Each tendon was sharply released from its bony insertions and subsequently divided transversely into proximal, central, and distal thirds; each of the latter portions was then divided longitudinally into lateral and medial halves for biochemical and histological analyses, respectively. For histology (all six specimens), tendons were processed for paraffin embedding [8] and stained with H&E and toluide blue to facilitate visualization of cell morphology and extracellular matrix. Histologic abnormalities were assessed on the basis of a previously reported classification system [1]. Biochemical characterization was performed as follows for the 26-y.o. female donor’s tissue: the lateral patellar and tibial segments (~100mg wet wt) were digested with proteinase K, and the soluble fraction was taken for GAG analysis using fluorophore-assisted carbohydrate electrophoresis (FACE). Portions (~250mg) of both the medial and lateral tibial and patellar segments were taken for PG core protein analysis. These samples were placed into PBS containing proteinase inhibitors and pulverized in liquid N₂, followed by extraction with 4M Gdm.HCl, dialysis, and PG purification fractionation by DE52 for Western blot analysis. All samples were analyzed both with and without Chondroitinase ABC digestion to confirm their identities as CS/DS-PGs. Antibodies used were anti-aggrecan (DLS), anti-versican (LF99), anti-ADAMTS5-cleaved aggrecan G1 (NITEGE), anti-ADAMTS-cleaved versican (DPEAAE), anti-decoggin (6d06) and anti-ADAMTS-5 (KNG). All samples were loaded on a tissue wet weight basis. Immunohistochemistry (IHC) was performed using anti-ADAMTS5 (KNG) [9].

RESULTS
Histology: All tendons appeared macroscopically normal with no evidence of inflammation or tearing. Histologic assessment revealed no evidence of degenerative changes in the central region whereas changes towards the patellar and tibial insertion sites were evident. Within the patellar insertional region, the posterior aspect of the tendon exhibited decreased collagen staining, rounded tenocyte nuclei, and increased cellularity. Distinct interfibrillar regions resembling granulation tissue were seen in three of the samples. At the tibial insertion, similar features were noted, but with granulation tissue and increased cellularity generally localized to the anterior aspect of the tendon. In the youngest tendon sample (26 y.o. female), both the cell type and density differed between the proximal and distal insertions (Fig 1, arrows), with chondrogenic, less organized cells found near the patellar insertion and linear arrays of fibroblast-like cells with rounded nuclei at the tibial insertion.

Biochemistry: FACE analysis (Fig.2) showed that both patellar and tibial insertions were similar in both GAG abundance and disaccharide composition. The chondroitin sulfate at both insertions was composed of about 60% C-4-S and 40% C-6-S, and the hyaluronan (HA) was similar in abundance to the C-6-S. The Western analysis for proteoglycan core proteins showed that decorin was equally abundant at the two insertion sites. In marked contrast, full-length aggrecan, and the aggrecanase-generated products, aggrecan G1-NITEGE and versican G1-DPEAAE, were highly abundant in the patellar but not the tibial insertion. Conversely, the tibial insertions were enriched in CS-substituted versican. Localization of ADAMTS5 by IHC showed that the protein was present at both insertion sites, but that the enzyme was largely intracellular in the tibial insertion region while associated with the ECM at the patellar insertion (images not shown).

DISCUSSION
In tendon-related human tendinopathy, clinical, imaging and matrix assessments reveal that the proximal-posterior aspects of the tendon are most commonly affected [5,6,7,10], implying higher functional (mechanical) demands, and possibly inferior reparative responses of these specific tendon regions. In the present study of macroscopically normal human patellar tendons, interestingly, each sample showed evidence of collagenous and cellular alterations at one or both insertion sites, consistent with the premise that microinjury accumulation precedes tendon rupture [11]. Histologic abnormalities, when present, tended to be localized in the posterior aspect of the proximal tendon, in contrast to the distal tendon, where changes tended to occur in the anterior aspect. Biochemical findings revealed an abundance of aggrecan and chondrocytes near the proximal insertion while versican, along with cells which did not appear fully differentiated into chondrocytes or fibroblasts, predominated at the distal tendon. The latter observation appears consistent with the role of versican in facilitating cell adhesion and differentiation. In addition, IHC staining results imply a differing role of ADAMTS5 at the proximal and distal tendon.

The spatially varying histologic and biochemical features of the patellar tendon strongly suggest differing mechanical environments at the proximal versus distal insertion sites. Suprisingly, very few studies have quantitatively compared tissue strain (e.g., optically measured surface strain) of the patellar and tibial insertions. However, on the basis of collagen fiber orientation, it is thought that the proximal tendon sustains considerable compressive forces in contrast to the shear forces sustained at the tibial insertion [12,13]. To our knowledge, regional “mapping” of the PG distribution and cellular phenotypes along the entire length of the human patellar tendon has not been reported. The histologic and biochemical results of human patellar tendons reported herein constitute baseline data for evaluation of matrix composition and structure of injured tendons.

REFERENCES

Figure 1: Cell morphology of different segments of patellar tendon: top: 200x, bottom: 400x

Figure 2: Left: CS/HA FACE analysis of 26-y female tendon; Right: Western Blots for Aggrecan (Ab- DLS), versican (LF99), and ADAMTS5 (Ab-KNG) (P= patellar insertion, C= central portion, T= tibial insertion)