Effect of collagen mutations on 3D fiber organization in Achilles and tail tendons

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INTRODUCTION: Osteogenesis imperfecta (OI) is a genetic disorder caused by mutations in collagen type I. Skeletal fragility and deformities are the main symptoms of OI, which also affects other collagen type I-rich soft tissues including ligaments and tendons generating hyperextension and laxity [1]. Although OI bone fragility has been extensively studied, there is a gap in the literature in terms of understanding the changes in soft tissues function affected by OI. Previous studies using second harmonic generation (SHG) microscopy have shown that, in OI, the mutated collagen is poorly organized at the fibril and fiber levels in tendon [2]. SHG images of the OI tendons were described to have increased waviness in their fibers, but there was no specific morphological assessment [2]. To increase our understanding of the effect of OI collagen mutations on tendons function, here we conduct a 3D visualization and morphological quantification of OI and healthy mouse tail and Achilles tendons using SHG microscopy and a novel collagen orientation assessment method. Tail and Achilles tendons in mice should have, as shown in rats [3], two different functions: structure bearing and energy storage, respectively. Here, we are interested in understanding how the fibers and fascicles are organized in mice related to their tissue function, and how collagen mutations as those observed in osteogenesis imperfecta (OI) alter the tendons fiber orientation.

METHODS: The Achilles tendon (AT) and tail tendon (TT) from a 14-week-old *oim/oim* (B6C3fe-a/acolla2$^{oim/oim}$) mouse and its wild-type (WT) counterpart were thoroughly isolated by exposing the collagen structure. SHG images of the tendon samples were collected at 2 mm from the muscle attachment for the Achilles tendon and 10 mm from the base for the tail tendon. The SHG microscope (Ultima 4 - Multiphoton Microscope, BRUKER) was operated with a 40X objective lens at immersion to acquire Z-stacks (composed of 400 slices) at 0.2 µm distance from each other with 1024 x 1024 pixel slides, and a field of view of 234.9 µm x 234.9 µm. The acquired stacks of images were used to reconstruct the tendons 3D volumes in the selected sections using the CTvox program. The acquired individual scans and Z-stacks were then run through a custom-made MATLAB code in order to evaluate the differences in collagen fiber orientation, by adapting a new 2D in-house code for 3D analysis [4]. The processed stacks of slides with the fiber orientation were then rendered in 3D using ImageJ.

RESULTS: SHG images showed fiber crimping in the *oim/oim* mouse tendons but not in WT, where fibers are more linear (Fig. 1A-D). 3D reconstructions of the tendon sections showed the volumetric organization of the fibers within the tendon. No fascicle organization is observed in the Achilles tendons (Fig. 1E-F). Smaller fascicles with larger spacing are observed in the *oim/oim* tails tendons (Fig. 1G-H). Fibers in the *oim/oim* mouse appear to be less densely packed. The analysis of the collagen fibers orientation showed fibers organized mainly in the longitudinal direction of the tendons in the WT mice, and instead a greater variation in the orientation for the *oim/oim* tendons (Fig. 1I-L).

DISCUSSION: This study reveals for the first time the 3D fiber structure and organization differences in the Achilles and tail tendon in mice with OI. Alterations in the distribution and orientation of collagen fibers as those observed here could give rise to the tendon and ligament hyper-extension and laxity typical of OI. Crimped fibers need to fully extend before becoming functional. Also, fibers that are not oriented in the tendon direction support less load than well aligned ones as those organized in small and spaced-out fascicles.

SIGNIFICANCE/CLINICAL RELEVANCE: OI-related alterations in the microstructure of type I collagen-rich soft tissues have not been extensively studied. The results of the present study serve as a starting point for understanding the effects of collagen alterations on tendons structure and mechanics in two tendons with different functions. This study helps to understand and explain laxity in OI tendons and ligaments.

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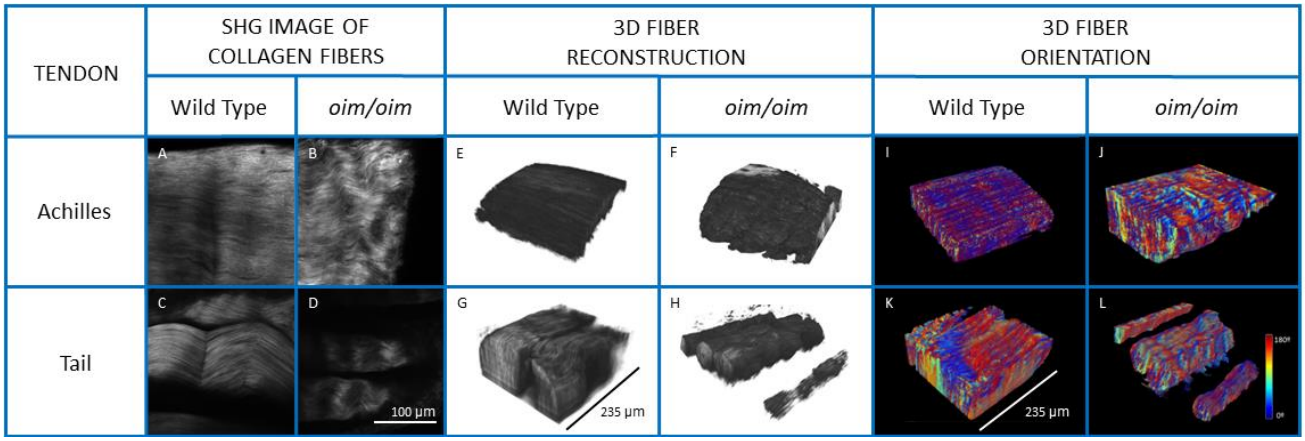

Figure 1 A–D) SHG images of tendon (234.9 µm x 234.9 µm) showing increased crimping and disorganization in the collagen fibers of *oim/oim* tendon. Tail tendons organization in fascicles is evident in both groups. **E-H)** 3D tendon sections (234.9 µm x 234.9 µm x 80 µm) showing the waviness and disorganization of the OI tendon fibers in volumetric space. Smaller fascicles with increased randomly distributed spacing are evident in the *oim/oim* tail tendons. **I-L)** 3D fiber orientation assessment in Achilles and tail tendons shows fiber organized mostly parallel to the direction of WT tendons but not in the *oim/oim* ones where fibers can often be perpendicular to the tendon direction.