

Pilot & Feasibility Program

A study of enthesopathy in X-linked hypophosphatemia

Carolyn Macica, PhD, Principal Investigator

Carolyn Macica's study focused on a particularly debilitating complication of XLH, enthesopathy. She studied the progression and pathogenesis of the calcification of tendon and ligament insertion sites.

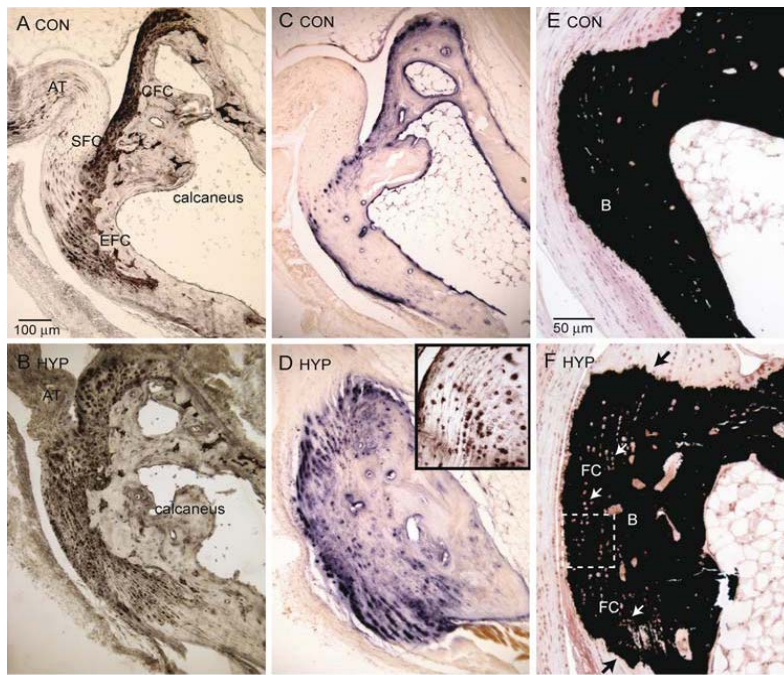
Dr. Macica states: "The formation of enthesophytes was our focus, with a major emphasis on characterizing the cellular changes that occur in enthesophyte formation using the murine model of XLH, Hyp mice. We have found that mineralization, while thought to originate from bone, is actually due to both an expansion of fibrocartilage cells that express the FGFR3 receptor and an increase in alkaline phosphatase activity.

We investigated the role of elevated circulating levels of FGF23 in fibrocartilage expansion using FGFR3 knockout mice on the Hyp background and in Dmp1 knockout mice, another model of osteomalacia characterized by elevated FGF23 levels, independent of the PheX mutation. We have evidence that an increase in chondrocyte-derived alkaline phosphatase is likely involved in the mineralization of both entheses and articular cartilage. We also sought to better characterize the dysregulation of alkaline phosphatase specific to cells of chondrocyte (vs. osteoblast) origin."

Findings To Date

Studies of fibrocartilaginous insertions

We completed studies of Hyp mice characterizing insertion sites most frequently affected in patients with XLH to determine if Hyp mice are a model of the extensive mineralizing enthesopathy observed in XLH patients. In contrast to control mice, fibrocartilage of Hyp mice was greatly expanded along the posterior calcaneal tuberosity, extending deep into the medial process of the tuberosity, and into the plantar fascia ligament attachment. Type II collagen staining is shown in panels A and B on the following page.



Cellular expansion of mineralizing fibrocartilage in Hyp mice at the Achilles tendon insertion into the calcaneal tuberosity in control and Hyp mice.¹

- In panel **A**, type 2 collagen (coll2) of the Achilles enthesis is confined in controls to calcaneal fibrocartilage (CFC) and enthesis fibrocartilage (EFC), whereas Hyp mice (panel **B**) demonstrate expansion of EFC into posterior and medial calcaneal processes.
- For alkaline phosphatase (ALP), activity is confined to the mineralized fibrocartilage in controls (panel **C**), but is expanded in Hyp mice (panel **D**), co-localizing with type 2 collagen-positive FC cells (Inset: FC cells are positive for type X collagen).
- Von Kossa staining corresponds with bone in controls (panel **E**), but in Hyp mice (panel **F**), the mineralization invades the Achilles insertion and plantar fascia ligament (black arrows). White arrows show FC cells.

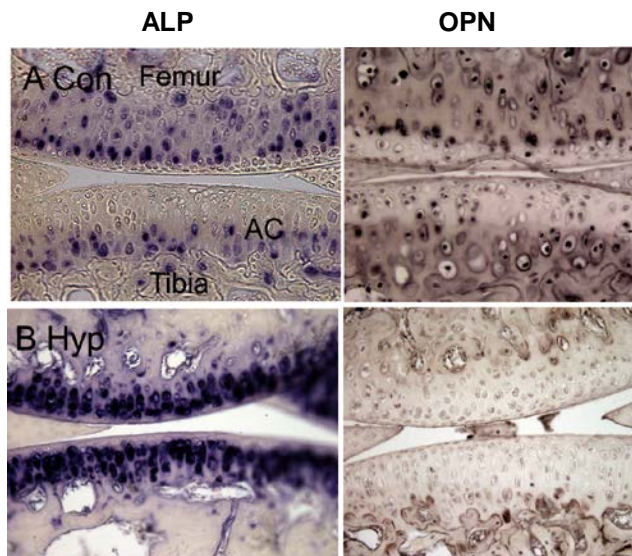
Generalized expansion of mineralized fibrocartilage was seen in Hyp mice as early as 10 weeks at a number of fibrocartilagenous sites, suggesting that cellular hyperplasia may be driven by changes in the cellular milieu. Thus we have determined that the increase in mineralization is due to an increase in mineralizing fibrocartilage and not to osteoblast-driven bone formation. Furthermore, the data are consistent with a role for FGF23 in mediating mineralized fibrocartilage hyperplasia. See: *Liang G, Katz LD, Insogna KL, Carpenter TO, Macica CM; Survey of the enthesopathy of X-linked hypophosphatemia and its characterization in Hyp mice; Calcif Tiss Int; 85:235-46, 2009.*

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Studies of articular cartilage

Degenerative joint disease in patients with XLH is characterized by articular cartilage thinning and subchondral sclerosis (*Radiology* 171:403, 1989; *Medicine* 68:336, 1989), yet there is little understanding of the cellular changes that accompany these pathologies. Thus, we have characterized articular cartilage of Hyp mice. In 10 week old Hyp mice we observe alterations in articular cartilage ECM proteins involved in cartilage remodeling. Our data suggest that ECM regulators of terminal differentiation of hypertrophic articular chondrocytes (such as matrix metalloproteinase 13) are required for maintenance of the normal phenotype. Disruption of these factors severely impacts the architecture of articular cartilage, which allows weight-bearing joints to accommodate mechanical force. These architectural changes also appear to be resistant to correction of hypophosphatemia and rickets.

Mineralization is defective despite elevated tissue alkaline phosphatase activity in Hyp mice. Articular cartilage chondrocyte ALP activity is elevated in 10 week old Hyp mice and encroaches upon the entire articular surface. Moreover, we found that osteopontin is downregulated in Hyp articular cartilage. In control mice, osteopontin (OPN) precisely co-localizes with the ALP-positive mineralized zone of articular cartilage. However, despite significantly expanded ALP activity in articular cartilage and growth plate chondrocytes of Hyp mice, OPN does not co-localize with ALP-positive cells and is down-regulated in the calcified zone.

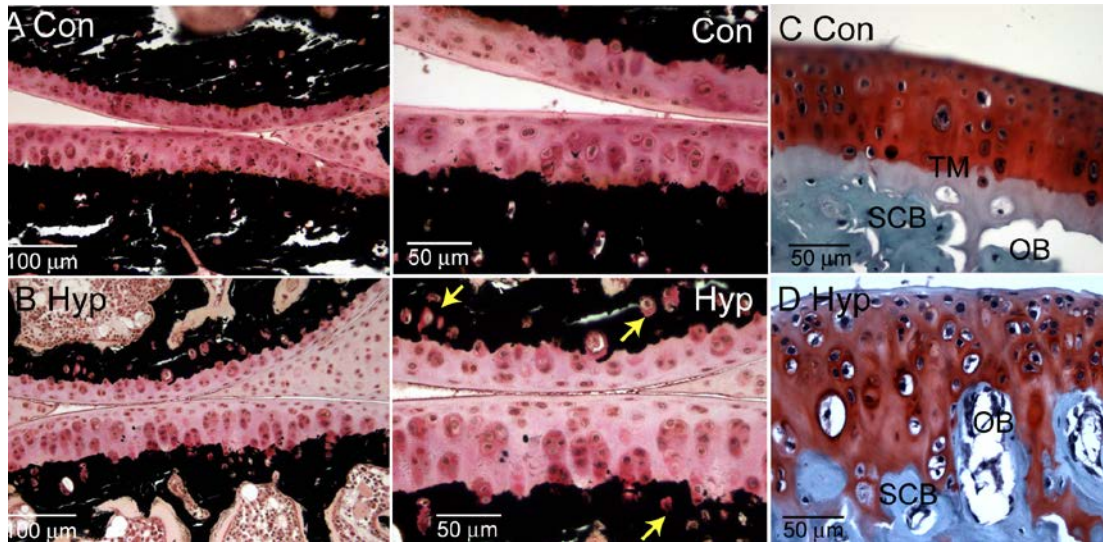


ALP and OPN immunoreactivity co-localize in hypertrophic articular cartilage chondrocytes of control mice, but not Hyp mice.

- In control mice (panel **A**), staining for ALP and osteopontin (OPN) are similarly distributed.
- In Hyp mice (panel **B**), the intense ALP staining is dissociated from limited OPN staining.

We also observed that despite elevated ALP activity throughout the cartilage, mineral deposition was absent in the upper zone of articular cartilage and diminished in the mineralized layer of 10 week old Hyp mice. Safranin O stain of proteoglycans reveals a typical pattern in control mice, staining heavily above the tidemark, where there is abundant proteoglycan. In contrast, staining in Hyp mice is diffusely distributed throughout the articular surface, intercalating with the

subchondral bone. Thus, the distinctive zones of articular cartilage were absent, and, in particular, the hypertrophic mineralized zone.



Decreased mineralization and altered proteoglycan (PG) distribution in Hyp articular cartilage.

- Von Kossa staining in 10 week old control (panel **A**) and Hyp (panel **B**) mice. Yellow arrows show demineralized areas around deep zone chondrocytes. Thickness of the Hyp mineralized zone is reduced. (Center panels at higher magnification.)
- Panels **C** and **D**: Safranin O/fast green stain showing diffuse PG staining in Hyp compared to controls. (OB, osteoblasts; SCB, subchondral bone; TM, tidemark (boundary between mineralized and unmineralized articular cartilage))