Type XI Collagen is Essential for Epiphyseal Growth Plate Cartilage Formation and Cartilage-to-Bone Remodeling

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INTRODUCTION: Type XI collagen is a well-known regulator of hyaline cartilage matrix assembly through the formation of collagens II/XI microfibrils [1]. In humans, mutations of collagen XI lead to chondrodysplasia (*Cho*) characterized by a range of skeletal abnormalities such as dwarfism and early onset osteoarthritis [2, 3]. Although previous studies on the constitutive *Col11a1*$^{+/-}$ murine model underscore the clear impact of collagen XI haploinsufficiency on cartilage and bone [4-7], it remains unclear how or whether collagen XI regulates skeletal tissue formation and turnover beyond its role in collagen II fibrillogenesis [8]. Also, collagen II and aggrecan are the major constituents of both articular cartilage and epiphyseal growth plate cartilage, which serve as the template for endochondral ossification [9]. It is unclear if collagen XI has similar roles in these two types of hyaline cartilages. This study, for the first time, queries the role of collagen XI in the formation and integrity of hyaline cartilages and cartilage-to-bone remodeling by studying the phenotype in our newly established cartilage-specific *Col11a1*$^{f/f}$/*AcanCre*^{ER} (*cKO*) model.

METHODS: Ablation of collagen XI was induced in *Col11a1*^{cKO} mice by tamoxifen (2 mg/25 g body weight) at postnatal day 1 (P1), and phenotypic changes were analyzed at P7 and P28 (approved by Drexel IACUC). No significant sex differences were observed. We applied μCT to analyze bone structure and Safranin-O/Fast Green histology for knee joint, tibia, and growth plate morphology. RNAscope and immunofluorescence (IF) were performed to assess gene expression and protein distributions of collagen XI. EdU and TUNEL assays were performed to assess proliferation and apoptosis. TEM was applied to quantify collagen fibril nanostructure in P28 joints, and AFM-nanoindentation was applied to 10 μm-thick, unfixed cryo-sections to quantify the tissue micromodulus, following our established methods [10]. Two-sample student's *t*-test was applied to detect genotype-associated differences at $\alpha = 0.05$.

RESULTS: At P28, μCT revealed pronounced skeletal defects in *cKO* mice, including shorter body length, tibia length, altered subchondral trabecular bone structure and a thickened growth plate (Fig. 1). At P7, collagen XI was highly expressed in the entire tibia epiphysis (Fig. 2a), and its ablation led to an overgrowth of cartilage near the growth plate "neck" (Fig. 2a), as well as decreased proliferation and increased apoptosis in the growth plate (Fig. 2b). At P28, collagen XI was present in articular cartilage but more highly expressed in the growth plate. The *cKO* growth plate chondrocytes lost their columnar organization (Fig. 2c, inset) and underwent both vertical and anterior overgrowth, concurrent with an overgrowth of the secondary ossification center (Fig. 2c, arrowhead). At the nanoscale, we found significant thickening of collagen fibrils in both articular cartilage and all zones of the growth plate at P28 (Fig. 3a,b). In the columnar and hypertrophic zones, the collagen fibrils lost their organized alignment and became more sparsely distributed and disorganized (Fig. 3a). Meanwhile, AFM-nanoindentation found reduced modulus for the hypertrophic, but not the columnar zone in *cKO* growth plates at P28 (Fig. 2c).

DISCUSSION: This study underscores an essential role of collagen XI for the formation and remodeling of the epiphyseal growth plate. The largely disrupted cellular proliferation and apoptosis essential for cartilage-to-bone remodeling as early as P7 indicate high sensitivity of resident chondrocytes to collagen XI-mediated collagen fibril integrity. It is possible that collagen XI is required for maintaining growth plate homeostasis, either by providing the proper matrix microniche or by directly interacting with cell surface receptors such as integrins, or both. In turn, the disrupted cartilage template leads to loss of proper zonal organization, impaired endochondral ossification in both the appendicular and axial skeleton, illustrating the essential role of collagen XI in overall cartilage-to-bone remodeling during skeletal development. Although canonical understanding of collagen XI suggests a major role for collagen XI in articular cartilage [11], the lack of histological defects in articular cartilage is in stark contrast to the disorganization and aberrant thickening of the growth plate (Fig. 2). This suggests that collagen XI plays differential roles in various hyaline cartilages and is likely a more crucial regulator of epiphyseal cartilage, expanding our understanding of this molecule beyond our canonical description [11]. Our ongoing studies aim to continue uncovering the impact of collagen XI loss on the cell-matrix interactions and mechanosensitive signaling pathways in articular versus growth plate cartilages.

SIGNIFICANCE/CLINICAL RELEVANCE: This study underscores an essential role of collagen XI in regulating growth plate cartilage integrity and cartilage-to-bone remodeling, advancing our fundamental understanding of this crucial regulatory cartilage in overall skeletal development.

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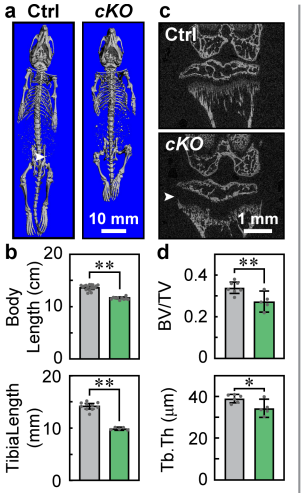


Fig. 1 a) Whole body 3D μCT of *Col11a1*^{cKO} (*cKO*) vs Ctrl mice. **b)** Body length (with tail) and tibia length. **c)** 2D knee μCT shows thickened growth plate (white arrowhead). **d)** BV/TV and Tb.Th of subchondral trabecular bone. ($n \geq 5$, *: $p < 0.05$, **: $p < 0.01$)

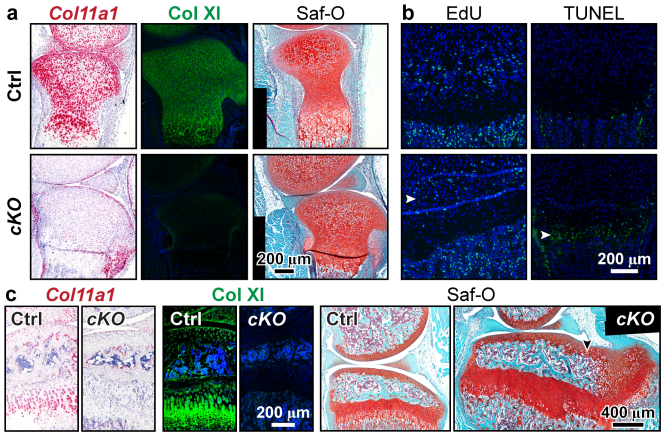
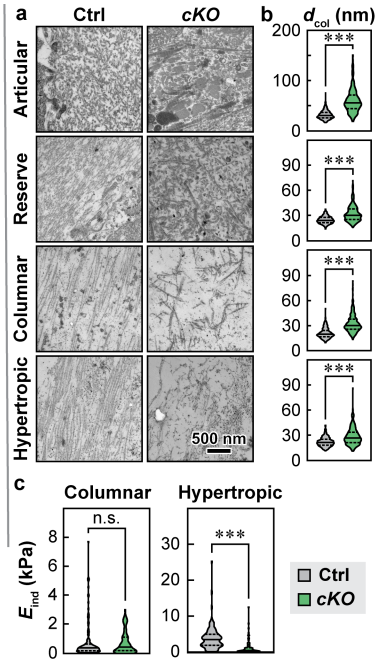


Fig. 2 a) RNAscope of *Col11a1*, IF of collagen XI, and histology of *cKO* vs Ctrl joints show collagen XI loss and altered epiphyseal cartilage at P7. **b)** EdU and TUNEL assays show decreased proliferation and increased apoptosis in P7 growth plate (white arrowheads). **c)** RNAscope, IF and histology show collagen XI loss led to aberrant subchondral bone overgrowth at P28 (black arrowhead).



→ **Fig. 3 a)** TEM images of articular and growth plate cartilage from *cKO* vs Ctrl mice at P28. **b)** Collagen fibril thickening was observed in both articular cartilage and all zones of growth plate (> 300 fibrils). **c)** AFM-nanoindentation shows lower modulus in hypertrophic zone but not columnar zone for *cKO* growth plate (≥ 80 locations from $n \geq 4$ animals). (***: $p < 0.001$)