Ablation of Type III Collagen Disrupts the Establishment of Various Hyaline Cartilage Tissues During Postnatal Growth

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INTRODUCTION: Type III collagen is the second most abundant collagen in the human body and plays a critical role in collagen fibrillogenesis [1]. It commonly forms heterotypic type I/III fibrils with collagen I, contributing to the regulation of fibril diameter and collagen cross-linking [2, 3]. Due to these essential functions, deficiency of collagen III in human is associated with an increased risk of vascular and organ rupture, as well as degenerative musculoskeletal conditions, as observed in patients with vascular Ehlers-Danlos Syndrome (vEDS) [4]. In our previous studies, we investigated the phenotypic changes of articular cartilage in collagen III haploinsufficient mice ($Col3a1^{+/-}$) and identified impaired tissue integrity hallmarked by increased fibril diameter, reduced modulus and morphological changes [5]. However, in this model, we were unable to evaluate the impact of near complete deletion of collagen III or delineate age-dependent roles of collagen III during postnatal growth. Furthermore, it is unclear if collagen III is also required for the proper formation of other hyaline cartilage tissues such as the growth plate. In this study, using our newly established inducible collagen III knockout murine model, we sought to investigate how the complete ablation of collagen III regulates collagen fibril formation and matrix integrity in articular cartilage, growth plate cartilage and formation of the secondary ossification center.

METHODS: Hind legs were harvested from $Col3a1^{F/F}/RosaCre^{ERT}$ ($Col3^{F/F}$) and control $Col3a1^{B6/B6}/RosaCre^{ERT}$ ($Col3^{B6/B6}$) mice at 3 weeks of age (P21), following the tamoxifen-induced knockout of *Col3a1* at P0 [6]. Safranin-O/Fast Green histology was applied to evaluate joint morphology and sulfated glycosaminoglycans (sGAGs) distribution in sagittal paraffin section of knee joints. Transmission electron microscopy (TEM) was performed on freshly dissected tibial articular cartilage and distinct regions of the growth plate (reserve, proliferative, and hypertrophic zones) to assess collagen fibril nanostructure in the matrix bulk. Scanning electron microscopy (SEM) was performed on the femoral condyle surface to assess surface collagen fibril diameter, following our established procedure [5]. Atomic force microscopy (AFM) nanoindentation was applied to quantify the indentation modulus (E_{ind}) of femoral condyle cartilage ($R \approx 5$ µm, $k \approx 8.9$ N/m) [5]. An unpaired two-sample Student's *t*-test was used to test the effect of genotype on fibril diameter and E_{ind} at the significance level of $\alpha = 0.05$.

RESULTS: In 3-week-old $Col3^{F/F}$ mice, we did not notice appreciable changes in the sGAG staining or morphology of articular cartilage compared to the $Col3^{B6/B6}$ control (Fig. 1a). At the nanoscale, however, we found significantly thickened collagen fibrils both on the surface and in the bulk matrix (Fig. 1b-c). In alignment with these structural defects, AFM-nanoindentation showed reduced tissue modulus, E_{ind} (Fig. 1d), illustrating impaired matrix formation and function with the loss of collagen III. In addition to articular cartilage, we also found thickened growth plate cartilage (Fig. 2a, images acquired at approximately the same sagittal plane for both genotypes) and altered fibril architecture in the growth plate, as marked by increased fibril diameters across the reserve, proliferative and hypertrophic zones, indicating that impaired matrix formation throughout all regions of growth plate cartilage with the loss of collagen III (Fig. 2b-c). Furthermore, despite the absence of joint morphological changes, we noted a reduction in the size of secondary ossification center concurrent with the growth plate thickening (Fig. 2a), suggesting that loss of collagen III also delays the remodeling of epiphyseal cartilage and associated secondary ossification center bone formation.

DISCUSSION: This study highlights a crucial role of collagen III in regulating the formation and turnover of various hyaline cartilage tissues during postnatal growth. Specifically, the fibril thickening and modulus reduction observed for articular cartilage in immature $Col3^{F/F}$ mice (Fig. 1) are aligned with our previous findings in adult $Col3a1^{+/-}$ mice [5], and indicate that collagen III is required for the proper matrix establishment during early postnatal growth. Besides articular cartilage, our findings also extend the role of collagen III to other hyaline tissues. In particular, the disrupted growth plate fibril structure and aberrant thickening (Fig. 2) also establish collagen III as a crucial regulator of its matrix fibrillar integrity and remodeling process. Indeed, the delayed formation of secondary ossification center suggests that ablation of collagen III disrupts the cartilage-to-bone development process involving the remodeling of epiphyseal cartilage. In cartilage, collagen III is enriched in the pericellular matrix, contributing to growth factor sequestration and assembly of nascent matrix molecules [7]. It is possible that collagen III is required for maintaining the cellular microniche in epiphyseal and growth plate cartilage, therefore mediating the cellular processes such as apoptosis, differentiation and osteoblastogenesis. Building on these findings, our ongoing studies are querying the impact of collagen III ablation on the structure, mechanics and cell mechanosensing of epiphyseal and growth plate cartilage, as well as the formation and structure of subchondral bone. We expect these findings to further advance our understanding of collagen III in the development and remodeling of hyaline cartilages and bone beyond its function in mediating articular cartilage.

SIGNIFICANCE: This study highlights collagen III as a critical regulator of cartilage matrix establishment and cartilage-to-bone remodeling during postnatal growth, supporting collagen III as an essential component for musculoskeletal tissue integrity and development.

REFERENCES: [1] Boudko+ 2008. [2] Niederreither+ 1995. [3] Liu+ 1997. [4] Malfait 2018. [5] Wang+ 2020. [6] Stewart+ 2025. [7] Hosseininia+ 2016.

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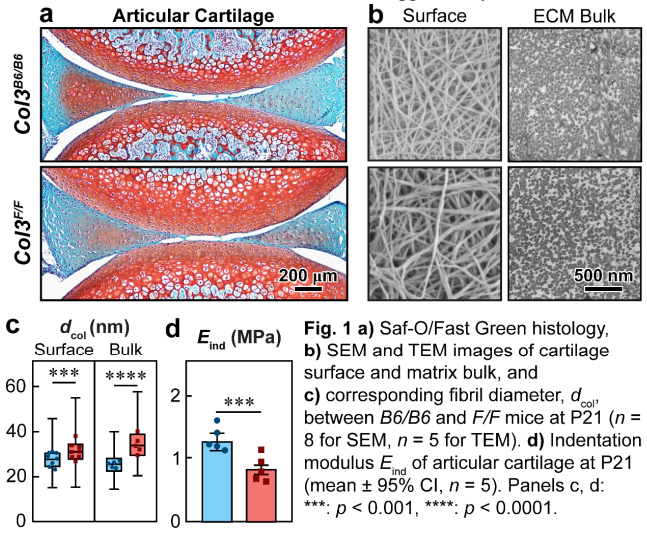

Fig. 1 a) Saf-O/Fast Green histology, **b)** SEM and TEM images of cartilage surface and matrix bulk, and **c)** corresponding fibril diameter, d_{col}, between *B6/B6* and *F/F* mice at P21 (n = 8 for SEM, n = 5 for TEM). **d)** Indentation modulus E_{ind} of articular cartilage at P21 (mean ± 95% CI, n = 5). Panels c, d: ***: $p < 0.001$, ****: $p < 0.0001$.

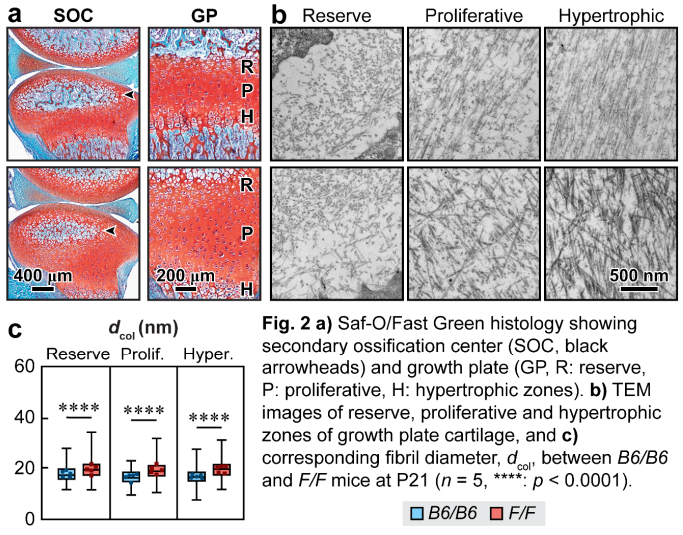

Fig. 2 a) Saf-O/Fast Green histology showing secondary ossification center (SOC, black arrowheads) and growth plate (GP, R: reserve, P: proliferative, H: hypertrophic zones). **b)** TEM images of reserve, proliferative and hypertrophic zones of growth plate cartilage, and **c)** corresponding fibril diameter, d_{col}, between *B6/B6* and *F/F* mice at P21 (n = 5, ****: $p < 0.0001$).