Investigating the roles of KMT2C/D in chondrocyte differentiation and endochondral ossification

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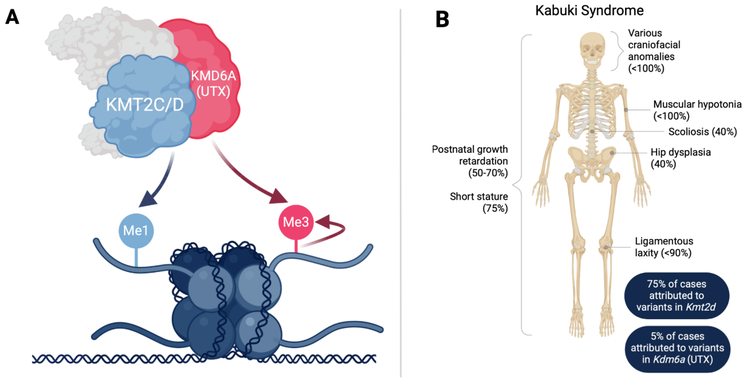
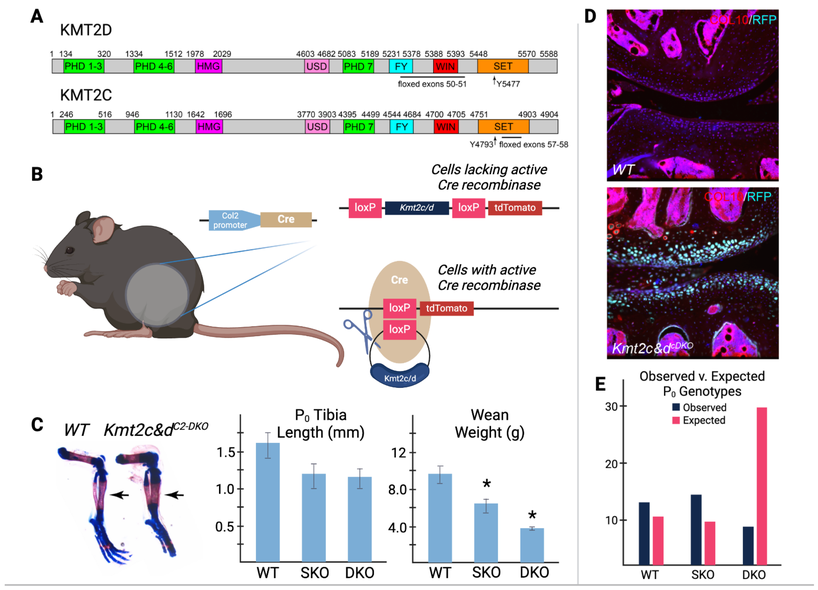
Abstract

Mutations in KMT2D, a histone 3 lysine 4 (H3K4) methyltransferase, have been implicated in Kabuki syndrome, a craniofacial development disorder. Patients often present with facial dysmorphology, in addition to skeletal abnormalities concerning spinal curvature, shortened peripheral bone length, and joint problems. Although the craniofacial irregularities have been explored in depth, the roles of KMT2D and its functional homolog KMT2C have yet to be thoroughly studied in the context of broader cartilage and skeletal morphology. Previously, our lab has observed that a chondrocyte-specific knockout of KMT2C and KMT2D leads to alterations in chondrocyte differentiation in shortened long bones when compared to wildtype samples. These observations implicate KMT2C/D more broadly in skeletal development. *Kmt2c&d* double knockout (DKO) mice display gross morphological changes in tibial growth plate thickening, lack of columnar chondrocyte organization, and overproliferation of articular cartilage as compared to wildtype samples. Altered expression of chondrocyte markers indicates altered chondrocyte differentiation in DKO long bone growth plates. This investigation will develop a stronger understanding of the cellular mechanisms associated with KMT2C and KMT2D, in the context of cartilage and skeletal development.

H3K4 methyltransferases are implicated in human disease

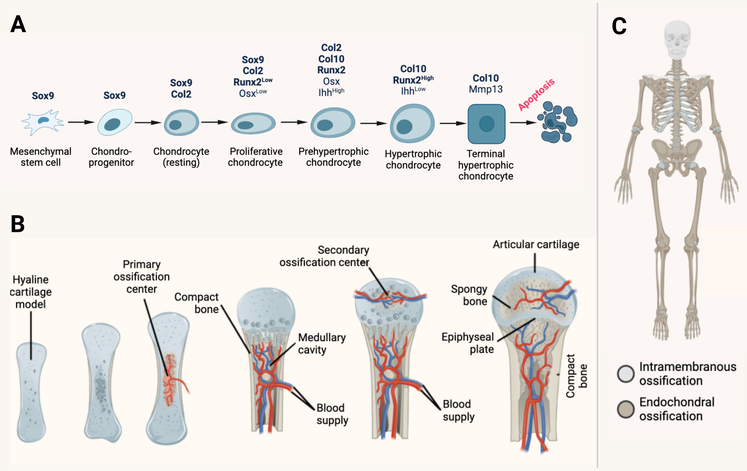


Chondrocyte-specific loss of KMT2C/D yields broad skeletal defects

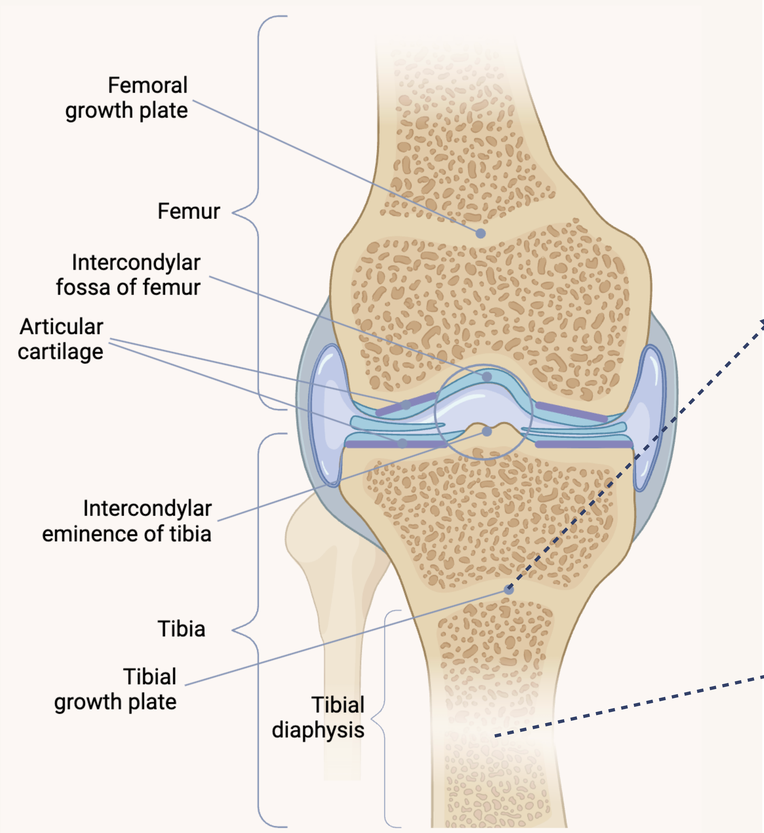


(A) Depiction of KMT2C/D protein. Floxed exons labeled. (B) Visualization of *Col2-Cre* system in mouse line. (C) Alcian blue and alizarin red stained P_0 hindlimb with quantification of tibia length and wean weight. (D) RFP for Tomato reporter localizes to Cre-expressing articular chondrocytes. (E) Observed vs. expected genotypes for P_0 mice.

Chondrocyte differentiation in endochondral ossification



Gross anatomy of the hindlimb



Acknowledgments

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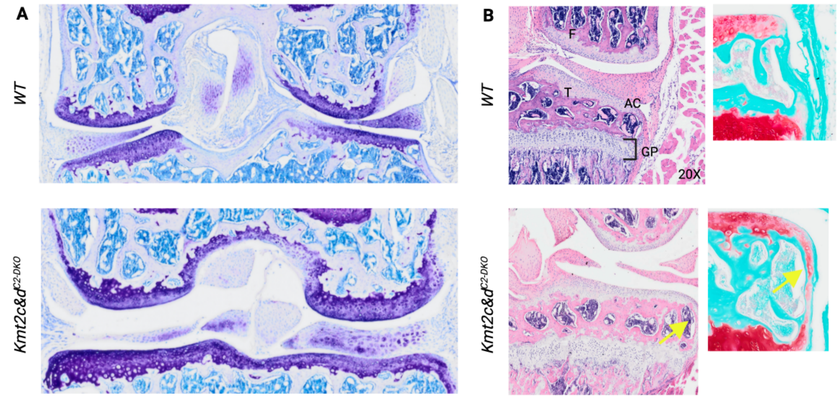
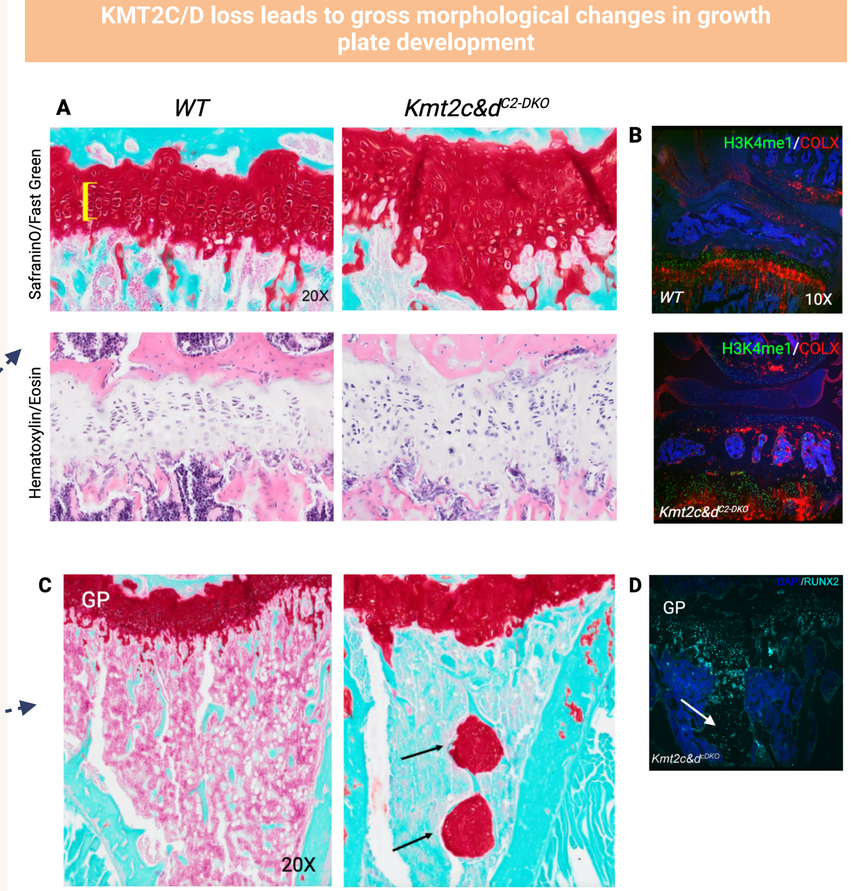
Overproliferation of articular cartilage occurs in absence of KMT2C/D



(A) Toluidine blue stained coronal section of 8-wk joint space for visualization of articular cartilage. (B) H&E stained tibial articular cartilage with enlarged SafraninO/FG stain both displaying overgrowth of articular cartilage and osteophyte progression.

KMT2C/D loss leads to gross morphological changes in growth plate development



(A) SafraninO/FastG and H&E stained coronal section of 8-wk tibial growth plate. (B) IF for H3K4me1 and hypertrophic COLX in proximal tibia. (C) SafraninO/FastG coronal section of 8-wk tibia displaying penetrance of cartilaginous matrix into diaphysis in *Kmt2c&d*$^{C2\text{-}DKO}$. (D) IF for RUNX2 in tibial diaphysis of 8-wk *Kmt2c&d*$^{C2\text{-}DKO}$.