Conditional knockout of Prdm16 leads to shortened and deviated nasal septal cartilage in mice

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INTRODUCTION: Epigenetic alterations contributing to craniofacial anomalies constitute more than half of congenital deformities, affecting approximately 35% of the birth defects[1]. A Genome-wide association study suggests that abnormal craniofacial developments in humans are associated with PRDM16, a histone methyltransferase and zinc finger transcriptional factor[2,3]. It has also been reported that mice with mutations in PRDM16 exhibited mandibular hypoplasia due to underdeveloped Meckel's cartilage, implying its potential role in chondrogenesis[4,5]. However, Prdm16 global knockout (KO) mice develop multisystem defects, including ventricular hypoplasia, and are neonatal lethal. Thus, the detailed molecular mechanism by which PRDM16 regulates craniofacial chondrogenesis and postnatal cartilage homeostasis remains poorly understood[5,6]. In the current study, we aimed to elucidate the functional role of PRDM16 in chondrogenesis during craniofacial development by using a novel cartilage-specific conditional knockout (KO) mouse model.

METHODS: All animal procedures were approved by UR IACUC. Cartilage-specific, Prdm16 KO mice (*Col2a1-Cre; Prdm16$^{flox/flox}$*) were generated. Cre-negative littermates were used as wild-type controls (WT). E18.5 WT and KO mice were stained with Alcian Blue and Alizarin Red (whole mount staining), and their nasal and cranial lengths were measured. To evaluate the craniofacial bone and cartilage development postnatally, skulls were harvested from 4-wk and 12-wk-old WT and KO mice and submitted for µCT (n = 5/ group) and Safranin O (Saf-O)/Fast green staining (n = 3/group). Both sexes were investigated, and data were analyzed with Student's t-test within the same sex and time point.

RESULTS: KO of PRDM16 protein was confirmed by Western blot analysis of E18.5 costal cartilage (data not shown due to limited space). The whole mount staining revealed that cartilage-specific deletion of Prdm16 KO mice exhibited a trend towards decreasing nasal ($p = 0.26$) and cranial lengths ($p = 0.06$) compared to WT mice at E18.5 (**Fig. 1A, B**). µCT analysis indicates shorter nasal and cranial length in both juvenile, 4-wk-old female and male KO mice than their corresponding control WT mice (**Fig. 2B**). However, no remarkable changes were observed in other craniofacial bones, including total mandible length, anterior to posterior mandible ratio, and condylar axis in both females and males at 4 wks of age (data not shown). At 12 wks of age, KO mice continued to exhibit significantly shorter nasal and cranial lengths relative to WT (**Fig. 2B**). While both female and male KO mice showed comparable nasal bone volume fraction (BV/TV) to WT at 4 wks of age; however, significantly higher BV/TV was observed in the KO mice as they reached maturity (**Fig. 2C**). Histological analysis showed that nasal septal cartilage exhibited decreased Saf-O staining in both sexes of KO mice at 4 wks of age, and length of nasal septal cartilage is also 37% and 29% shorter versus WT male and female mice, respectively (**Fig. 3B**). Furthermore, the KO exhibited severe deviation in the septal cartilage compared to WT (**Fig. 3C**). The deviation point was observed at the junction between the nasal bone and the septal cartilage[7,8]. Note only male mice shown in **Fig. 2** and **Fig. 3** due to limited space.

DISCUSSION: Using a cartilage-specific KO model, we unveiled that Prdm16 KO mice exhibited shorter nasal lengths and increased BV/TV, independent of sex. The nasal septum consists of a heterogeneous structure and is responsible for sagittal and vertical maxillary growth. In particular, while posterior nasal septal cartilage (a hyaline cartilage) forms perpendicular plate of the ethmoid via endochondral ossification, the anterior portion of nasal septal cartilage remains cartilaginous throughout one's life[7]. The observed shortened nasal length in the KO mice implies disrupted endochondral ossification process during which posterior nasal septal cartilage transforms into the perpendicular plate of the ethmoid. Furthermore, KO mice also exhibited severe nasal septal deviation vs. WT mice. Interestingly, the midface abnormalities in our cartilage-specific Prdm16 KO model resemble brachycephaly in humans and Apert Syndrome (AP) mouse model (*EIIa-cre; Fgfr2$^{S252W/+}$*; where mutant Fgfr2 was knock-in using a ubiquitous Cre driver) that displayed underdeveloped midface with septal deviation as well as respiratory issues[9]. In the AP mouse model[7], the authors suggest that *Fgfr2* mutation-mediated septal deviation could be due to premature chondrocyte hypertrophy, which resulted in altered septal matrix, impinging upon nasal bone and vomer[9]. Nevertheless, whether there is a molecular link between PRDM16 and FGFR2 in regulating chondrocyte phenotypic changes and endochondral ossification of nasal septal cartilage warrants further investigation. Additionally, a recent study using *Sox2-Cre; Prdm16$^{flox/flox}$* mice reports the involvement of Prdm16 in chondrogenesis in neural crest cell lineage[4]. Currently, our lab is exploring the functional role of PRDM16 in chondrogenesis and osteogenesis in the cranial neural crest cell lineage as well as how PRDM16 module endochondral ossification of nasal septal cartilage using CRISPR-Cas9 gene editing technology and human iPSC models.

SIGNIFICANCE/CLINICAL RELEVANCE: Abnormal nasal septum development contributes to a variety of craniofacial anomalies. Our study will elucidate the regulatory role of PRDM16 in craniofacial chondrogenesis and cartilage homeostasis and advances, facilitating future tissue engineering for septal correction.

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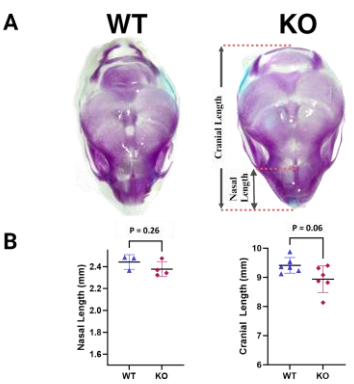

Fig. 1: (**A**)Whole mount skeletal staining of E18.5 embryos. (**B**) Measurement of Nasal and Cranial lengths of E18.5 embryo. While no statistically significant difference was observed in nasal and cranial lengths between WT and KO mice at E18.5, a decreasing trend was evident in both nasal and cranial lengths in KO mice compared to WT mice.

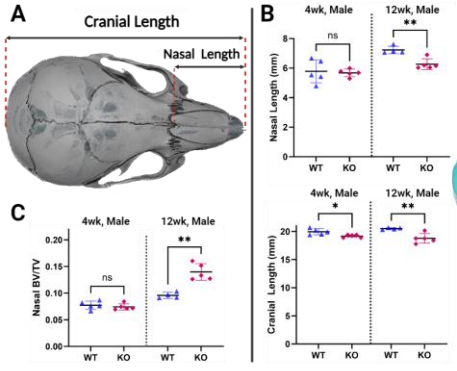

Fig. 2: (**A**) Linear measurements of mouse skull. (**B**) Comparison of Nasal and Cranial lengths between WT and KO of Males at 4 wks and 12 wks age. Significantly shorter Nasal and Cranial lengths are observed at 12 wks. (**C**) Bone volume fraction of nasal region (* p <.05, ** p <.01). No difference is observed at 4 wks, but at 12 wks, KO mice exhibit higher BV/TV than WT mice. Female images not included due to space.

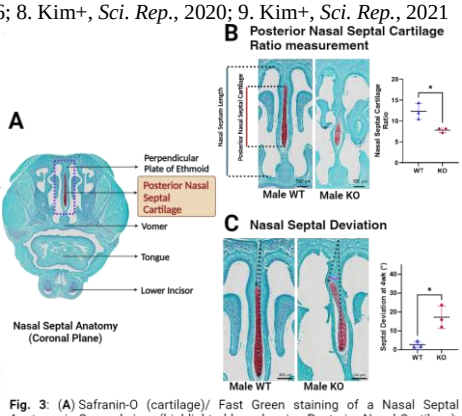

Fig. 3: (**A**) Safranin-O (cartilage)/ Fast Green staining of a Nasal Septal Anatomy in Coronal view; (highlighted box showing Posterior Nasal Cartilage). (**B**) Measurement of Nasal Septal Cartilage Ratio of Males at 4 wks. (**C**) Measurement of Septal Deviation of 4 wk Males (* p <.05). The angle of septal deviation between the most deviated point of the septum and the midline. Female images not included due to space.