**Integration of comprehensive 3D microCT and signaling analysis reveals differential regulatory mechanisms of craniofacial bone development**

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**ABSTRACT**

Growth factor signaling regulates tissue-tissue interactions to control organogenesis and tissue homeostasis. Specifically, transforming growth factor beta (TGFβ) signaling plays a crucial role in the development of cranial neural crest (CNC) cell–derived bone, and loss of *Tgfbr2* in CNC cells results in craniofacial skeletal malformations. Our recent studies indicate that non-canonical TGFβ signaling is activated whereas canonical TGFβ signaling is compromised in the absence of *Tgfbr2* (in *Tgfbr2*$^{fl/fl}$*;Wnt1-Cre* mice). A haploinsufficiency of *Tgfbr1* (aka *Alk5*) (*Tgfbr2*$^{fl/fl}$*;Wnt1-Cre;Alk5*$^{fl/+}$) largely rescues craniofacial deformities in *Tgfbr2* mutant mice by reducing ectopic non-canonical TGFβ signaling. However, the relative contributions of canonical and non-canonical TGFβ signaling in regulating specific craniofacial bone formation remain unclear. We compared the size and volume of CNC–derived craniofacial bones (frontal bone, premaxilla, maxilla, palatine bone, and mandible) from E18.5 control, *Tgfbr2*$^{fl/fl}$*;Wnt1-Cre,* and *Tgfbr2*$^{fl/fl}$*;Wnt1-Cre;Alk5*$^{fl/+}$mice. By analyzing three dimensional (3D) micro-computed tomography (microCT) images, we found that different craniofacial bones were restored to different degrees in *Tgfbr2*$^{fl/fl}$*;Wnt1-Cre;Alk5*$^{fl/+}$ mice. Our study provides comprehensive information on anatomical landmarks and the size and volume of each craniofacial bone, as well as insights into the extent that canonical and non-canonical TGFβ signaling cascades contribute to the formation of each CNC–derived bone. Our data will serve as an important resource for developmental biologists who are interested in craniofacial morphogenesis.