Kir2.1 Potassium Channels and Bone Morphogenic Proteins in Craniofacial Development

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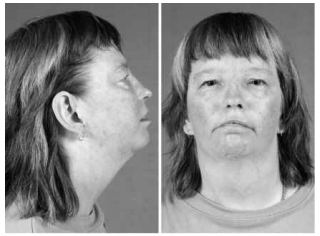
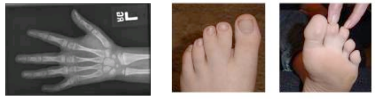
ABSTRACT

It is well known that craniofacial development relies on signaling molecules such as Bone Morphogenic Protein (BMP). More recently, it has become apparent that ion channels are also critical for craniofacial development. However, how ion channels contribute to canonical developmental signaling remains mysterious. Loss of the K^+ Inwardly Rectifying Channel Kir2.1 (*Kir2.1*$^{KO/KO}$) phenocopies loss of BMP2/4 signaling from the cranial neural crest cells (cNCCs) of mice. Kir2.1 is also required in the CNCCs for secondary palate closure. Furthermore, BMP signaling is reduced in the developing palate of *Kir2.1*$^{KO/KO}$ mice. To understand how Kir2.1 contributes to BMP signaling, we knocked out one copy of Kir2.1 and turned on a constitutively active BMP receptor in the cranial neural crest. We then quantified changes in craniofacial development. In *Kir2.1*$^{KO/+}$ mice that express a constitutively active BMP receptor (*caBMPR1a/+*) in the cNCC, we found an exacerbation of phenotypes including a shortened premaxilla, shortened nasal bones, widened fontanelle, and decreased mandible height and length. Mice lacking one copy of Kir2.1 (*Kir2.1*$^{fl/+}$) and one copy of the BMP4 ligand (*BMP4*$^{fl/+}$) in the cNCC showed a tendency towards rescuing the craniofacial defects of the *BMP4*$^{fl/+}$ in the cNCC alone. *BMP4*$^{fl/+}$ alone show craniofacial defects at embryonic day 18.5, as noted by increased fontanelle area, decreased mandible length, and decreased mandible height. While the *Kir2.1*$^{fl/+}$*; BMP4*$^{fl/+}$ also showed craniofacial phenotypes, they are less severe. Data from our lab shows that depolarization can induce BMP4 release. Loss of Kir2.1 should depolarize cells and could lead to a constant release of BMP4. Together, these results suggest a negative feedback loop in BMP4 signaling in which constant release of BMP4 is detrimental to the efficiency of BMP4 signaling.

KIR2.1 Mutations Cause Developmental Defects

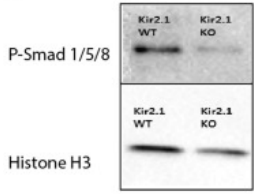


Individuals with Anderson Tawil Syndrome (KIR2.1 mutation) show characteristic skeletal features, including cleft palate, wide set eyes, low set ears, small jaw, clinodactyly of the 5th digit, brachydactyly, and syndactyly of digits 2 and 3.



Yoon *et al*, 2006; Katz, *Arch Neurol.* 1999

BMP signaling is decreased in *Kir2.1*$^{KO/KO}$ mice

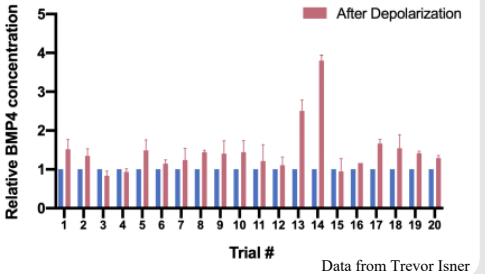


Belus et al. 2018

BMP signaling, including the phosphorylation of Smads 1, 5 and 8, are decreased in *Kir2.1*$^{KO/KO}$ animals.

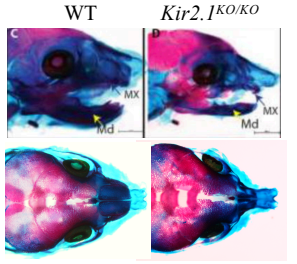
BMP Release is Induced by Depolarization in Cranial Neural Crest

In Immortalized Mouse Embryonic palatal Mesenchyme (iMEPM) cell culture, BMP release is responsive to cellular depolarization.

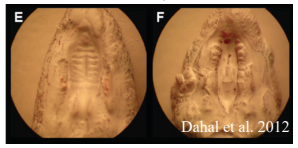


Data from Trevor Isner

Loss of Kir2.1 in mice phenocopies BMP loss of function craniofacial and digit defects

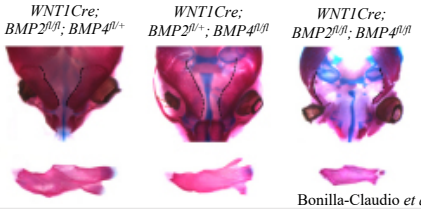


Belus et al., 2018



Dahal et al. 2012

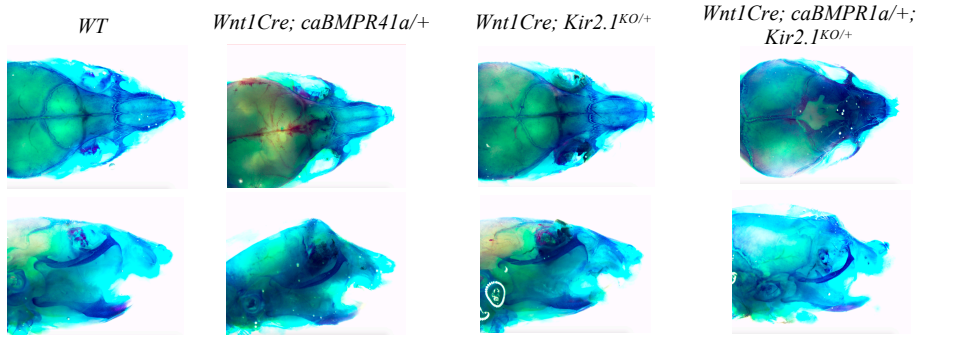
KIR2.1$^{KO/KO}$ mice and loss of BMP2/4 in cNCCs show craniofacial defects such as micrognathia, widened fontanelle, and clefting of the secondary palate. BMP loss of function is combinatorial, with the most severe phenotypes present in the skulls missing both BMP2 and BMP4

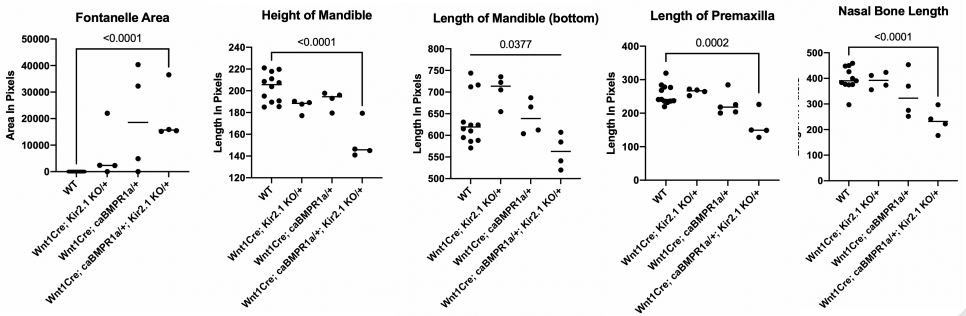


Bonilla-Claudio *et al.*, 2012

Loss of Kir2.1 combined with constitutively active BMP receptor yields exacerbated phenotypes

The combination of constitutively active BMP receptors with the loss of one copy of *Kir2.1* shows an exacerbation of craniofacial phenotype. *Wnt1Cre; caBMPR1a; Kir2.1*$^{KO/+}$ have the most severe defects including widened fontanelle area, reduced mandible height and length, reduced length of the premaxilla, and reduced length of the nasal bones.





Loss of Kir2.1 with loss of BMP4 ligand appears to rescue phenotypes

At embryonic day 18.5 (E18.5), skulls missing one copy of *Kir2.1* and one copy of the ligand *Bmp4* trend toward rescuing phenotypes compared to skulls missing *Bmp4* alone.