Developmental Biology 417 (2016) 4–10

Contents lists available at ScienceDirect

Developmental Biology

journal homepage: www.elsevier.com/locate/developmentalbiology

# A novel ciliopathic skull defect arising from excess neural crest

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ARTICLE INFO

*Article history:*
Received 1 July 2016
Accepted 1 July 2016
Available online 6 July 2016

*Keywords:*
Cilia
Ciliopathy
*Fuz*
*Fgf8*
Neural crest
Craniofacial
Skull
Calvaria
Coronal suture
Greig cephalopolysyndactyly
Morphogenesis
Craniosynostosis
*Wnt1*
*Mesp1*
Mouse

ABSTRACT

The skull is essential for protecting the brain from damage, and birth defects involving disorganization of skull bones are common. However, the developmental trajectories and molecular etiologies by which many craniofacial phenotypes arise remain poorly understood. Here, we report a novel skull defect in ciliopathic *Fuz* mutant mice in which only a single bone pair encases the forebrain, instead of the usual paired frontal and parietal bones. Through genetic lineage analysis, we show that this defect stems from a massive expansion of the neural crest-derived frontal bone. This expansion occurs at the expense of the mesodermally-derived parietal bones, which are either severely reduced or absent. A similar, though less severe, phenotype was observed in *Gli3* mutant mice, consistent with a role for Gli3 in cilia-mediated signaling. Excess crest has also been shown to drive defective palate morphogenesis in ciliopathic mice, and that defect is ameliorated by reduction of *Fgf8* gene dosage. Strikingly, skull defects in *Fuz* mutant mice are also rescued by loss of one allele of *fgf8*, suggesting a potential route to therapy. In sum, this work is significant for revealing a novel skull defect with a previously un-described developmental etiology and for suggesting a common developmental origin for skull and palate defects in ciliopathies.


## 1. Introduction

Craniofacial defects are among the most common and varied human congenital anomalies, affecting at least 1 in 600 live births (Mossey, 2003). While some classes of skull defect are increasingly well understood, there are many for which the etiology remains largely unknown, and even unexplored. For example, the most common skull vault defect has been comprehensively studied: Craniosynostosis is a premature fusion of the cranial sutures for which several causative genes are known and for which mouse models are available (Mossey, 2003; Twigg and Wilkie, 2015; Johnson and Wilkie, 2011). By contrast, craniofacial phenotypes such as acalvaria, calvarial thinning and collapsed calvaria remain only very poorly understood as a result of the paucity of human genetic studies and/or mouse models (Moore et al., 1999; Tokumaru et al., 1996). A deeper etiological understanding of the full spectrum of skull defects is an important challenge for developmental biologists, and could inform individual treatment paradigms and comfort both patients and their families.

This diversity in human skull anomalies reflects the complexity of mammalian skull morphogenesis. For example, two of the major bone pairs in the neurocranium, the frontal and parietal bones, are derived from different embryonic lineages. Both of these bones are required to protect the forebrain, and while the frontal bones are neural crest-derived, parietal bones arise from paraxial mesoderm (Jiang et al., 2002; Yoshida et al., 2008). Previous lineage analyses have shown that neural crest- and mesoderm-derived skull mesenchyme maintain their boundary at the coronal suture until birth (Merrill et al., 2006; Yoshida et al., 2008; Jiang et al., 2002). In addition to maintaining lineage boundaries, the initial positioning of neural crest- and mesoderm-derived mesenchyme must also be tightly regulated relative to the underlying brain. Initially, the entire forebrain is encased by neural crest, however, the caudal half later is covered by mesoderm (Jiang et al., 2002; Yoshida et al., 2008) (see Fig. S1). As such, the border between neural crest- and mesoderm-derived skull mesenchyme must reposition during cranial morphogenesis. Strikingly, however, the developmental time window in which such re-positioning occurs has not been characterized.

Here, we report a novel skull phenotype in a ciliopathic mutant mouse. We show that only a single calvarial bone plate encases the forebrain in mice lacking *Fuz*, an essential regulator of ciliogenesis (Park et al., 2006; Gray et al., 2009). To elucidate the etiology of

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http://dx.doi.org/10.1016/j.ydbio.2016.07.001
0012-1606/

this defect, we characterized early morphogenesis of the frontal and parietal bones. We find that *Fuz* mutants develop a novel skull phenotype in which the neural crest-derived frontal mesenchyme is enlarged at the expense of the parietal mesenchyme, and thus mutants develop only a single calvarial bone pair. We previously showed that Gli3 processing was disrupted in *Fuz* mutant mice, and accordingly, we now show that neural crest-derived frontal mesenchyme is also expanded in *Gli3*$^{xt\text{-}j}$ mutant mice at the expense of the parietal bone. Finally, parietal bone formation was rescued when *Fgf8* was genetically reduced in *Fuz* mutants, suggesting that expansion of *Fgf8* in *Fuz* mice is responsible for increased frontal mesenchyme. These findings provide new insights into pathological skull development generally, and potentially shed light on ciliopathies, Gli3-related Grieg Cephalopolysyndactyly and FGF-related craniofacial syndromes.

## 2. Results and discussion

### 2.1. Only a single calvarial bone pair develops in Fuz mutant mice

Previously, we reported that no coronal suture was evident at

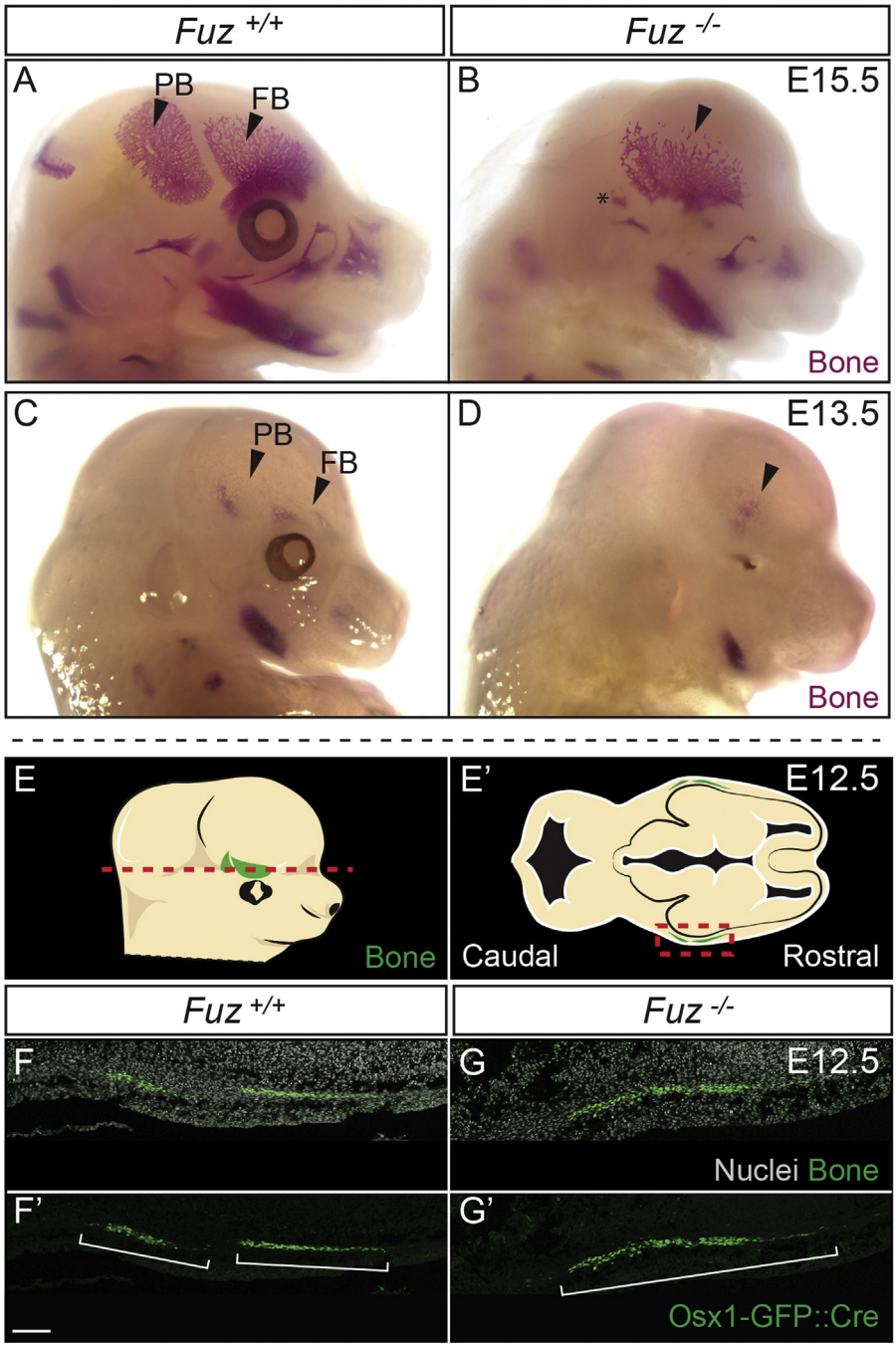


**Fig. 1.** *Fuz* mutants form a single calvarial bone pair and no coronal suture. (A-B) Alizarin red staining of E15.5 embryos. (A) *Fuz*$^{+/+}$ embryo shows frontal (FB) and parietal bones (PB) separated by the coronal suture (CS) (n=5). (B) *Fuz*$^{-/-}$ embryo showing a single bone plate (black arrowhead) (*n*=4). (C-D) Alizarin red staining of E13.5 embryos. (C) Two mineralization centers are observed in *Fuz*$^{+/+}$ embryos (*n*=12). (D) *Fuz*$^{-/-}$ embryo showing a single mineralization site (black arrowhead) (*n*=6). (E-E′) Schematics indicating sectional plane and anatomy represented in (G-J′). (F-G′) Horizontal sections of E12.5 embryos showing *Osx1-GFP::Cre* expression (green) and nuclei (gray). (F-F′) Two domains of GFP expression correspond to the frontal and parietal bones in *Fuz*$^{+/+}$ embryos (*n*=4). (G-G′) A single GFP domain is observed in *Fuz* mutant (*n*=3).