



# TGF- $\beta$ Signaling and Aplasia Cutis Congenita: Proposed Animal Model

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**ABSTRACT** TGF- $\beta$  plays a role in cell migration, proliferation, and differentiation during embryonic development. This study investigated the effect of neural crest- or mesoderm-specific loss of TGF- $\beta$  type II receptor in mice. These conditional knockout mice both exhibit skin defects of the skull associated with an underlying bone defect, a phenotype consistent with the human disorder aplasia cutis congenita. The authors suggest that TGF- $\beta$  type II receptor gene is a candidate gene for aplasia cutis congenita.

## AUTHORS

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**C**ongenital syndromic disorders frequently include craniofacial anomalies. For instance, cleft palate, the most common craniofacial disorder, occurs in Treacher-Collins syndrome.<sup>1</sup> The investigation of the etiology of these malformations will hopefully result in the ability to treat and/or prevent these conditions in the future.

Numerous studies have revealed that specific genes are related to particular familial disorders. In addition, mouse knockout models containing disruptions of genes implicated in human disorders often reproduce the human phenotypes of human. For instance, *Tbx1* null mice exhibit the DiGeorge syndrome phenotype seen in humans with haploinsufficiency of the *TBX1* gene.<sup>2</sup>

Some knockout mice die prematurely at an early developmental stage because

of a constitutional defect, such as in vascular system, making it impossible to analyze later developmental events. Therefore, conditional knockout mice have been developed using the Cre-LoxP system.<sup>3</sup> The Cre-LoxP system is a powerful tool to control the function of a specific gene in temporal and spatial specific manner. Briefly, the Cre gene is inserted under the regulation of a specific gene promoter and loxP sites are engineered around a gene of interest. When this promoter is activated, Cre protein excises the gene region between the loxP sites. For instance, *Wnt1* is expressed in the neural crest cell lineage during embryonic development and can be used to limit Cre expression temporally and spatially.<sup>3,4</sup>

The authors have previously reported that *Wnt1-Cre;Tgfb2* lose Transforming Growth Factor- $\beta$  (TGF- $\beta$ ) signaling in the

neural crest cell lineage, resulting in severe craniofacial defect.<sup>5</sup> TGF- $\beta$  signaling is involved in important biological functions such as cell migration, proliferation, and differentiation.<sup>6</sup> During embryogenesis, there are two mesenchymal cell lineages in the craniofacial region, cranial neural crest- and mesoderm-derived cells.<sup>7</sup> In this study, the authors analyzed two kinds of conditional knockout mice, lacking TGF- $\beta$  specifically in their neural crest- or mesoderm-derived cells in order to investigate the contribution of these lineages to craniofacial development. The conditional knockout mice exhibit skin defects of the skull associated with an underlying bone defect.

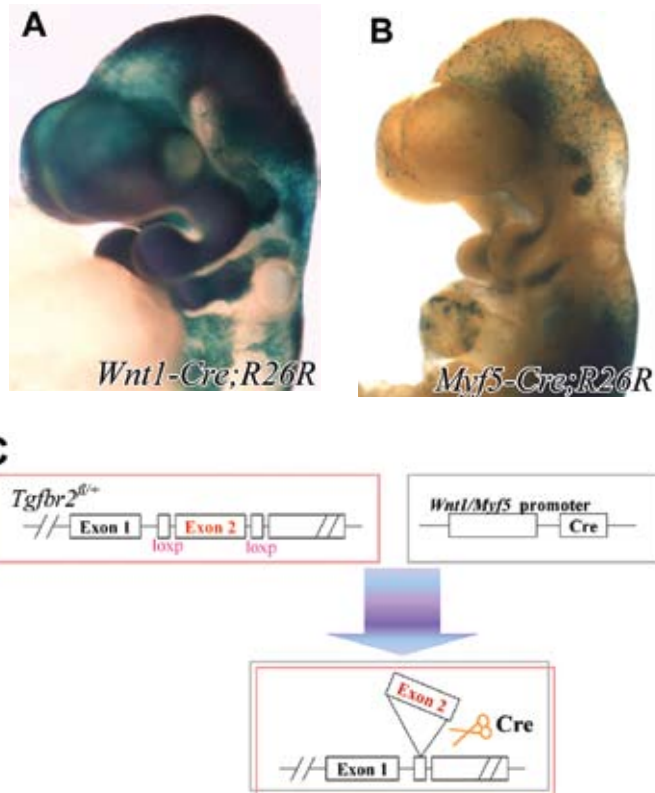
Aplasia cutis congenita, ACC, is a human disorder characterized by a localized defect of skin structure at birth, usually occurring on the scalp.<sup>8,9</sup> This disorder is frequently complicated by defects of the muscle and bone beneath the affected skin and sometimes associated with a defect of the skull.<sup>10,11</sup> ACC is divided into nine groups.<sup>11</sup> The phenotype of group 4 of ACC, in particular, is very similar to that of both *Myf5-Cre;Tgfb2<sup>fllox/fllox</sup>* and *Wnt1-Cre;Tgfb2<sup>fllox/fllox</sup>* mouse models, suggesting that *Tgfb2* is a potential candidate gene for ACC.

## MATERIALS AND METHODS

All animal studies were performed according to IACUC guidelines.

### Two-component Genetic System for Marking the Progeny of Somite-derived Cells

The *R26R* conditional reporter allele has been described previously.<sup>12</sup> The authors mated *Wnt1-Cre* or *Myf5-Cre* and *R26R* mice to generate *Wnt1-Cre;R26R* or *Myf5-Cre;R26R* embryos. Detection of  $\beta$ -galactosidase activity in whole embryos (Embryonic 9.5 day) was carried out as previously described.<sup>4</sup>



**FIGURES 1A-B.** Whole mount  $\beta$ -galactosidase activity staining at embryonic day 9.5 of *Wnt1-Cre;R26R* mouse (a) and *Myf5-Cre;R26R* (b) mice. **FIGURE 1C.** Schematic diagram illustrating the conditional knockout mouse strategy.

### Generation of *Myf5-Cre;Tgfb2<sup>fllox/Flox</sup>* Mutant Mice

*Wnt1-Cre* or *Myf5-Cre* transgenic mice have been described previously.<sup>5,13</sup> The authors crossed *Wnt1-Cre;Tgfb2<sup>fllox/+</sup>* or *Myf5-Cre;Tgfb2<sup>fllox/+</sup>* with *Tgfb2<sup>fllox/fllox</sup>* mice to generate *Myf5-Cre;Tgfb2<sup>fllox/fllox</sup>* or *Myf5-Cre;Tgfb2<sup>fllox/fllox</sup>* null alleles that were genotyped using PCR primers as previously described to produce 36 offspring with similar characteristics.<sup>5</sup>

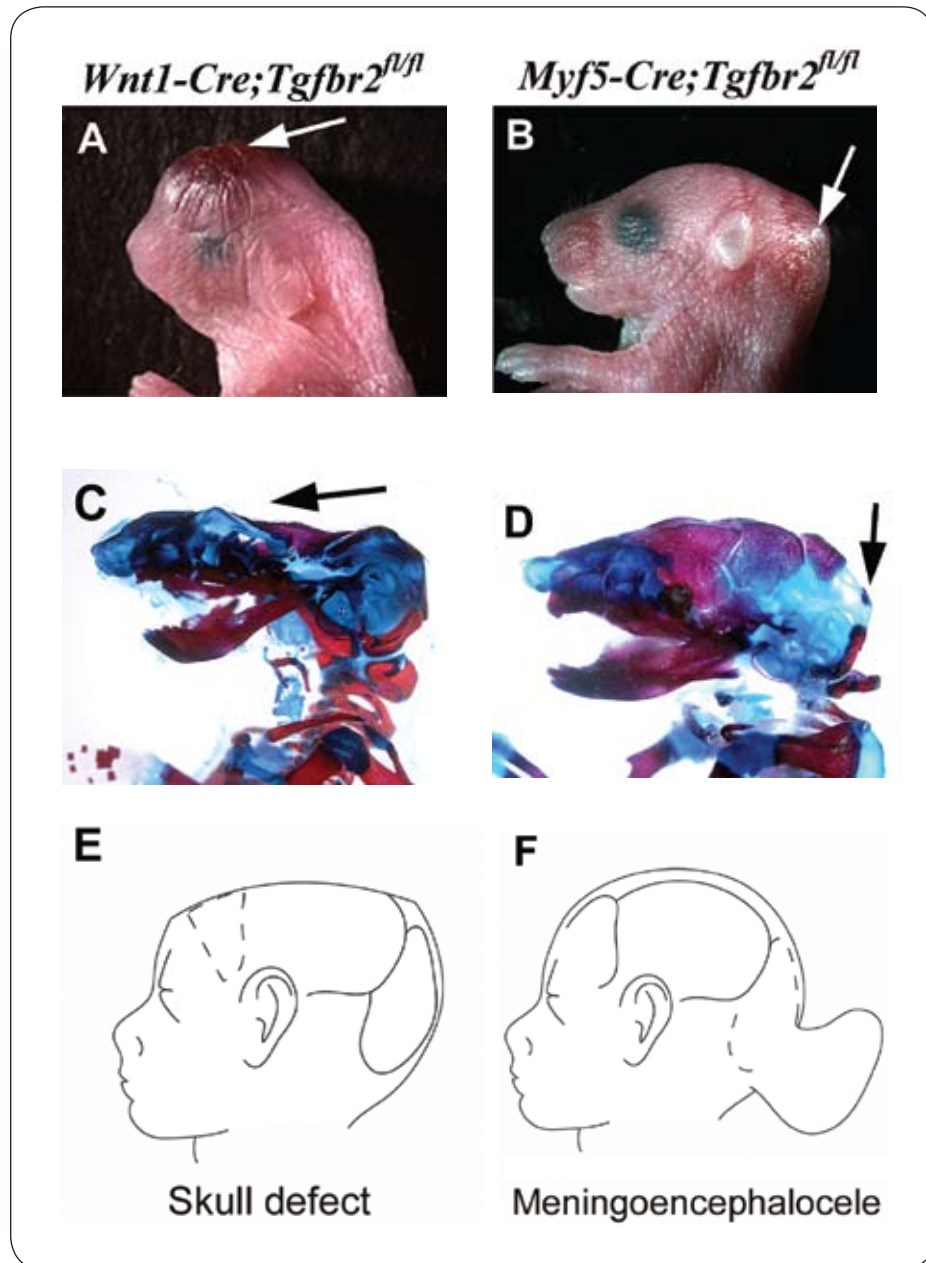
### Staining of Whole Skeleton

Whole skeletal preparations of newborn mice were prepared and stained with Alizarin Red and Alcian Blue as previously described.<sup>14</sup>

## RESULTS

### Two Mesenchymal Cell Lineages Are Involved in Craniofacial Development

Two cell lineages are involved in craniofacial development: cranial neural crest-derived and mesoderm-derived.<sup>4,7</sup> The Cre-LoxP recombination system is a powerful tool to investigate the fate of specific cell lineages when Cre transgenic mice are crossed with *Rosa26R* mice.<sup>12</sup> The offspring produce  $\beta$ -galactosidase activity in the Cre expressing cells, which allows the investigator to detect the specific cell lineage. For instance, the neural crest cell lineage can be indelibly marked in *Wnt1-Cre* transgenic mice.<sup>4</sup> At E10.5, cranial neural



**FIGURES 2A-B.** Macroscopic appearance of newborn *Wnt1-Cre;Tgfb2<sup>fl/fl</sup>* (a) and *Myf5-Cre;Tgfb2<sup>fl/fl</sup>* (b) mice. **FIGURES 2C-D.** Alizarin Red and Alcian Blue whole mount bone staining of *Wnt1-Cre;Tgfb2<sup>fl/fl</sup>* (c) and *Myf5-Cre;Tgfb2<sup>fl/fl</sup>* (d) mice. **FIGURES 2E-F.** Schematic drawing of human patients with frontal bone defect (e) and meningoencephalocele (f).

crest cells populate the branchial arches and front nasal process (blue, **FIGURE 1A**).

Mesenchymal cells are detectable in *Myf5-Cre;R26R* transgenic mice.<sup>13</sup> Mesoderm cells are visible in the core of the branchial arches and somite (blue, **FIGURE 1B**). The blue staining provides a

visualization of the cells that will lose TGF- $\beta$  signaling when Cre transgenic mice are crossed with *Tgfb2*-floxed mice. **FIGURE 1C** diagrams the authors' strategy for making conditional knockout mice. Cells that express *Wnt1* (cranial neural crest) or *Myf5* (mesoderm) will induce

the Cre protein that will excise Exon 2 of the *Tgfb2* gene. Thus, the creation of *Tgfb2* conditional knockout mice that lack TGF- $\beta$  specifically in either the cranial neural crest or the mesoderm.

### *Tgfb2* Conditional Knockout Mice Exhibit a Defect in Skull Development

*Tgfb2* conventional knockout mice die prematurely at an early embryonic stage.<sup>15</sup> Therefore, the authors made *Tgfb2* conditional knockout mice specific for the neural crest or the mesoderm.<sup>5,16</sup> *Wnt1-Cre;Tgfb2<sup>fl/fl</sup>* mice showed apical swelling and hemorrhage of the head (**FIGURE 2A**, white arrow).

Whole mount bone staining revealed retarded development of the frontal bone (**FIGURE 2C**, black arrow). On the other hand, *Myf5-Cre;Tgfb2* mice showed swelling and hemorrhage around the occipital area of the head (**FIGURE 2B**, white arrow) and whole mount bone staining revealed a defect of the supraoccipital bone (**FIGURE 2D**, black arrow). These phenotypes of the *Tgfb2* conditional knockout mice were seen in the human disorder aplasia cutis congenita, such as meningoencephalocele (**FIGURES 2F, E**).

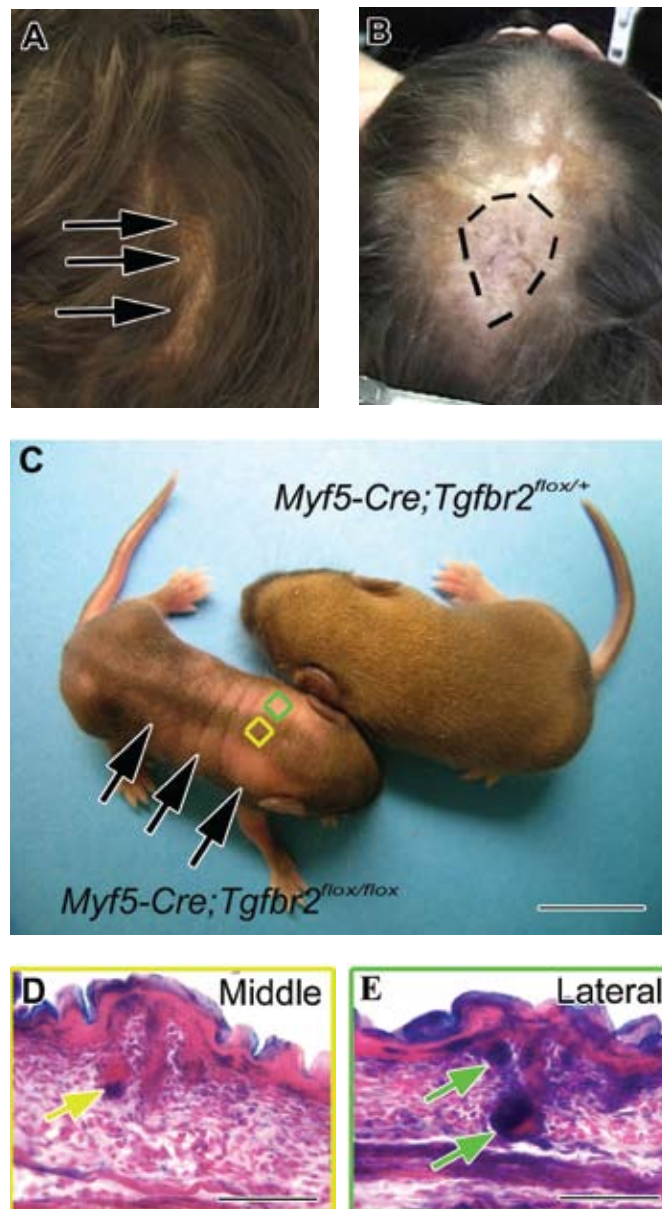
### TGF- $\beta$ Type II Receptor Conditional Knockout Mice Reproduce Phenotypes of Aplasia Cutis Congenita Patients

ACC patients have a congenital skin defect on the skull (**FIGURES 3A, B**). In the few conditional knockout mice that could survive after birth, the authors observed cutis verticis gyrate (**FIGURE 3C**, compared with **FIGURE 3A**, arrows), a phenotype characterized by deep furrows and linear folds on the skin.<sup>17,18</sup> The authors hypothesized that Cre recombination might have occurred in the lateral area without hair, but not in the middle area with hair. To test this hypothesis, the

authors assessed lacZ expression in the skin of *Myf5-Cre;R26R<sup>lox/+</sup>* mice skin at the newborn stage (FIGURES 3D, E). Indeed, hair follicles in the lateral-dorsal area contained only lacZ positive cells (FIGURE 3E). In the mid-dorsal area, the hair follicles contained a mixed population of *Myf5-Cre* positive and negative cells (FIGURE 3D).

## DISCUSSION

The conditional knockout of *Tgfbr2* in mouse neural crest- and mesoderm-derived cells resulted in the disorganization of connective tissue above the defective bone area.<sup>5,16</sup> Aplasia cutis congenita is a rare human congenital skin condition associated with bone and skeletal muscle defects beneath affected skin and is strikingly similar to the phenotype observed in the authors' *Tgfbr2* conditional knockout mouse model (FIGURE 2, FIGURES 3A-C). ACC appears to show dominant inheritance but many details remain unknown.<sup>19,20</sup> For instance, one ACC patient has a frontal bone defect associated with a skin defect, but another patient has a parietal bone defect associated with a skin defect.<sup>11,10</sup> ACC is also accompanied by cutis verticis gyrate that is characterized by the appearance of deep, linear skin folds in the scalp<sup>17,18</sup> (FIGURE 3A). TGF- $\beta$  isoforms and TGF- $\beta$  type II receptor are expressed in hair follicles during embryonic and neonatal stages.<sup>21,22</sup> TGF- $\beta$  is a critical factor in the formation of connective tissue and promotes the synthesis of extracellular matrix in dermal tissue.<sup>23,24</sup> The cause of the skin defect in our *Tgfbr2* mouse model may be the loss of TGF- $\beta$  signaling in mesenchymal and epithelial cells in hair follicles. These skin structure and hair follicle defects result in a phenotype remarkably similar to ACC. Thus, the authors suggest that *Tgfbr2* is a candidate gene for aplasia cutis congenita. ■■■■



**FIGURES 3A-B.** Aplasia cutis congenita patients. Arrows indicate cutis verticis gyrate. Dotted circle highlights the congenital skin defect. **FIGURE 3C.** Macroscopic appearance of *Myf5-Cre;Tgfbr2<sup>flx/flx</sup>* and *Myf5-Cre;Tgfbr2<sup>flx/+</sup>* mice at postnatal day 10. **FIGURES 3D-E.** Enlarged view of yellow and green boxes in C. Arrows indicate the  $\beta$ -galactosidase activity.

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