Determining how a novel mutation in Fzd6 alters Planar Cell Polarity

Maria Rickman, Connie Corcoran, and Dr. Maureen Cetera
University of Minnesota, Department of Genetics, Cell Biology and Development

Background & Significance

Planar cell polarity (PCP) refers to the uniform orientation of cell polarity that specifies the orientation of cells in the plane of a tissue.[4] Cell behaviors and cell movements are oriented along a tissue axis during development directing structural formation, body axis elongation, and neural tube closure.[14] When cells are unable to orient along a tissue axis, severe developmental defects occur (Fig. 1).[2,8]

Proper morphogenesis requires cells to sense direction along the body axis

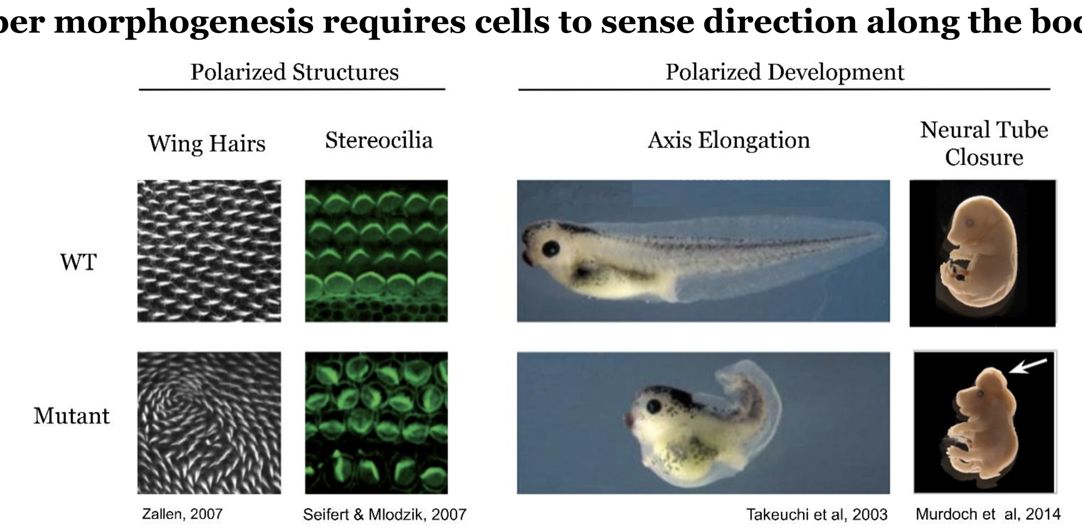


Fig 1. Planar cell polarity mutants disrupt developmental processes.

Cells segregate PCP proteins to opposing regions of the cell to establish an axis of polarity

The PCP pathway is regulated by an intercellular signaling complex composed of the core set of PCP membrane proteins: Frizzled (Fzd), Vangl, and Celsr.[10] These proteins are embedded within the cell membrane and are localized asymmetrically (Fig. 2).[11]

Oriented cell behaviors are patterned by the PCP system

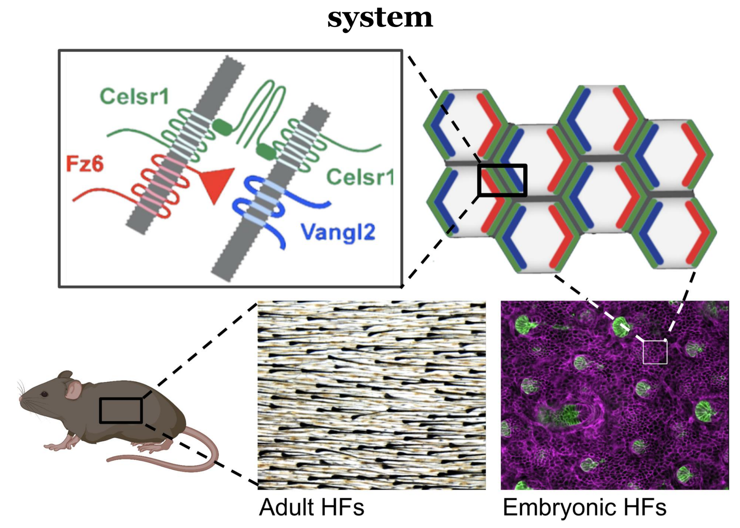


Fig 2. PCP proteins direct the orientation of hair follicles (HFs) in the mouse skin. PCP acts in the epidermis to pattern embryonic HFs.

PCP knock-out (KO) mutants disrupt hair follicle orientation

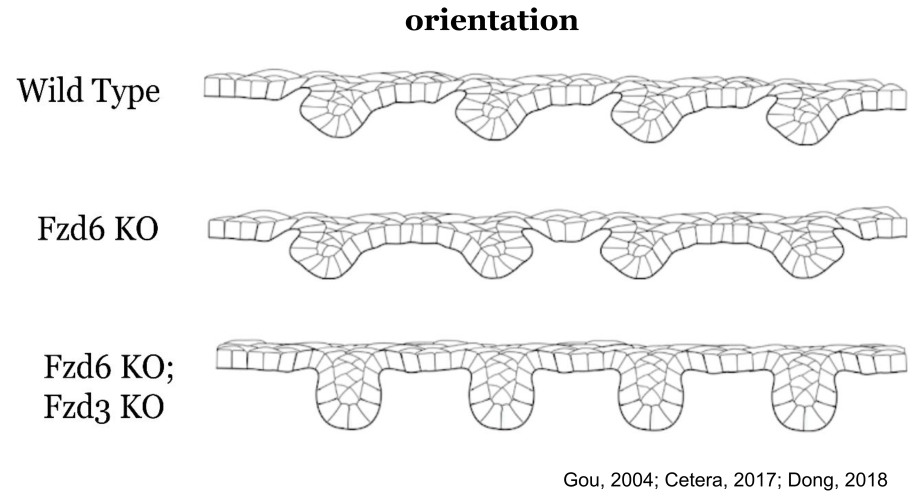


Fig 3. Sagittal schematic of embryonic hair follicles. Wild type HFs are anteriorly oriented, Fzd6KO mutant HFs are randomized, and Fzd6KO; Fzd3KO double mutant HFs grow straight down.

Of the Fzd proteins, Fzd6 and Fzd3 are responsible for controlling PCP signaling. Fzd3 and Fzd6 act redundantly in regulating the polarity of skin.[15] Loss of both Fzd3 and Fzd6 completely eliminates PCP function. Without PCP function, neural tubes remain open, stereocilia are disordered, and hair follicle growth is abnormal where follicles grow straight down (Fig. 3).[12,15]

The fancy mouse *rosette* shows unique defects in hair follicle orientation

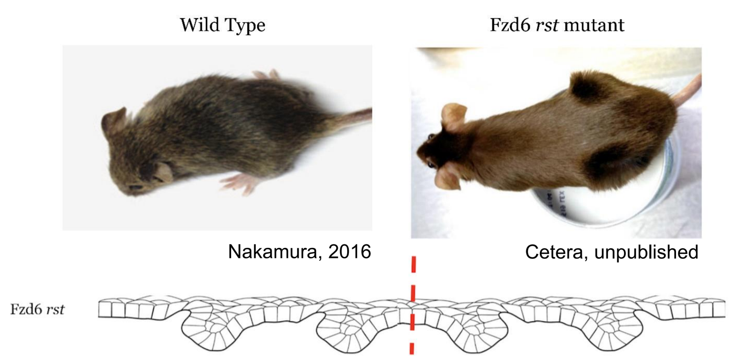


Fig 4. A spontaneous mutation called rosette developed in a fancier's colony in the 1960s. The mutation causes whorls in the posterior of the mouse skin. During embryonic stages, HF orientation is reversed in the posterior region of the epidermis (right side of red line).

The *rosette* mutation blocks N-linked glycosylation which is predicted to block protein trafficking

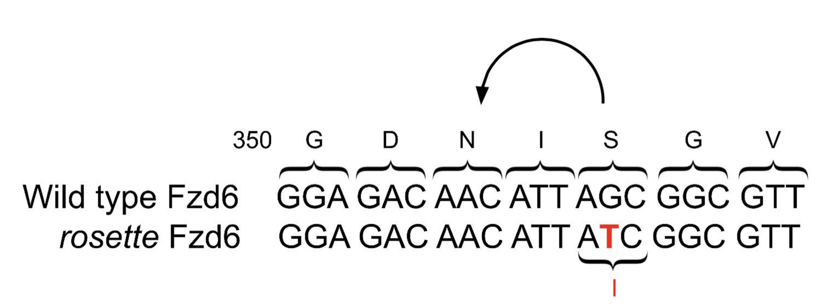


Fig 5. We mapped the mutation to amino acid 354 resulting in a Serine to Isoleucine substitution. Serine at position 354 is predicted to be required for N-linked glycosylation at amino acid 352. Glycosylation is predicted to control protein export from the ER. Thus, this mutation could inhibit Fzd6 localization to the cell membranes.

Mutations in Fzd6 alone also impact hair follicle orientation. Specifically, the Fzd6 point mutation (S354I) found in *rosette* mice (Fig. 5) causes coordinated mis-orientation of hair follicle development. Hair follicle growth in *rosette* have reversed rather than randomized hair follicle orientation demonstrated in Fzd6 KO mice (Fig. 3).[7] This mutation prevents protein maturation and Fzd6 exportation from the ER to the cell membrane (Fig. 6).

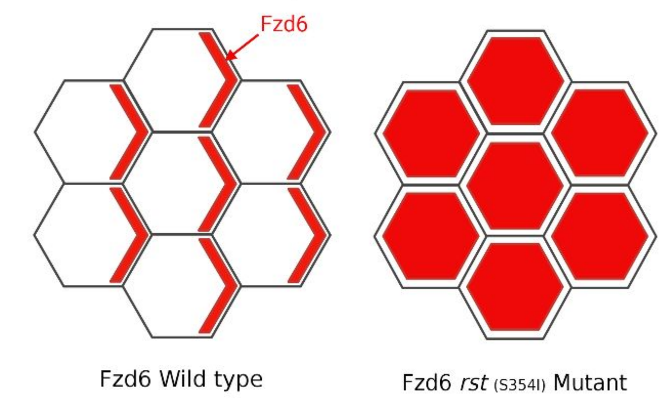


Fig 6. Staining for Fzd6 protein in wild type and rosette mutants revealed that Fzd6 is cytoplasmic in the epidermis of rosette mutants. Thus it would be unlikely to participate in PCP signaling at the cell membranes.

To determine the impact of the point mutation on Fzd6 function, our goal was to quantify and compare PCP defects in *rosette* against Fzd6 KO backgrounds without any Fzd3 activity. Quantification of PCP defects can be done through measuring hair follicle orientation, neural tube closure defects, and stereocilia orientation

Hair Follicle Orientation

Methods & Results:

The embryonic Fzd6KO and rst hair follicle patterns were first determined by confocal microscopy (Fig. 7). Our goal is to find markers that allow us to quantify hair follicle phenotypes in Fzd6KO and rst mutants in a Fzd3 null background with a widefield system. Confocal microscopy focuses the light at the plane of interest while a widefield system does not (Fig. 8). For this reason, we need to look for markers that are specifically expressed in the hair follicles, with little expression in other cell types, and low background staining.

Confocal microscopy

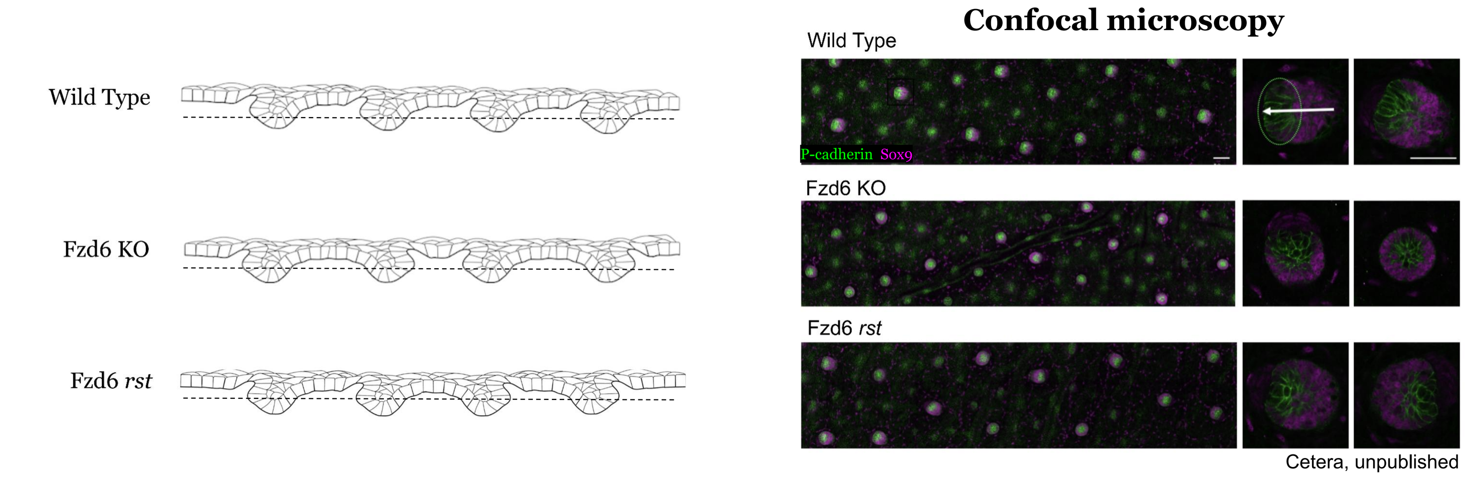
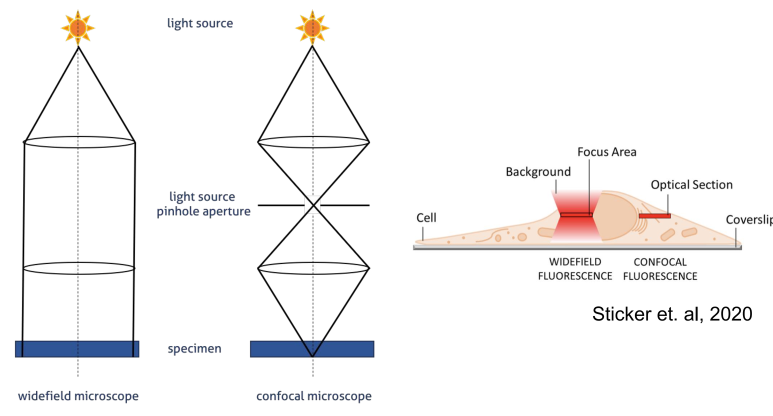


Fig 7. Confocal imaging revealed the embryonic phenotypes of Fzd6KO and Fzd6 rst animals. P-Cadherin labels the anterior of the HF and Sox9 labels the posterior. In WT, follicles all point anteriorly. In Fzd6KO embryos, HFs are randomly oriented. In Fzd6 rst mutants, anterior follicles are correctly oriented and posterior follicles are reversed.

To establish a high throughput screening method and characterize hair follicle distribution patterns, various molecular markers are used (Fig. 9). P-Cadherin antibodies label the anterior of hair follicles. While E-Cadherin and Sox-9 antibodies label the posterior of hair follicles. Phalloidin labels all cells.[3]

Widefield imaging vs Confocal imaging



Widefield microscopy

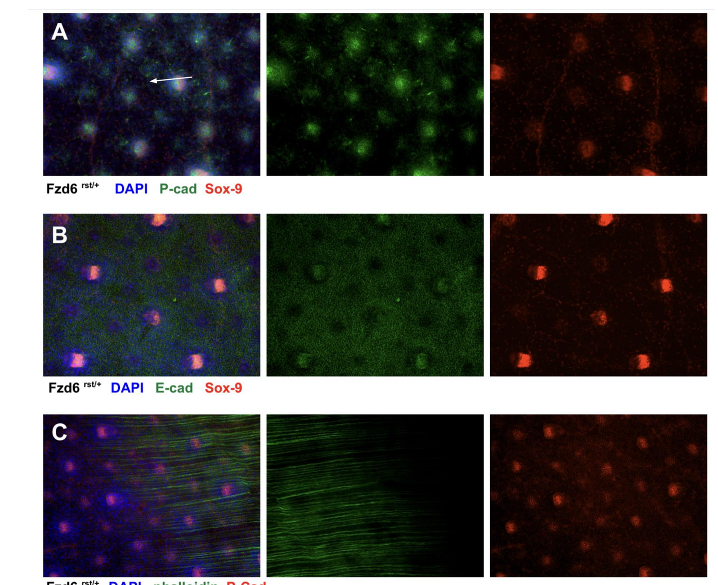


Fig 9. Embryonic hair follicles stained with the indicated markers. Images acquired with a widefield microscope.

Conclusions:

P-Cad and Sox-9 in skin 1 (Fig. 9-1) imaging show the cleanest signal when compared to E-Cad and Sox-9 (Fig. 9-2). These molecular markers will be used during high throughput screening on a wide field scope for future experiments.

Future Directions:

To determine if the Fzd6 point mutation found in *rosette* has any function, the Fzd6 rst mutation will be combined with Fzd3 KO. To combine the mutations, various matings will be arranged (Fig 10A). The Fzd6KO will be paired with the Fzd3 KO creating a double knockout via a series of matings (Fig 10B). We will measure hair follicle orientation across the back skin using the markers P-Cad and Sox-9. If the *rosette* mutant combination has the same hair follicle pattern and phenotypic characteristics as the double knockout, the point mutation has no function. However, if the phenotypes are different, the *rosette* point mutation has a function in the planar cell polarity pathway.

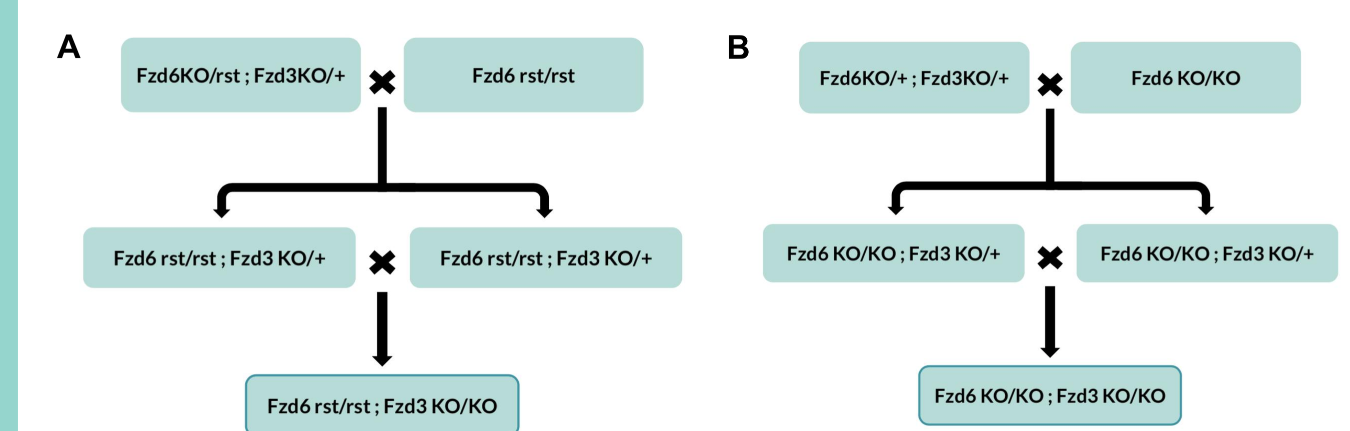


Fig 10. Cross scheme to generate animals of interest.

Neural Tube Closure Defects

Methods & Results:

In order to analyze neural tube defects, embryos were imaged for different backgrounds (Fig. 11C,D). Fzd6 KO/KO had no impact on neural tube closure, while the double knockout resulted in an open neural tube defect (Fig. 11A,B).[15]

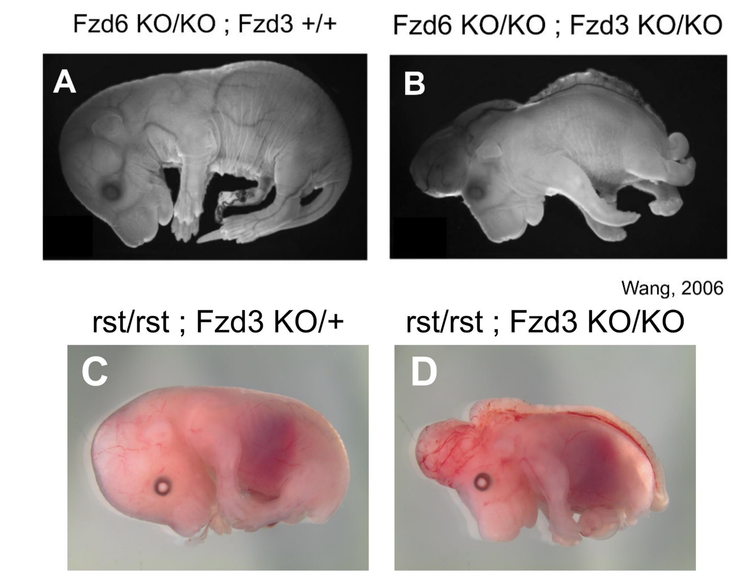


Fig 11. Neural tube defects in Fzd6 and Fzd3 double mutants.

Fzd3 KO/KO resulted in a open neural tube defect when paired with a Fzd6 rst mutation (Fig. 11C).

Conclusions:

The Fzd6 rst mutation inhibits Fzd6 function during neural tube closure. The phenotype observed is equivalent to the double knockout mutation (Fzd6 KO/KO; Fzd3 KO/KO) (Fig. 11B).

Future directions:

Perform additional matings (Fig. 10) and analyze neural tube closure defects. We will determine when neural tube defects occur and image embryos to determine the placement and severity of the defect.

References

1. Aw, W. Y., & Devenport, D. (2017). Planar cell polarity: global inputs establishing cellular asymmetry. *Current opinion in cell biology*, *44*, 110–116. https://doi.org/10.1016/j.ceb.2016.08.002
2. Butler, M. T., & Wallingford, J. B. (2017). Planar cell polarity in development and disease. *Nature reviews. Molecular cell biology*, *18*(6), 375–388. https://doi.org/10.1038/nrm.2017.11
3. Cetera, M., Leybova, L., Joyce, B. *et al.* (2018). Counter-rotational cell flows drive morphological and cell fate asymmetries in mammalian hair follicles. *Nat Cell Biol* 20, 541–552. https://doi.org/10.1038/s41556-018-0082-7
4. Cetera, M. (2021). Bad Hair Days: Reversing the Axis of Epidermal Polarity Presentation.
5. Devenport D. (2014). The cell biology of planar cell polarity. *The Journal of cell biology*, *207*(2), 171–179. https://doi.org/10.1083/jcb.201408039
6. Dong, B., Vold, S., Olvera-Jaramillo, C., Chang, H. (2018). Functional redundancy of frizzled 3 and frizzled 6 in planar cell polarity control of mouse hair follicles. *Development*; 145 (19): dev168468. doi: https://doi.org/10.1242/dev.168468
7. Guo, N., Hawkins, C., Nathans, J. (2004). Frizzled6 controls hair patterning in mice *Proceedings of the National Academy of Sciences*, 101 (25) 9277-9281; DOI: 10.1073/pnas.0402802101
8. Matis, M., & Axelrod, J. D. (2013). Regulation of PCP by the Fat signaling pathway. *Genes & development*, *27*(20), 2207–2220. https://doi.org/10.1101/gad.228098.113
9. Murdoch, J. N., Damrau, C., Paudyal, A., Bogani, D., Wells, S., Greene, N. D. E., Stanier, P., & Copp, A. J. (2014). Genetic interactions between planar cell polarity genes cause diverse neural tube defects in mice. *Disease Models & Mechanisms*, 7(10), 1153– 1163. https://doi.org/10.1242/dmm.016758
10. Peng, Y., & Axelrod, J. D. (2012). Asymmetric protein localization in planar cell polarity: mechanisms, puzzles, and challenges. *Current topics in developmental biology*, *101*, 33–53. https://doi.org/10.1016/B978-0-12-394592-1.00002-8
11. Struhl, G., Casal, J., & Lawrence, P. A. (2012). Dissecting the molecular bridges that mediate the function of Frizzled in planar cell polarity. *Development (Cambridge, England)*, *139*(19), 3665–3674. https://doi.org/10.1242/dev.083550
12. Tang, X., Zhang, L., Ma, T., Wang, M., Li, B., Jiang, L., Yan, Y., & Guo, Y. (2020). Molecular mechanisms that regulate export of the planar cell-polarity protein Frizzled-6 out of the endoplasmic reticulum. *The Journal of biological chemistry*, *295*(27), 8972–8987. https://doi.org/10.1074/jbc.RA120.012835
13. Torban, E., Patenaude, A.-M., Leclerc, S., Rakowiecki, S., Gauthier, S., Andelfinger, G., Epstein, D. J., & Gros, P. (2008). Genetic Interaction between Members of the Vangl Family Causes Neural Tube Defects in Mice. *Proceedings of the National Academy of Sciences of the United States of America*, *105*(9), 3449–3454. http://www.jstor.org/stable/25461255
14. Vladar, E. K., Antic, D., & Axelrod, J. D. (2009). Planar cell polarity signaling: the developing cell's compass. *Cold Spring Harbor perspectives in biology*, *1*(3), a002964. https://doi.org/10.1101/cshperspect.a002964
15. Wang, Y., Guo, N., Nathans, J. (2006).The Role of *Frizzled3* and *Frizzled6* in Neural Tube Closure and in the Planar Polarity of Inner-Ear Sensory Hair Cells *Journal of Neuroscience,* 26 (8) 2147-2156; DOI: https://doi.org/10.1523/JNEUROSCI.4698-05.2005

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