The Role of Cilia in Left-Right Patterning in Lateral and Oblique Facial Clefts

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Objectives: Lateral and oblique facial clefts (LOFC) are rare unilateral orofacial clefts with detrimental impact on affected patients. *SPECC1L* haploinsufficiency can cause LOFC. However, the etiology and pathophysiology remain mostly unknown due to a lack of animal models and genetic testing. We observed left-right asymmetry in *Twist1*$^{+/-}$*; Specc1l*$^{+/dEx4}$ and *IRF6*$^{+/-}$*; Specc1l*$^{+/\Delta C510}$ mutant mouse embryos exhibiting LOFC. This study investigates how actin and tubulin cytoskeletal filaments disrupt primary cilia homeostasis in migratory cranial neural crest cells (CNCC). We posit that the interaction between *Twist1* and *Specc1l* is crucial for cytoskeletal organization, and that cytoskeletal disruption affects primary cilia development, leading to alternations in signaling pathways essential for CNCC guidance and left-right patterning.

Experimental Methods: H&E staining was used to compare left-right craniofacial asymmetry in mouse embryos. RT-qPCR analysis quantified the expression of potential target genes for *Twist1* and *Specc1l.* Immunofluorescence staining determined the expression and organization of cytoskeletal filaments in *Twist1* and *Specc1l* double heterozygous embryos.

Results: *In vivo* tracing of CNCC migration revealed significant left-right patterning disruption, with more CNCC migrating to the right side of pharyngeal arches. Quantitative analysis showed significant increase of ciliary proteins *Fgf8*, *Fgfr1*, *ICK*, *Kif3a*, and *IFT88* in *Twist1*$^{+/-}$*; Specc1l*$^{+/dEx4}$ mutant embryos, while *Fgf3* expression showed no significant difference. Additionally, there was a marked reduction in actin and tubulin levels in *Twist1*$^{+/-}$*; Specc1l*$^{+/dEx4}$ mutant embryos, indicating compromised cytoskeletal regulation of ciliogenesis. Immunofluorescence staining revealed aberrations in primary cilia morphology, including decreased cilia length, further supporting the hypothesis that cytoskeletal dynamics are essential for maintaining ciliary structure and function.

Conclusion: Our findings suggest that haploinsufficiency of *Twist1* and *Specc1l* causes LOFC, indicating that these genes are critical for left-right patterning of CNCC, cytoskeletal homeostasis, and primary cilia development. The alterations in primary cilia provide a mechanism for the compromised left-right symmetry in the LOFC mutant mice.

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