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Organized cell movement is a major mechanism underlying facial morphogenesis

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Objectives: The morphogenesis of the midface involves narrowing of the medial mesenchyme to form the nasal septum, columella, philtrum and premaxilla. The decrease in width could be driven by extrinsic factors such as growth of the brain and eyes or by intrinsic movements of mesenchymal cells. Here we tested these hypotheses in the chicken embryo using organ culture techniques combined with live cell imaging.

Methods: Frontonasal mass and lateral nasal prominences were dissected from stage 25 embryos and photographed for 48h. A subset of cultures was treated with ROCK inhibitor (10 µM Y27632) to inhibit reorganization of the cytoskeleton. Measurements of width between the nasal slits were made. Other cultures were imaged over time using confocal microscopy. Hoechst dye was added to visualize the cell nuclei. Movies were captured at 10-minute intervals for 3-4h and cell nuclei were manually tracked using Fiji software (N = 11 controls and 9 ROCKi). Mathematical analyses of the xy positions for each timepoint and each cell were carried out.

Results: In vitro culture of the showed that the brain and face were not required for midfacial morphogenesis. ROCKi completely blocked narrowing in vitro, supporting the role of the cytoskeleton and individual cell movement. Mathematical analysis of cell tracking data indicated dynamic fluxes in the degree of tissue organization over time. There were consistent peaks of order and troughs of disorder. Surprisingly, there was coordination of the direction of cell movement in areas of 400 microns in diameter. This coordination was highly symmetrical, suggesting strong genetic control. Y27632 abolished symmetry and decreased order. We also found there were areas undergoing convergence and divergent and these were temporally regulated.

Conclusion: We conclude that intrinsic cellular movement is the major mechanism underlying ordered cell movements in the midface. Our novel live imaging methods allow direct observation of growing facial mesenchyme in normal and disease models.