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PERSPECTIVE

In preprints: Shh signaling activity predicts cardiac laterality in *Astyanax mexicanus* populations

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In vertebrates, heart asymmetry is biased toward the left side and orchestrated by signals from the embryonic left–right (L–R) organizer (LRO) (Grimes and Burdine, 2017). In fish, the LRO features a fluid-filled cavity, the Kupffer's Vesicle (KV), located in the tail bud. The fluid is set in motion by motile cilia, which leads to the right-sided inhibition of the Nodal family gene *spaw* by the antagonist Dand5, and the activation of target genes in the lateral plate mesoderm (Hashimoto et al., 2004). In comparison with humans, which display laterality disorders in only 1 of 5000-7000 births (Shiraishi and Ichikawa, 2012), the teleost *Astyanax mexicanus*, a single species consisting of two phenotypically distinct surface- and cave-dwelling populations, exhibit remarkable differences in their heart L–R asymmetry frequency. Cavefish display up to 20-30% of right-oriented hearts, compared with <5% for surface fish (Ma et al., 2021). In Ng et al. (2023 preprint), the authors examine the role of Sonic hedgehog (Shh) signaling in L–R patterning in the two populations of *Astyanax mexicanus*. Previously shown to be responsible for the environmental adaptation of the cavefish, Shh increases the olfactory sense and contributes to eye degeneration (Yamamoto et al., 2004; Menuet et al., 2007). The authors propose that the enhancement of Shh also leads to structural changes in the LRO and subsequent L–R axis reversion.

Natural differences in the morphology of the KV or its precursors, the dorsal forerunner cells (DFCs) are associated with laterality defects in zebrafish (Moreno-Ayala et al., 2021). Thus, Ng and colleagues started by comparing the KV structures between the surface fish and two cavefish families exhibiting high or low cardiac-looping defects. They found that the cavefish family with high cardiac L–R defects exhibits larger KV or more than one KV, potentially as a result of lumen formation defects. Specifically, a large KV area correlates with looping defects in the cavefish family with laterality defects. Cilia number and length in the KV, which are associated with L–R robustness in fish, can vary considerably among wild-type and transgenic strains (Gokey et al., 2016). In the cavefish family with high cardiac-looping defects, cilia length was shown to be increased and cilia density decreased. It will be interesting to evaluate in future studies whether these natural changes in KV ciliation are associated with different KV flow patterns (Sampaio et al., 2014) or if the ratio between motile and immotile cilia (Ferreira et al., 2017) is altered in this cavefish family.

KV cells are a coalescence of the DFCs, which are considered a subset of the endoderm (Warga and Kane, 2018). KV area correlates with DFC cell numbers and fewer DFCs leads to cardiac-looping defects. Ng and colleagues quantified the number of DFCs and noticed that, consistent with the enlarged and multiple KV, the cavefish with high L–R defects exhibited more DFCs. In parallel, they found that the master regulator of motile ciliogenesis *foxj1a* (Tavares et al., 2017) is enriched in the tailbud of the cavefish with high L–R defects. The authors suggest that those differences may account for the structural differences in KV morphology in this cavefish family.

Upstream of *foxj1a*, Shh signaling is a modulator of ciliogenesis and is associated with L–R asymmetry establishment in vertebrates (Negretti et al., 2022). In cavefish with high L–R defects, the expression of *shh* and its receptor *ptch2* expand toward the posterior midline, where the notochord meets and shapes the KV (Compagnon et al., 2014). Using the Smoothened agonist SAG, the authors found that Shh overexpression in surface fish recapitulates the cavefish phenotype with regressed eyes as well as increased KV area, cilia size and cardiac-looping defects.

The hallmark of laterality initiation is the right-sided enrichment of the Nodal antagonist *dand5*, expressed in KV cells (Hashimoto et al., 2004). Ng et al. analyzed the expression of *dand5* in the cavefish with higher L–R defects and found a higher percentage of abnormal expression, which correlates with large KVs. These observations on *dand5* laterality can be recapitulated by Shh overexpression, which leads to the bilateral or right-sided expression of *spaw*, responsible for propagating the L–R information from the posterior to the anterior mesoderm. Overall, these results suggest that the structural KV variations, including enlargement and cilia patterning, are responsible for the observed L–R defects, under the control of Shh signaling.

The pre-chordal plate at the anterior midline is crucial to understanding the role of Shh in cavefish adaptation (Yamamoto et al., 2004; Menuet et al., 2007). This research by Ng and colleagues offers insights into the role of Shh signaling in L–R asymmetry by showing that it is also enhanced posteriorly. It will be interesting to investigate how a higher DFC number and larger KVs in the cavefish populations with high L–R defects could be responsible for this phenotype. Although no other natural occurrence of multiple KV has been reported, loss-of-function conditions of *pk1a* and *myo1d* can lead to fragmented KV lumens (Oteiza et al., 2010; Saydmohammed et al., 2018) and could be compared to the one present in this study. The analysis of fluid flow patterns in large KVs could also contribute to understanding how *dand5* degradation only occurs on the left side (Sampaio et al., 2014; Juan et al., 2018). Moreover, Shh has been reported on extracellular vesicles in the mammalian LRO (Tanaka et al., 2005). However, the role of these vesicles or similar extracellular granules (Tanaka et al., 2023) in controlling *dand5* expression remains to be investigated. Finally, it will be interesting to determine whether Shh levels are also predictive of L–R robustness in other vertebrate species. In summary, these

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findings offer valuable insights into how morphological traits can appear through modifications in key genetic pathways and can serve as a model to investigate the impact of Shh signaling on development and evolution.

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