How cilium dysfunction in the zebrafish central nervous system triggers spine deformities: implications for the etiology of human idiopathic scoliosis.

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Institute and group

The group belongs to the Developmental Biology department of the Institut de Biologie Paris-Seine (IBPS, http://www.ibps.upmc.fr/en), a large Institute devoted to life sciences within the Science and Engineering Faculty of Sorbonne Université, Paris. IBPS advances knowledge in basic science and investigates issues related to the environment, ageing, and developmental, neurodegenerative and behavioral diseases. A key aspect of the IBPS research strategy lies in the development of novel methodologies at the interface between Biology and Mathematics or Biology and Physics.

Our group is interested in the molecular and cellular mechanisms underlying morphogenesis of the vertebrate central nervous system (CNS), and in the perturbations of these processes in human diseases affecting CNS development. The main project of the group aims at dissecting the functions of the cilium in CNS morphogenesis using the mouse and zebrafish as model systems. Cilia are motile and/or sensory organelles formed from a centriole-derived basal body. They also modulate signal transduction and their dysfunctions cause rare human diseases, the ciliopathies that affect multiple organ functions (1). We have previously identified *Ftm/Rpgrip1l* as a causal gene in severe ciliopathies with associated brain abnormalities, Meckel and Joubert syndromes (2). Using animal models of Rpgrip1l dysfunction, we have shown that cilia are involved in multiple aspects of forebrain morphogenesis via modulating the Hedgehog/Gli pathway (3-5). We also studied the mechanisms by which cilia become polarized along the plane of epithelia, a process known as planar cell polarity (PCP). We have uncovered a role of the Rpgrip1l protein in this process (6) and have shown that it requires apical junction maturation downstream of the PCP pathway (Donati et al., submitted for publication).

PhD project

The present PhD project aims at understanding how neural cilia and cerebrospinal fluid determinants control axis morphogenesis. Idiopathic scoliosis (IS) is defined as a 3D torsion of the spine in the absence of obvious underlying anatomical or physiological defect (7). 40% of IS cases are hereditary and genetic studies identified the first IS causative gene, POC 5 (8). POC5 encodes a protein involved in centriole maturation, a prerequisite for the formation of cilia. This, together with recent data from zebrafish studies, has led to the exciting idea that cilia defects can cause IS. Indeed, several zebrafish lines with spine curvatures display mutations in ciliary genes (9). Among the possible causes of IS, neurological deficits associated or not with abnormal cerebrospinal fluid (CSF) flow are put forward but no clear data are available in humans (10), while recent zebrafish and murine data favour this hypothesis (7,11,12).

To investigate these mechanisms, we have produced a null mutant for the zebrafish *rpgrip1l* gene, which develops severe spine deformities during the phase of accelerated body growth (juvenile period) (C. Vesque et al., in preparation). In humans, *RPGRIP1L* is involved in severe ciliopathies with nervous system abnormalities, some of them associating scoliosis (2). *rpgrip1l* mutant fish are thus an ideal model to study how cilia defects lead to IS. We will perform a detailed study of the mutant phenotype at early stages of curve initiation, analyze transcriptomics data obtained from the comparison of scoliotic versus straight animals to identify IS mechanisms downstream of cilia, use genetics and drug treatments to correct the phenotype.

1) Detailed analysis of the interface between CNS and CSF: tissue defects, cilia integrity and CSF composition in the scoliotic *rpgrip1l* mutant

Our µComputed Tomography (µCT) analysis showed correct formation of adult mutant vertebrae in *rpgrip1l*$^{-/-}$ fish. Concerning the nervous system, ventricle volumetric analysis on transparized brains have shown that the mutant brains are hydrocephalic, even before scoliosis onset, especially at the brain-spinal cord junction. Histology and immunofluorescence studies reveal an abnormal morphology of the central canal where the CSF circulates and the presence of ectopic cells in its lumen. Further immunofluorescence (IF) and in situ hybridization (ISH) studies will determine whether there is normal production of neurons and glia contacting the central canal (13, 14) and whether the ectopic cells present in the central canal are delaminating neural cells or invading macrophages or microglia.
Cilia are present in lower number along the CNS cavities of scoliotic juveniles than of controls, and display an abnormal morphology. To test their motility, cilia movements will be visualized by confocal live imaging using transgenic lines (15). To analyze CSF flow direction and speed, we will track fluorescent beads injected in the ventricle (16). Finally, we will investigate by IF the persistence of the Reissner fiber, a CSF component whose loss at embryonic stage leads to axis curvature and whose initial polymerization requires cilia motility (16) and determine if its loss observed in scoliotic animals precedes or follows the scoliosis onset.

2) Transcriptomic analysis to identify molecular cues involved in IS

To identify molecular mechanisms involved in IS downstream of cilia, we have undertaken a global analysis of the transcriptional program of mutant versus wild type tissues at the onset of spine deformities. We are currently analyzing the changes in the transcriptome of either brain or whole trunk. Our preliminary analysis suggests that the ROS pathway (17) and neuropeptide signaling (18) are both activated at scoliosis onset. The objectives will be 1) to perform a detailed bioinformatic comparative analysis of the wild type and mutant transcriptomes to identify the major deregulated pathways; 2) to confirm and further analyze these pathways by a combination of Q-PCR, in situ hybridization (ISH) transgenic reporters and fluorescent probes, with particular interest for neuropeptide and ROS signaling

3) Rescuing or alleviating the scoliotic phenotype through genome engineering and drug treatments

We will try to rescue the scoliotic phenotype of juvenile zebrafish by introducing transgenes to restore normal levels of activity of the deregulated pathway(s) identified at step 2 or by applying drugs. Our preliminary bio-informatic analysis has shown that the URP (Urotensin Related Peptide, a conserved family of neuropeptides) pathway (18) is up-regulated at scoliosis onset. We will try to lower-down its activity either by removing one or two copies of a receptor for this pathway (19) thanks to genetic crossing (in collaboration with G. Pezeron et H. Tostivint, MNHN, Paris) or by performing CRISPR/Cas9 mosaic inactivation of the URP receptor in the *rpgrip1l*$^{-/-}$ background. We will also test whether upregulating the URP pathway is sufficient to induce scoliosis by expressing URP2 cDNA under the control of a promoter active in ciliated cells of the central canal, Foxj1.

Another pathway that is up-regulated in mutant juvenile at scoliotic onset is the oxidative stress (14). If the previous analysis (step 2) determine that this effect precedes scoliosis, we will try to lower ROS levels, for example by mutating one key producing enzyme that is highly up-regulated in the mutant: ACOD1/irgl1 or by inhibiting its activity with drugs (etomoxir, GR6001) or with the more global OXPHOS inhibitor rotenone (20). Finally, we will try to decipher if the ROS and URP pathways are part of the same molecular cascade leading to scoliosis, for example if one pathway is downstream of the other, with the hope to identify the initial pathogenic event(s) produced by cilia dysfunction.

These studies will help uncover novel functions of cilia that affect directly or indirectly axis morphogenesis at puberty and should help improve IS patients care on a long-term basis.

References

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