

## SUMMARY FOR GENERAL PUBLIC

Motile cilia are cell protrusions found on the surface of epithelial cells (up to 300 per cell), which are able to move. They are present in various tissues and play a key role in many processes, such as mucociliary clearance in airway epithelium (AE), oocyte transport in fallopian tubes, sperm motility, cerebrospinal fluid flow in brain ventricles, or establishing organ symmetry during embryonic development.

Proper function of motile cilia is very important to the body, as evidenced by the inborn condition caused by cilia motility defects, known as primary ciliary dyskinesia (PCD). Symptoms of PCD often begin soon after birth and include recurrent respiratory infections that lead to lung function impairment, and even to lung transplantation. The patients may also be infertile or have reduced fertility, due to lack of sperm movement or defective egg transport in the fallopian tube. Less frequent, but very interesting symptom of the disease is the altered position of organs inside the body or even complete inversion of the viscera (*situs inversus*). To date, no drug has been found to cure PCD, and the goal of the therapy is mainly improvement of airway mucus clearing and early treatment of respiratory infections.

Unfortunately, the clinical picture and severity of symptoms often vary from patient to patient, making the diagnosis difficult and thus often delayed. This is due to the large number of genes involved in the disease. To date, about 50 genes have been identified in which mutations cause PCD. However, they only allow to explain ~70% of the PCD cases. Therefore, it is important to search for new causes of PCD.

The most important process whose disruption can lead to inappropriate ciliary motility is multiciliogenesis, the process of creating motile cilia. Multiciliogenesis is a complex, multiprotein-dependent process that is still not fully understood. However, it is known that it is regulated not only at the level of protein-protein interactions, but also at the post-transcriptional level, i.e. at the stage of protein transcripts. Such regulation involves regulation of the protein transcript levels dispensable for multiciliogenesis by specific microRNA (miRNA) molecules. At the same time, levels of protein transcripts that are necessary for proper multiciliogenesis must be increased, thus miRNAs interacting with them need to be silenced.

Recently, it has been shown that the members of the microRNA(miR)-34/449 family play a key role in multiciliogenesis. Cells with silenced expression of these miRNAs are unable to produce functional motile cilia. Studies in mice have shown, that knock out of all miRNA-34/449 family members leads to respiratory and fertility symptoms, similar to those seen in PCD patients. However, our understanding of this miRNA regulation in multiciliogenesis is still limited. For example, it is not known what is the exact function of each miR-34/449 family member, what transcripts they target, and in what order they are activated during cilia biogenesis. It is also unclear whether apart from the miR-34/449 family, other, yet unknown miRNA families are involved in cilia formation regulation.

To gain a better understanding of the multiciliogenesis process, we would like to analyze the expression of miRNAs in *in vitro* cultured AE cells from healthy individuals. We will characterize miRNA expression profiles during ciliary biogenesis through miRNAseq, look for miRNAs whose expression changes during multiciliogenesis and the ciliary gene transcripts they target. We will verify whether the identified miRNAs actually act on ciliary genes and examine how changes in the levels of specific miRNAs affect the process of multiciliogenesis.

The results of this project will allow to better characterize the expression of the miR-34/449 family and to identify other miRNAs involved in multiciliogenesis. In addition to expanding our knowledge of post-transcriptional gene regulation in cilia formation, results of this project may serve as a reference in the studies of gene expression in patients with PCD. They thus may allow the identification of new causes of the disease in PCD patients in whom no disease-causing mutations have been found so far.