Amino acid substitutions as the molecular basis for primary ciliary dyskinesia (PCD)

Primary ciliary dyskinesia (PCD), whose main symptoms include recurrent respiratory infections, male infertility and situs inversus, belongs to a class of inherited diseases known as ciliopathies. In the case of PCD, the disease is related to the dysfunction of motile cilia - projections found on the surface of respiratory epithelial cells, in sperm and in the embryo. PCD is a genetically highly heterogeneous disease (50 genes have been published so far). It is caused by recessive mutations in the genes encoding proteins that either form the structural/functional elements of the motile cilia or are essential for their biogenesis.

PCD is most commonly caused by defects in the outer dynein arms (ODA), elements of the motile cilia ultrastructure responsible for generating ciliary beating. To become functional components of the cilium, multi-protein ODAs are pre-assembled in the cytoplasm with the help of other proteins. The majority of pathogenic mutations in PCD genes result in the absence of full-length proteins. Missense mutations resulting in amino acid (aa) substitutions are less common. The mechanisms by which such mutations disrupt the assembly, structure and function of ODA in cilia of epithelial cells are poorly understood. DNAI1, an intermediate dynein chain, is part of ODA and one of the two proteins that initiate the pre-assembly process of ODA in the cytoplasm. DNAI1 is one of the eight PCD genes in which mutations are frequently detected in Polish PCD patients. Among the pathogenic variants of DNAI1, the participation of aa substitutions is more frequent than in most other PCD genes.. Most of the pathogenic aa substitutions in DNAI1, which manifest as the absence of ODA, occur in exons that encode an important WDR40 domain, known for its function in protein interactions.

In the proposed project, we will study how the pathogenic effects of aa substitutions in the DNAI1 protein impair its structure, stability and/or protein-protein interactions, important for the correct pre-assembly and functioning of ODA, ultimately leading to defects in ciliary motility. The project involves: 1. In silico prediction of the effect of DNAI1 aa substitutions detected in PCD patients on the structure and stability of the WDR domain. 2. Biophysical analyzes of isolated variants of DNAI1 protein to verify in silico models. 3. Analysis of the effect of aa substitution in DNAI1 on its binding to the known DNAI2 interactor. 4. Analysis of the effect of aa substitution in DNAI1 on the functioning of airway epithelial cells. 5. Analysis of the effect aa substitution in DNAI1 on its interactions with other proteins in airway epithelial cells.

In silico modeling of pathogenic aa substitutions in DNAI1 will provide a comprehensive picture of the predicted impact of such changes on protein stability; biophysical analyzes will validate in silico models and enable an unprecedented comparison of DNAI1-DNAI2 binding in wild-type (wt) or mutant (aa) DNAI1 variants. Global analysis using tandem mass spectrometry will identify a spectrum of proteins whose interaction with DNAI1 is altered due to the presence of aa substitutions. DNAI1, which is both part of the ODA structure and an essential partner in cytoplasmic pre-assembly of ODA, provides a good model to study such interactions. The study will provide information relevant to studies on the effect of aa substitutions on the WDR domain in various proteins. A comprehensive analysis of the impact of aa substitutions on the stability and interactions of DNAI1, allowing for experimental confirmation of their pathogenicity, will increase the certainty of PCD diagnosis in patients with this type of mutations.