Ciliopathies are a growing group of inherited diseases caused by dysfunction of primary or motile cilia, evolutionary conserved organelles present on the surface of many eukaryotic cells. During the recent years, intensive research efforts have been directed to understand genetics and molecular basis of ciliopathies, but so far, many areas remain unknown or unexplored. Primary ciliary dyskinesia (PCD), whose symptoms include recurrent airway infections, male infertility and *situs inversus*, is a flagship ciliopathy caused by a hereditary dysfunction of motile cilia. Genetically heterogeneous, PCD is caused by mutations in genes encoding proteins, which either form structural/functional elements of motile cilia, or are essential for cilia formation. Among hundreds of cilia-related genes (ciliome genes), in more than 40, were found mutations which cause PCD (further referred to as PCD genes). In spite of a substantial progress achieved in elucidating molecular basis of PCD pathogenesis, mutations underlying the disease remain unknown in ~30% of patients. It is still not known, whether this is due to the presence of unknown pathogenic variants, lying outside the usually tested positions in the known PCD genes, or due to the presence of mutations in unknown novel PCD candidate genes.

Application of modern sequencing techniques, such as whole exome sequencing (WES) technology, to the unsolved PCD cases is not always conclusive. In some cases, other techniques have to be employed, to understand the genetic cause of the disease in patients. Large genomic indels can be detected using copy-number sensitive techniques (e.g. high-coverage whole genome sequencing, WGS). Some pathogenic variants related cannot be identified by any genomic sequence analysis alone, and analysis of the ciliated cells transcriptome (RNAseq) needs to be employed; proper interpretation of these findings requires comprehension of the role of ciliary gene transcripts isoforms at different stages of ciliated cells differentiation in healthy individuals.

Based on our results obtained during the previous project on WES in PCD patients, we would like to study in detail the genome and transcriptome of those PCD patients, in whom WES did not show conclusive results. Our goals include: identification of pathogenic variants resulting in structural changes of the genomic sequence, identification of aberrant splicing events in PCD genes; examination of the relevance of different transcript isoforms of PCD genes at different stages of motile cilia biogenesis.

Material to be analyzed (ciliated cells from respiratory tract epithelium) will be collected from selected PCD patients, in whom previous genome analyses did not explained the genetic cause of the disease, and from healthy individuals. The cells will be cultured in special media, to multiply the cells and to achieve their differentiation (ciliation) without the interference of any environmental factors. The project will use high throughput sequencing technologies: whole genome sequencing an whole transcriptome sequencing. Further analyses will be performed to validate and interpret sequencing data.

Explaining the genetic basis of PCD in patients with WES-unsolved mutations is essential for achieving full molecular diagnostics in these patients. Estimating the frequency of large genomic indels and sequence variants affecting splicing in PCD genes will allow to design better schemes of molecular PCD diagnostics in Polish and Slavic population. A better understanding of the role of ciliary gene transcript isoforms in healthy human RE differentiation is essential to extend our understanding of motile cilia biology. This knowledge is also indispensable for the development of new therapeutic strategies in PCD (for example, emerging RNA-based therapies).