The role of DNA methylation in therapy that increases CFTR channel activity in cystic fibrosis

Cystic fibrosis (CF) is the most common life-limiting autosomal recessive disorder characterized by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene, which has an serious impact on the respiratory, gastrointestinal, endocrine, reproductive and skeletal systems. The average life expectancy in 2018 for Polish patients was calculated at 24.5 ± 8.9 years. This was mainly due to low use of health resources, since the CFTR modulators drug programme was implemented only recently in 2022. Interestingly, CF severity varies among subjects, even in siblings and twins with the same mutations and the modifier genes and other factors have impact on the disease progression. While the genetic basis of CF is well-established, the molecular mechanisms of other factors contributing to CF are still unknown. The scientific hypothesis of this project assumes that epigenetic modifications, particularly DNA methylation, may explain the phenotypic variation in CF and changes in methylation may be responsible for the different disease progression and severity. Moreover, we also hypothesize that treatment with CFTR modulators might further alter methylation pattern.

This project aims to comprehensively investigate methylation patterns in different groups of CF patients (pediatric and adults) and healthy controls to elucidate the epigenetic factors influencing disease severity, progression and therapy response. The patients groups are as follows: (1) homozygous for the F508del mutation before and after 12-month treatment with CFTR modulators (Kalydeco with Symkevi or Kaftrio) (2) heterozygous for the F508del mutation (a) before and after 12-month treatment with CFTR modulators (Kalydeco with Symkevi or Kaftrio) (b) without CFTR modulators treatment (3) mutations other than F508del without CFTR modulators treatment. By comparing samples collected from healthy controls and patients with different mutations, as well as before and after treatment with CFTR modulators, we aim to identify specific epigenetic changes associated with the phenotypic variability, disease progression and therapeutic response, providing insights into the molecular mechanisms underlying CF pathogenesis.

We plan to isolate DNA from peripheral blood samples from healthy controls and the discovery group of pediatric patients and perform whole-genome bisulfite sequencing (WGBS). Data obtained from WGBS will be validated with pyrosequencing on other sample material (nasopharyngeal swabs) to confirm whether the DNA methylation is also changed locally in the airways. Moreover, we plan to analyze with pyrosequencing the selected regions/genes with changed methylation on bigger replication group, consisting of both children and adults, as well as healthy controls. We also plan to check whether the change in methylation influences the expression of chosen genes and proteins. Finally, we plan to study the effect of reactive oxygen species and inflammation on DNA methylation in cell culture model of human bronchial epithelium and whether CFTR modulators influence this process.

The novelty of this project is an assumption that DNA methylation may explain the phenotypic variation in CF, whereas the changes in methylation may be responsible for the different disease progression and severity. To our knowledge, no previous studies have analyzed if the treatment with CFTR modulators affect whole-genome methylation patterns. Additionally, most investigations were performed on adults, while our study group includes also children, that enables to study the early effects of the disease and its treatment on methylation changes

This project will uncover the dynamic changes in DNA methylation that are associated with disease progression, severity and its treatment in CF patients. Early diagnosis of cystic fibrosis and identifying factors that might influence its treatment (both clinical, and molecular, especially epigenetic markers) would improve early therapy, facilitate further development of personalized therapeutic strategies and prevent or delay the irreversible changes in the airways, which is crucial to extending the lifespan of the patients and improving their quality of life.