Mucopolysaccharidoses (MPS) are a group of genetically determined metabolic diseases classified as LSD. MPS is caused by a complete or partial lack of activity of specific lysosomal enzymes which causes intracellular accumulation of glycosaminoglycans (GAGs). Due to the type of stored GAG and the enzyme affected by the defect, there are 13 types/sub-types of the disease. Additionally, the absence/presence of symptoms from the central nervous system allows to distinguish between neuronopathic (MPS I, MPS II, MPS III and MPS VII) and non-neuronopathic forms (MPS IV, MPS VI and MPS IX).

Despite our knowledge about the etiology of MPS, understanding of pathomechanisms is still not enough to develop therapies for all types of this group of diseases. Although the underlying cause of the disease has been known for many years, the molecular mechanisms of this group of diseases still remain unclear. The possibility of the influence of other factors i.e gene expression disorders or organelle dysfunctions on the development and progression of the disease is being considered. One of such factors may be the disrupted expression of the PFN1 gene, which encodes profilin small proteins that interact with the cell cytoskeleton). Preliminary studies have shown that among hundreds of genes whose expression is altered in cells of individuals with MPS, only one gene, PFN1, is commonly changed across almost all human types and subtypes of MPS. Profilin is a protein that interacts with actin and influences the cytoskeletal structure, which is disrupted in MPS. Furthermore, cytoskeletal abnormalities are a common cause neurological disorders which are typical for MPS types I, II, III, and VII, as well as skeletal abnormalities, characteristic of MPS types I, II, IV, VI, and IX, and cardiovascular disorders observed in all types of MPS.

Preliminary studies conducted by the project manager confirmed the results of the transcriptomic analysis (elevated PFN1 protein levels in cells of diseased compared to healthy subjects). It was preliminarily evaluated whether PFN1 could affect one of the underlying disease markers, which is GAG accumulation. After silencing the PFN1 gene, it was tested whether the level of accumulated GAGs would change in MPS I, IIIA and IVA. The results of this experiment showed that downregulation of PFN1 influences a significant decrease in GAGs in the cells of affected individuals. Taking into account the significance of (I) results of preliminary study (II) cytoskeletal abnormalities in the development of neurological disorders, (III) the impact of changes in the expression level of the PFN1 gene on the development of numerous musculoskeletal abnormalities, (IV) the disturbances in the expression level of the PFN1 gene in cells of individuals with each type of MPS, the aim of this project is to test the hypothesis that changes in the expression level of the PFN1 gene may be a significant factor in the disturbances observed in the course of MPS, and that profilin itself may prove to be a promising therapeutic target. Research under this project is to include 1)testing the effect of PFN1 gene silencing on disease markers: GAG levels, lysosome morphology, and dysfunctional enzyme activity, will be evaluated. 2)Investigating the impact of PFN1 silencing on cytoskeletal structure. 3)Evaluation of the effect of PNF1 overexpression on healthy cells. Overexpression of the PFN1 gene will be induced in the cells of healthy subjects, which will allow us to check whether raising the level of the protein will cause changes such as those observed in the cells of diseased subjects. GAG levels, assessment of lysosomes, evaluation of enzymatic activity of relevant hydrolases and analysis of cytoskeletal structure will again be performed. 4)Evaluation of the effects of temozolomide and butyrate on PFN1 levels in a cellular model of MPS. Both compounds show the ability to downregulate the expression of the gene under study , but their effects have so far been studied in cancer. The tested cell lines will again be analyzed for disease markers. 5)The compound with the best effect will be used in studies on a mouse model of MPS I to evaluation of the effect in improving the disturbances observed in the course of MPS.

The obtained results will allow for the verification of the hypothesis that disrupted expression of the PFN1 gene, which encodes profilin, a protein involved in cytoskeletal organization, plays a significant role in the pathological changes observed in mucopolysaccharidoses. The analysis of cytoskeletal structure will provide insights into the impact of PFN1 silencing on cellular morphology and the organization of actin filaments, providing valuable information about the role of PFN1 in maintaining cytoskeletal integrity.