PACS2 syndrome is an ultra-rare disease that manifests primarily in a neurological form. Typical symptoms include seizures in the early years of life, global developmental delay, often with autistic features, facial dysmorphic changes, and cerebellar dysgenesis. Some patients have additional symptoms such as fingers bone malformations, ocular problems, cardiological disorders, or problems with the urogenital system. The syndrome was first described in 2018 by Olson and her team, and it has been diagnosed in approximately 100 people worldwide to date.

The cause of PACS2 syndrome is *de novo* mutation in the *PACS2* gene, specifically the E209K or E211K variants. These mutations lead to changes in the amino acid sequence of the PACS2 protein, altering its charge, which in turn affects its functions. PACS2 plays the role of a intracellular transporter of other proteins, indirectly regulating many key processes, such as apoptosis (programmed cell death), cellular stress response, and interactions between the endoplasmic reticulum (ER) and mitochondria. To date, only three papers have been published that have analyzed the impact of these mutations on the molecular properties of the protein. Researchers have observed increased interaction of the mutated PACS2 protein with other proteins, such as the 14-3-3ε protein, sirtuin 1, and TRPV1. Additionally, increased susceptibility of cells to apoptosis induced by staurosporine (a reticular stressor) and disturbances in the flow of calcium ions from the ER to the mitochondria, leading to their accumulation in the cytoplasm, have been observed.

The mentioned studies provided valuable information on the pathomechanism of PACS2 syndrome. However, they do not well reflect the actual condition of patients. These studies used cancer cells with overexpression of the mutated protein. Therefore, in our research, we propose a new, more accurate model using induced pluripotent stem cells (iPSCs) obtained from 3 patients with PACS2 syndrome and their clones, in which the mutated gene has been replaced with the correct one. This is a model that has not been previously used in research on this disease.

In our previous studies, on a mouse model of PACS2 syndrome, we observed reduced expression of tight junction proteins in the hippocampus, which may indicate a disruption of blood-brain barrier. Damage of this barrier leads to an increase in the amount of albumin in the central nervous system, changes in the expression of potassium channels, and activation of astrocytes. We were able to observe this mechanism in our data, so we decided to differentiate iPSCs into astrocytes. Astrocytes play a key role in maintaining central nervous system homeostasis and appear to be important in the pathogenesis of PACS2 syndrome. Activated astrocytes can induce neuroinflammation and exacerbate seizures. Data from the literature and our results suggest that mitochondria and their dysfunction may play a key role in astrocyte activation and underlie the pathogenesis of PACS2 syndrome. We have demonstrated, among other things, a reduction in the contact sites between the ER and mitochondria, changes in the expression of mitochondrial anchoring proteins, and a decrease in the flow of calcium ions to the mitochondria.

Therefore, in this project, we propose to determine the co-localization of ER-mitochondria and the level of calcium ions in the cytoplasm. The accumulation of calcium ions can lead to the activation of astrocytes, which in turn can secrete numerous cytokines and chemokines. One of our hypotheses is that astrocytes with the PACS2 gene mutation will have an altered secretome profile, therefore we plan to assess the levels of selected pro-inflammatory, chemotactic factors, astrocyte activation markers, and potassium ion levels, which can also significantly modulate seizures. In addition, we intend to investigate the susceptibility of cells with PACS2 syndrome to pro-apoptotic stimulation dependent on death receptors and the PACS2 protein, as well as to stimuli independent of these proteins that cause direct mitochondrial effects.

Currently, there is no causal treatment for PACS2 syndrome, which highlights the urgent need for research aimed at developing effective therapies. Therefore, we plan to assess the effect of dimethyltryptamine (DMT) on cells with PACS2 syndrome and the effect of this compound on cells additionally treated with apoptosis stimulators. DMT is a natural compound that has the ability to regulate mitochondrial function. Recent studies using DMT in models of neurodegenerative diseases have shown improved cognitive function and a positive effect on processes related to mitochondria. Our group is authorized to conduct research using neuroplastogens, which gives us a unique opportunity to investigate their effects in the context of PACS2 syndrome. Moreover, in the event of obtaining positive *in vitro* results, we are ready to quickly move on to studies on animal models of PACS2 syndrome, which are also available to our team. The proposed activities therefore have not only scientific significance but also a potential social impact, especially for patients with PACS2 syndrome and their families.