

Spinocerebellar ataxia type 3 (SCA3) is an incurable genetic neurodegenerative disease caused by a special type of mutation, which results in an increased number of CAG nucleotide repeats in the *ATXN3* gene sequence. As in the case of other neurodegenerative disorders, such as Parkinson or Huntington disease, the symptoms of SCA3 occur later in life and include severe motor disturbances, such as gait ataxia, motor imbalance, spasticity, oculomotor dysfunction, and mental impairment resulting from neuronal damage. The mutation in the *ATXN3* gene leads to the formation of defective ataxin-3 protein, which forms toxic aggregates in the cell. Ataxin-3 plays an important role in regulating which proteins should be removed in the cell by different cellular mechanisms of controlled removal of cell fragments (proteins and organelles). Ataxin-3 recognizes a tag on proteins called "ubiquitin" that directs a protein or cell fragment to degradation. Ataxin-3 is able to detach this tag from the protein, thereby preventing premature or incorrect removal of proteins from the cell. The mutation in the gene results in the altered function of the ataxin-3 protein, which could no longer perform its role properly. Moreover, the toxic aggregates formed by mutant ataxin-3 could bind to various cellular structures and organelles, leading to further damage in the cell. For instance, cellular organelles that lead to the removal of cell fragments can be "clogged" by aggregates of ataxin-3.

The detailed mechanisms that result from *ATXN3* mutation and cause the SCA3 disease are not known yet. Therefore, in this project, we will examine if enforcing the controlled removal of certain cell fragments will repair the disrupted cellular processes resulting from the *ATXN3* mutation. We hypothesize that mutant ataxin-3 disrupts specific elements of the process of removing cell fragments, and correcting this error with small molecules will lead to a beneficial reduction in the level of mutant ataxin-3. That way, the cellular homeostasis will be restored. We will use the SCA3 mouse model, which contains 150 CAG repeats (named Ki150), and control mice with 21 CAG repeats (named Ki21) in the human part of the *ATXN3*, which we obtained in our previous projects. The Ki150 model presents a disease phenotype similar to patients suffering from SCA3, which involves motor incoordination, loss of balance, and gait disturbances. Importantly, the symptoms occur early in 1-month-old animals, which enables for fast evaluation of the effect of the proposed treatment.

We will begin by determining the role of normal and mutant ataxin-3 in regulating the removal of cell fragments by studying in detail the ubiquitin tags generated on proteins obtained from Ki150 and Ki21 mouse brains. Ubiquitin can be attached to proteins as a single molecule or in the form of a chain of ubiquitin molecules. Various ubiquitin modifications lead to different outcomes in cells. Therefore, this specific ubiquitin code will give us clues of which degradation pathways are impaired in SCA3. We will examine the activity of the proteasome, the content of vesicles in which proteins are removed, and the interaction of ataxin-3 with the cellular structures responsible for removal. Next, we want to examine the effect of various small molecules and the exact mechanism of lowering ataxin-3 in the cell culture (mouse and human). We will investigate how these small molecules affect mutant ataxin-3 and determine the basic parameters of treatment with these molecules, such as frequency and duration of incubation with the molecules. Furthermore, using a highly advanced microscopic system, cryogenic electron microscopy, we will determine the complete structure of ataxin-3 for the first time and the structure of the mutated fragment of this protein bound to other molecules, which remove proteins in the cell. Finally, we will examine the effect of small molecule administration in mice modeling human disease on the important disease hallmarks, such as aggregates formed by ataxin-3, markers of inflammation, motor symptoms, and others.

We hope that thanks to this project, we will learn more about the special role of one of the key pathogenic processes in SCA3 related to the dysfunction of protein clearance mechanisms. A detailed study of this mechanism will provide a better understanding of SCA3 and other neurodegenerative diseases, which will enable designing a proper treatment.

Research on neurodegenerative diseases and their influence on the human brain is important given the severe consequences for patients, high social burden, and growing medical costs. The approach proposed in this study can lead to the development of treatment for SCA3 and possibly other neurodegenerative disorders.