The role of DNA methylation and alternative splicing involvement in pathogenesis of spinocerebellar ataxia type 8 (SCA8) – the neurodegenerative disorder caused by microsatellite repeats expansion.

Spinocerebellar ataxia type 8 (SCA8) is a rare neurodegenerative disorder, one of the most frequently observed spinocerebellar ataxias in Poland and still not well known. The genetic cause of this disorders is multiplication of the CTG microsatellite repeat region located in the *ATXN8OS* gene. Such expansions embracing also other microsatellite repeats are called as dynamic mutations and are known to cause a number of neurodegenerative disorders. The *ATXN8OS* gene containing such expansion expresses transcript with elongated CUG tract. It is known that the increasing number of microsatellites repeats in non-coding regions leads to the RNA-toxic gain of function mechanism because the hairpins are formed in RNA with expansion region, RNA – binding proteins (often being splicing factors) are sequestrated within these RNA foci and exacerbation of alternative splicing events is observed.

Thus, we are aimed to broad the research on the expanding CTG repeat structure in the *ATXN8OS* gene and epigenetic modifications of this region as the methylation. We suppose that the methylation may have the impact on the expression level of the *ATXN8OS* and *ATXN8* genes and on the further consequences of the mutation. Using novel high-throughput technology called long reads next generation sequencing we plan to demonstrate that the length and composition of the expanded microsatellite region within the *ATXN8OS* and *ATXN8* gene may influence the DNA methylation level of this region. Moreover, we plan to indicate that in SCA8 patients the disturbed splicing factors level leads to the increase in alternative splicing events and abnormal proteins' forms – similarly to myotonic dystrophy type 1 (DM1). As the consequence of the increased alternative splicing the occurrence of the other proteins isoforms may influence the mutation penetrance and progression of the disease.

Clinical studies will be conducted by experienced clinicians in 70 patients with spinocerebellar ataxia type 8 and will be based on validated neurological scales. As a control group for RNA sequencing, the results from GEO (an international public repository) of the transcriptomic RNAseq data of 100 healthy individuals to economize the financial aspects of the project will be used. DNA and RNA sequencing and methylation level will be performed as a wet laboratory tasks and extensive bioinformatic and statistical analyses will be then provided.

We also plan to **develop the first human SCA8 iPSC model** derived from SCA8 patients' cells and developing SCA8-derived neurons to perform the transcriptomic and RNA-binding proteins analysis in neuronal tissue. The reprogramming of the human somatic cells to the human induced pluripotent stem cells (hiPSC) are in line with last new directives of European Union that aims to advance the development of alternative model systems to replace animal studies.

The results of the project may have the potential impact on societal and economic issues. They may bring the new perspectives for the disease course prediction and age of onset. The developments in the field of rare disorders will contribute to EU rare diseases policy and Polish Plan for Rare Disorders which aim at improving patients' access to diagnostics and knowledge. The subject of the project is in line with the interest of the European Reference Network for Rare Neurological Disorders (ERN-RND) on which the IPiN is a member. Moreover, the research team members are also involved in activities of Ataxia Global Initiative. The society of these international associations will implement the project results as they are obliged to promote sharing data and providing information about scientific research to people with ataxia and the public. The results of the project will be disseminated at national and international conferences, meetings of ERN-RND and AGI, and through scientific articles in Open Access journals. Cooperation with patient associations and providing the demonstration lessons in schools during World Ataxia Day will complement the ways of disseminating knowledge.

The project goals are planned to be performed by the scientific team of professionals: The clinical, molecular, and epidemiological aspects of neurodegenerative disorders have been studied in the IPiN for the last 25 years. The research strategy is to organize two cooperating subgroups responsible for 1) clinical (Neurologists and Genetic Counsellors) and 2) molecular studies (Molecular Geneticists). Furthermore, medical students from Lazarski University, Faculty of Medicine will be involved in the project to facilitate their start of scientific careers.