Amyotrophic lateral sclerosis (ALS) is the most common degenerative disorder of the motor neuron system. The disease is characterized by motor neuron degeneration in the primary motor cortex, brainstem and spinal cord.
Understanding of ALS pathogenesis comes from molecular genetics. First ALS-causative gene *SOD1* encodes superoxide dismutase, which main function is the removal of free radicals. In recent years several other genes causing ALS or ALS-like phenotypes have been revealed. Although the genetics of ALS has been partially defined, it is currently not known whether the roles of aforementioned proteins in motor neuron degeneration is through one common or numerous separate pathways.
For several decades extensive studies have been conducted on ALS pathology, resulting in a lot of data that contributed to understanding of the genetic and biochemical causes of most cases of FALS and their underlying pathophysiological consequences.
In the last few years, a number of transcriptome studies were performed using a limited number of post mortem samples from ALS patients and controls. Though the samples used were selected for post-mortem intervals prior to freezing not exceeding 24 h, it could be speculated that this lag-time could affect the results.
Experiments with animal mutant genes model can help to explain pathomechanism but often models do not replicate disease phentotype, are time and cost consuming. Not only mutation but also the full genetic background in patients is important especially in multigenetic diseases like ALS which could not be present in animal models. Thus, we believe that the use of primary cell lines of human fibroblast derived directly from patients with genetic background of ALS represents a great advantage. Moreover, the use of fibroblasts in these studies possesses multiple technical advantages. This is mainly due to the fact that fibroblasts are one of easiest type of cells to grow in a culture, and their durability makes them amenable to a wide variety of manipulations (transcriptomics, epigenetics, proteomic tests using various molecular biology methods, including whole RNA sequencing).

The main objective of the project is to determine the biological effects of mutations and novel rare genetic variants, especially those not linked previously to amyotrophic lateral sclerosis (ALS). The long-term goal of the project is to identify new cellular pathways dysfunction of which may be involved in ALS pathophysiology. The practical aim of the project is to develop a method combining the detection of causative genetic variants with the determination of their functional effect at the level of the cell and the organism and with their impact on clinical phenotype. The molecular and cellular effects of putative causative sequence variants will be based on whole-transcriptome study, followed by biochemical and molecular functional tests, using primary cell lines of skin fibroblasts isolated from the ALS patients.
We expect that analysis of transcriptome from the ALS patients with novel or rare genetic variants co-segregating with the disease phenotype could result in identification of novel cellular pathways, whose dysfunction is involved in ALS pathophysiology. We plan to determine the biochemical and cellular effects of mutations and novel genetic variants (especially those not linked previously to ALS) using primary fibroblasts cultures isolated from 40 ALS patients and RNAseq method.
In particular , in our study we will focus on the transcriptome and signalosome branches that have been pursued by the latest (the newest) research on ALS. Namely, we will zoom in these fragments of the transcriptome and signalosome, which may be associated with the gene: TBK1, TUBA4A, ErbB4.
However various NGS techniques results in a successful diagnostic tool in study genetic disease the diagnosis often is only possible if the gene has been previously reported to be implicated in a similar condition. Bioinformatic prioritizations tools provide pointers toward phenotype expansion of known genes and novel disease genes but cannot be the last step of research, because do not explain mechanism leading to disease.

NGS analysis of cases without known disease-causing mutation could identify a number of rare variants that cluster in known genes or in novel disease associated genes. Further, besides genetic variants detected using whole exome or whole genome sequencing, RNA sequencing in using primary cell lines of skin fibroblasts isolated from the ALS patients will identify transcriptomic profiles and expression quantitative trait loci (eQTL), as well as splicing abnormalities, not detected in whole exome analysis.

The novelty of the proposed research project is based on the determination of whole transcriptome profile of 40 patients with potentially ALS-causative mutations and, subsequently, on the bioinformatic analysis and identification of a functional relationship between gene mutations and resulting from a disease transcriptome differences and changes in various signaling pathways.