PROJECT 1

**DoS:** Nilo Riva

**Title:** Disentangling phenotypic and molecular heterogeneity in Motor Neuron Disease.

**Curriculum:** Neuroscience and Experimental Neurology

Link to OSR/UniSR personal page:

**Project description (Number of characters, including spaces: 2.000 - 3.000):**

Motor Neuron Diseases (MND), is a group of neurological disorders defined by the degeneration of motor neurons (MN) in the brain and spinal cord. Amyotrophic lateral sclerosis (ALS) is the most common and severe form, leading to death within 3-5 years from onset. Despite extensive research, there is no effective treatment up to date, with Riluzole being the only drug with a recognized small effect for modestly slowing the relentless neurodegenerative process (1). Within this context, the heterogeneity of this the disease is increasingly recognized as a major determinant for the failure of clinical trials and translation of preclinical therapeutic studies to the human disease. About 10% of ALS cases are familial (fALS), whereas the remaining 90% apparently occur sporadically (sALS). To date, more than 30 genes have now been reproducibly implicated in ALS, while more than 120 have been proposed as potentially related to ALS, even if most are still of uncertain and often not replicated significance (1). In about 60–80% fALS patients, a gene mutation can be identified, being SOD1, C9orf72, TARDBP and FUS the four major ALS genes by frequency [1]. While less well understood, the genetic hereditability of sALS has been estimated to be more than 60%. Therefore, disentangling disease heterogeneity, both at a phenotypic and genotypic levels, remains a major issue for a better comprehension of disease complexity as well as the development of future, more personalized therapeutic approaches for subgroup of patients. We will therefore recruit a large cohort of ALS patients in order to perform detailed genotype-phenotype correlations. We already have stored in our tissue Bank (INSPE) DNA and serum samples from more than 700 patients, along with detailed clinical data. However, the recruitment will continue during the study. Genetic evaluation will be performed with Next generation technologies (NGS). The Neurodegenerative ILLUMINA panel, encompassing more than 100 genes related to neurodegenerative diseases, will be employed. Therefore, we will also be able to explore the oligeneic hypothesis of ALS. Indeed, the contribution of rare variants with intermediate to large effects is increasingly recognized in the context of a multistep model of disease.[2] According to this hypothesis, each co-occurring variant alone could be tolerated but when combined with a second variant would exceed the threshold required for neurodegeneration. Another major issue in ALS is the development of novel, reliable biomarkers, both for diagnosis, prognosis and monitoring disease progression. We will therefore explore whether specific subgroups of patients, accordingly with their phenotype or genotype. In this context, the measure of neurofilaments is considered as the benchmark biomarker in ALS, reflecting the levels of axonal degeneration. However, correlation with patient's genotype or phenotype are still lacking. We previously shown that motor nerve biopsy may allow to differentiate between motor neuron disease (MND) and motor neuropathies (MN) (2). Moreover, we previously defined, for the first time, specific gene expression changes in human motor nerve biopsies, differentiating ALS from motor neuropathy patients. We identified 815 differentially expressed genes (DEGs), of which 529 were up-regulated and 286 down-regulated in ALS (3). Starting from our unique gene expression data raised in human tissues, we will strive to identify novel biomarkers and ALS gene variants leveraging on a combined gene expression, pathway analysis and exome sequencing pipeline. Expression and localization of novel candidate biomarkers will be assessed in both human tissues
from ALS patients compared with controls and rodent models of ALS. Novel candidate biomarkers will be evaluated in a discovery cohort and then replicated in a second ALS cohort and compared to current benchmark biomarkers (e.g. NFs), stratifying for disease genotype.

**Skills to be acquired by the student:**

Perform independent literature search, study planning, pose a research question/problem, examine the range of available modes of inquiry, identify the appropriate research mode and procedure, identify a data collection strategy, analyze and interpret data, draw conclusion from the data and write research paper. In parallel, the student will also perform investigate in the lab by NGS analysis (including DNA and RNA extraction, preparation of libraries, filtering, annotation and interpretation of variants, including bioinformatics), Sanger sequencing, real-time PCR, RNA-seq, NF assay, ELISA; and bioinformatics analysis, confocal microscopy, immunohistochemistry and immunofluorescence staining.

**References** (max. 3)