PASTEUR PARIS UNIVERSITE (PPU) INTERNATIONAL DOCTORAL PROGRAM 2020

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PROJECT

- **FILE #08**
- **ACRONYM:** MUTALS
- **TITLE:** From novel mutations to development of animal models in Amyotrophic Lateral Sclerosis

LABORATORY

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LABORATORY PRESENTATION AND RESEARCH TOPICS

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DESCRIPTION OF THE PROPOSED PROJECT

- **KEYWORDS:** ALS, C9orf72, neuron-glia interactions, drug screening
- **ABSTRACT**

Amyotrophic Lateral Sclerosis (ALS) is an incurable devastating neurodegenerative disease that cause death in 3 to 5 years after disease onset. A G4C2 repeat expansion in the C9ORF72 gene is the prime genetic cause of ALS. (Gitler e Tsuiji 2016; Taylor et al. 2016). Although many progresses have been accomplished to understand how these repeats are pathogenic, the molecular and cellular mechanisms underlying motor neuronal cell death is still unclear (Ling et al. 2013). Furthermore, there is no treatment for this devastating disease. Importantly, the consortium has identified novel missense mutations in C9ORF72, as well as in its protein partner, SMCR8. Our preliminary results indicate that overexpression of these mutations may cause a deleterious gain-of-function of the C9ORF72/SMCR8 complex resulting in alteration of the autophagy clearance pathway, ultimately resulting in neuronal cell death (Amick et al. 2016; Jung et al. 2017; Sullivan et al. 2016; Webster et al. 2016; Yang et al. 2016). We propose to confirm these important findings and to explore further the pathogenicity of these mutations. We will notably develop state-of-the-art cell and animal models of these novel mutations to investigate how they may cause ALS-FTD. Furthermore, we will test whether G4C2 expanded repeats may induce expression of aberrant variants of C9ORF72, which would also alter autophagy through a deleterious gain-of-function mechanism. Also, the role of the

glia participation in neuronal degeneration upon mutant C9orf72 and members of this complex will be investigated.

- **DESCRIPTION OF THE PROJECT**

The PhD candidate will be focused on developing zebrafish models to study the hypothesis that the C9orf72 mutation leads to neurodegenerative features *in vivo*. Our previous studies indicate that zebrafish is a suitable animal model to study C9ORF72 functions (Ciura et al. 2013; Sellier et al. 2016). We will take advantage of the CRISPR/Cas9 gene editing and homologous recombination to develop knockin zebrafish expressing C9ORF72 or SMCR8 mutations inserted into the endogenous zC9orf72 or zSmcr8 locus. Following characterization of the evoked and spontaneous motor parameters of heterozygote and homozygote animals at the F2 generation, these animals will be crossed with HB9-RFP zebrafish to study the motoneurons branching, eventual axonal degeneration and to sort these cells for further molecular characterization. Also, juvenile and aged animals will be assessed for any neuronal and behavioral deficits. Molecular coupled with omics analysis will be performed in the variety of these animal models to unravel the pathophysiological mechanisms affected. Finally, the versatility of zebrafish for drug screening will be optimal for testing pharmacological compounds that could represent initial leads for pre-clinical testing in ALS and related neurological disorders. Development of animal models is clearly complex, expensive and time consuming, but note that we have already developed zebrafish models to investigate C9ORF72 function and related motor neuron dysfunctions (Ciura et al. 2013; Lattante et al. 2015).

The hexanucleotide (GGGGCC/CCCCGG) repeat expansions (HREs) result in three potential pathogenic hallmarks of disease. First, decreased C9orf72 mRNA expression levels in patients suggest a loss-of-function mechanism (Ciura et al. 2013; Therrien et al. 2013). Second, RNA transcripts from the HREs potentially gain a toxic function by sequestering RNA-binding proteins in foci (Gendron et al. 2014) and/or inhibiting transcription through the formation of RNA-DNA hybrids (Gitler e Tsuiji 2016). Lastly, both sense and antisense RNA transcripts can undergo non-canonical repeat-associated non-ATG translation (RAN-T), generating five potentially toxic dipeptide repeat protein species (DPRs): poly(glycine-alanine) [poly(GA)], poly(glycine-proline) [poly(GP)], poly(glycine-arginine) [poly(GR)], poly(proline-alanine) [poly(PA)], and poly(proline-arginine) [poly(PR)] (Gitler e Tsuiji 2016). DPR inclusions were reported in different CNS areas of C9orf72-ALS/FTD patients (Ash et al. 2013; Gendron et al. 2013; Mori, Arzberger et al. 2013; Mori, Weng et al. 2013; Zu et al. 2013). Recently, nuclear import and/or export defects especially caused by arginine-containing poly-GR or poly-PR

have been proposed as signifcant contributors to pathogenesis based on disease models (Saberi et al. 2018; Schludi et al. 2015). In addition, pervasive DPR pathology is found during presentation of initial symptoms of disease, preceding onset of pathology such as TDP-43 inclusions (Baborie et al. 2015; Proudfoot et al. 2014). DPRs alter cellular functions and induce toxicity in different ways in various models (Westergard et al. 2016).

Could DPRs undergo cell-to-cell transmission between CNS-resident cell types? Seeking evidence of DPR transmission, we can test whether transmission of the different DPRs could occur between neurons and glial cells (microglia and astrocyte). Two different experimental approaches can be conducted: Co-culture experiments, and conditioned media experiments using cortical and spinal cord neurons. Our objective is to investigate alterations in the transmission mechanism of these DPRs in the cellular and animal models of these novel mutations obtained during this project.

Furthermore, we believe that our project will reveal novel mechanisms explaining neuronal cell degeneration in patients, and may have significant implications beyond ALS-FTD. This is a crucial point as neurodegenerative diseases represent an increasing burden and challenge to our society and are one of the main causes of death in Europe. In that aspect, it is striking that autophagy mis-regulation is suspected to be involved in an increasing number of neurodegenerative diseases, including ALS with known mutation in including TBK1, UBQLN2, VCP, P62/SQSTM1, Optineurin and CHMP2B, but also Huntington's disease, Parkinson's disease, Alzheimer's disease and other dementia. Thus, our proposed research on the regulation of autophagy by C9ORF72/SMCR8 will enrich our knowledge on other neurodegenerative disorders. Finally, our proposal is highly significant to public health since it will open new lines of research toward identifying a treatment for these devastating neurodegenerative diseases. In short, if successful, our project will help to better understand the causes of ALS-FTD and open novel routes to develop innovative therapy for that devastating disease.

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EXPECTED PROFILE OF THE CANDIDATE

- **EXPERIENCE REQUIRED**
  Student that has completed the Master or MD with neuroscience experience and background. The candidate should also possess experience with animal experimentation, cellular models, genetics and molecular biology. Prior experience with disease models is highly recommended.