**PROJECT**

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| **Supervisor:** | ALESSANDRA BOLINO |
| Title: | AAV-Based Gene Therapy Approaches for Inherited Demyelinating Charcot-Marie-Tooth type 4B1 and B2 Neuropathies. |
| *Curriculum*: | Neuroscience and Experimental Neurology |
| Link to the personal page of the University or relevant hospital site website: | https://www.unisr.it/docenti/b/bolino-alessandra___ |

**Description of the Project (max 3,000 characters including spaces)**

**Background/gap of knowledge**

Charcot-Marie-Tooth type 4B (CMT4B) is a severe autosomal recessive demyelinating neuropathy with childhood onset characterized by the presence of aberrant myelin in the nerve that degenerates causing axonal problems and functional impairment. CMT4B neuropathies are caused by loss-of-function mutations in the myotubularin-related 2 (*MTMR2*, CMT4B1), *MTMR13* (CMT4B2), and *MTMR5* (CMT4B3) genes. All these proteins belong to a broad family of protein tyrosine phosphatase/dual specificity-like phosphatases (PTP/DSP). Whereas catalytically active MTMR2 dephosphorylates the phosphoinositides PtdIns3*P* and PtdIns(3,5)$P_2$, MTMR5 and MTMR13 are catalytically inactive. MTMR13 and MTMR2 form heterodimers, which have higher enzymatic activity and a different sub-cellular localization as compared to MTMR2 homodimers.

**Rationale and hypothesis**

Our lab generated several *in vitro* and *in vivo* models of CMT4B1, whereas a *Mtmr13* KO mouse model is already available in our lab. *Mtmr2* KO mice recapitulate CMT4B1 and CMT4B2 *Mtmr13* KO mouse model shows reduced levels of Mtmr2, suggesting that a low MTMR2 activity is responsible for CMT4B diseases. Consistent with this, lentiviral vector-mediated expression of MTMR2 in *Mtmr2* KO Schwann cell/DRG neuron primary co-cultures effectively reversed aberrant myelin. As these neuropathies result from the direct (CMT4B1) or indirect loss (CMT4B2, CMT4B3) of MTMR2 protein activity, expressing an active form of MTMR2 should be sufficient to prevent the disease.

**Objectives and specific aims**

In this project, we propose to assess efficacy of a therapeutic strategy for CMT4B neuropathies based on AAV9-mediated gene therapy. We propose to replace MTMR2 in both Mtmr2 and Mtmr13 knock out mice by means of AAV9 vector mediated transduction.

**Expected outcomes**

Our preliminary data already showed high transduction efficacy of AAV9 in Schwann cells, the target cells in these neuropathies. Moreover, we already obtained proof-of-concept data that MTMR2 replacement by means of AAV9 vector delivery ameliorates the CMT4B1 phenotype in the Mtmr2 KO mouse model.

**Skills that the student should acquire** (max. 600 characters including spaces):

Expand and consolidate the knowledge of laboratory techniques, which involve mouse genetics; morphological analysis in vivo; immunofluorescence in vivo and confocal microscopy; biochemistry; and molecular biology. Acquire a consistent know-how on the PNS in health and disease. Learn how to critically evaluate experiments, how to plan experiments, the design of a scientific strategy to answer compelling scientific questions, to give public seminars and write papers.

**References** (max. 15)

Bolino A, et al., Journal of Cell Biology 2004
Bolis A et al., Journal of Neuroscience 2009
Vaccari I et al., PLoS Genetics 2011
Sawade L et al., Nature Communications 2020
Guerrero-Valero M. et al., PNAS 2021
Bolino A, D'Antonio M. J Periph Nervous System 2023
Cipriani S et al., Brain Comm 2025