Boosting MFN2 levels in neurons using adeno-associated virus (AAV)as a therapy for Charcot-Marie-Tooth disease type 2A

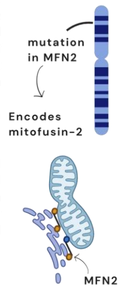
Marine Tessier, Nathalie Bonello[1], Nathalie Roeckel-Trévisiol[1], Marc Bartoli[1], Valérie Delague[1], Bernard Schneider[2], Nathalie Bernard-Marissal[1]

[1] Aix Marseille Univ, INSERM, MMG, U 1251, Marseille, France ; [2] Brain Mind Institute, Ecole Polytechnique Fédérale de Lausanne (EPFL), Station 19, 1015 Lausanne, Switzerland

marine.tessier@univ-amu.fr

INTRODUCTION

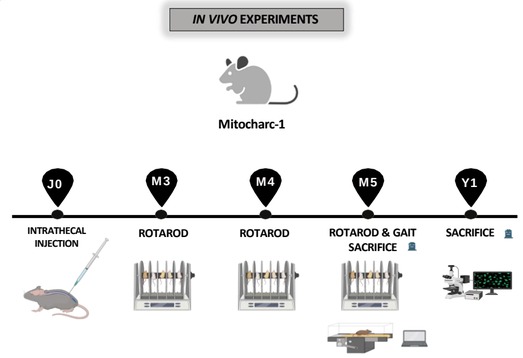
Charcot-Marie-Tooth disease (CMT) is the most common inherited disorder of peripheral nerves with a prevalence of 1:2500 [1]. Patients typically present distal muscular weakness and atrophy, gait impairment, foot deformities as well as sensory loss. CMT are mostly defined in two categories depending on the cell type primarily affected: either the Schwann Cells (SC) in demyelinating CMT forms (CMT1) or the motor and sensory neurons in axonal CMT forms (CMT2). The genetic heterogeneity of this disease and the diversity of the affected cell types make the prospect of a common therapy difficult to envisage. CMT2A is the most common axonal form, and is linked to mutations in the *MFN2* gene encoding Mitofusin-2 (Mfn2). More than 100 mutations were reported so far in CMT2A. Mfn2 has two main functions : it promotes mitochondrial fusion and modulates endoplasmic reticulum–mitochondria tethering called MAMs. The abnormal expression of Mfn2 in CMT2A patients is associated with mitochondrial dysfunction and axonal degeneration [3]

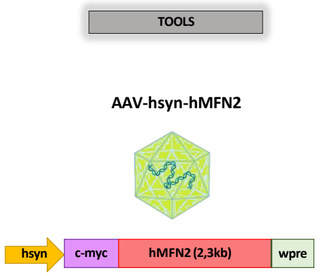


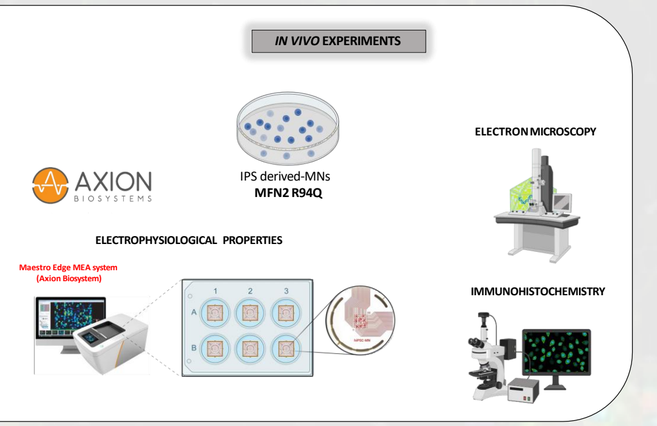
Our aim is to alleviate CMT pathology by designing viral vector strategies dedicated to restore the altered function of the mutated protein in the appropriate cell type. We are working *in vitro* on IPS-derived motorneurons from CMT2A patients in which we explore the effect of the overexpression of WT-MFN2 on different parameters (electrophysiological properties, neuritic length, MAMs..). *In vivo* we deliver our construct in mitocharc-1 mice by lumbar intrathecal injection, allowing us to study motor function, axonal degeneration and mitochondrial dynamics.

Our data provide proof-of-concept evidence that specific gene therapy approach may serve as a therapeutic strategy for CMT and potentially other inherited autosomal dominant neurological diseases.

METHODS

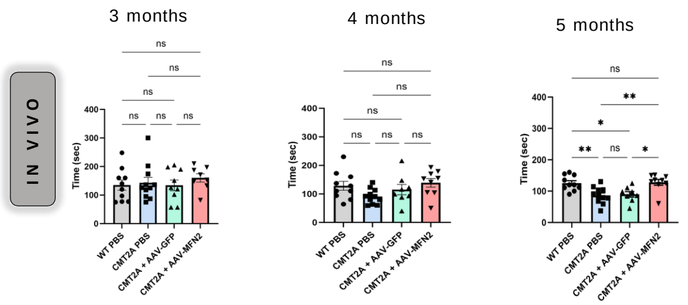






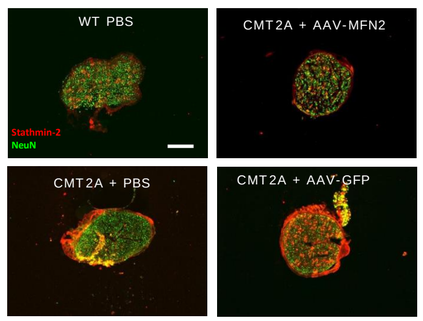
RESULTS

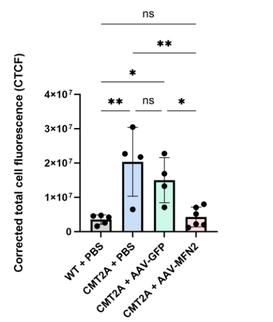
MOTOR FUNCTION



Overexpression of WT-MFN2 prevents motor impairments onset

AXONAL REGENERATION

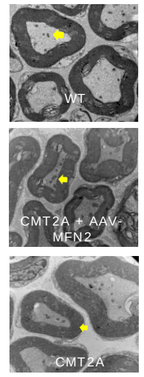


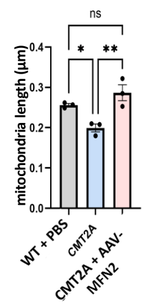


There is a **significant increase** of **STMN2 expression** in the tibial nerve of **CMT2A + PBS** and **CMT2A + AAV-GFP** mice

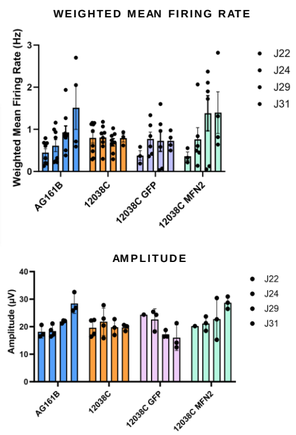
Overexpression of WT-MFN2 prevent distal axonal degeneration

MITOCHONDRIAL DYNAMICS





ELECTROPHYSIOLOGICAL PROPERTIES



Increase of control firing rate and amplitude over time

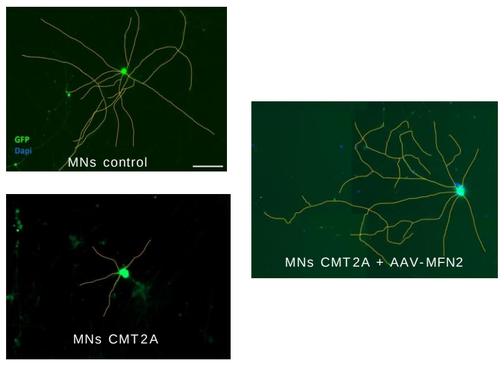
GOOD MATURATION OVER TIME

No change in patient firing rate and amplitude over time

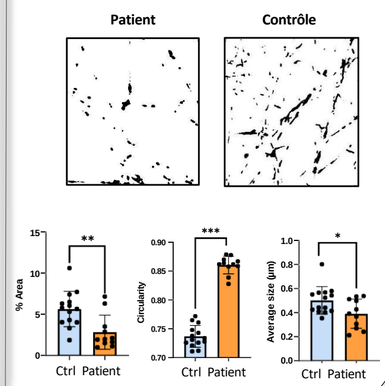
MATURATION DEFECT

Overexpression of WT-MFN2 prevents maturation defect in vitro

NEURITIC LENGTH



MITOCHONDRIAL DYNAMICS



Overexpression of WT-MFN2 rescues neurtic length and mitochondrial fusion in vitro

CONCLUSION

MFN2 gene therapy exerts positive effects on disease onset. It leads to :

- a decrease of axonal degeneration
- a normalization of neuronal activity
- the prevention of motor dysfunctions

Our data provide proof- of- concept evidence that specific gene therapy approach may serve as a therapeutic strategy for CMT and potentially other inherited autosomal dominant neurological diseases.

REFERENCES

[1] H. Skre, *Clinical Genetics*. **6**, 98–118 (1974).
[2 Kulkarini et al., 2023
[3] Chandhok et al., 2018).