Role of mitophagy in *CHCHD10*-related motor neuron disease

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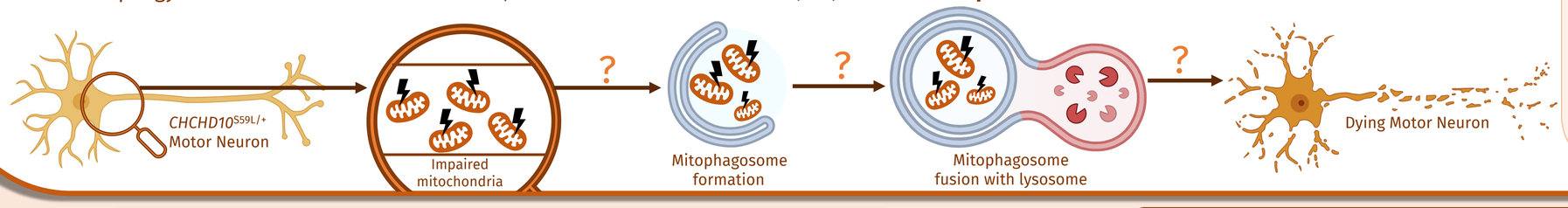
Background

In neurons, complex mechanisms are required to manage energetic demand through mitochondria renewal.
Mitophagy, the selective clearance of damaged mitochondria, is essential for mitochondrial quality control, and its dysregulation contributes to neurodegeneration. The identification of a point mutation (p.S59L) in the *CHCHD10* gene was the first genetic evidence that **mitochondrial dysfunction can trigger motor neuron disease (MND)** (Bannwarth et al., 2014). We generated ***Chchd10*$^{S59L/+}$ mice** that reproduce key ALS features of amyotrophic lateral sclerosis (ALS) and crossed them with **MitoQC reporter mice** to visualize mitophagy *in vivo* (Ganley et al., 2016). To further investigate potential mitophagy defects in our ALS motor neurons, we also used **motor neurons (MN) cell lines and patient iPSC-derived motor neurons.**



Aim of the Study

In *CHCHD10*$^{S59L/+}$ models, mitochondria are impaired. Typically, damaged mitochondria are selectively removed through mitophagy, a process in which an autophagosome engulfs the impaired mitochondria, forming a mitophagosome that fuses with a lysosome to degrade its contents. **We aim to investigate whether the accumulation of dysfunctional mitochondria observed in *CHCHD10*$^{S59L/+}$ models results from a defect in mitophagy processes.**

Impacts on Mitophagy *In Vivo*

Model : S59L_MitoQC Mice

Modeling Human Pathology

ALS
MN & neuromuscular junction (NMJ) degeneration, TDP43 aggregates

Chchd10$^{S59L/+}$

Mitochondrial Myopathy

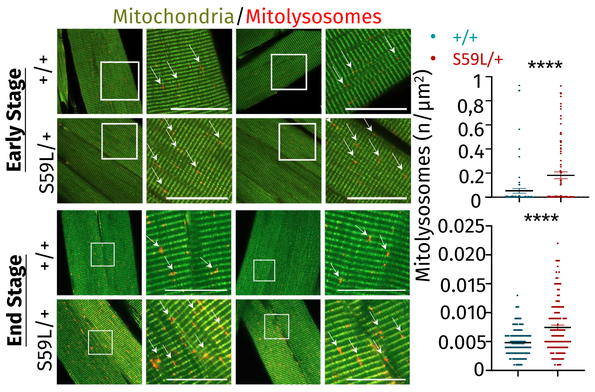
X

Mitophagy Visualization

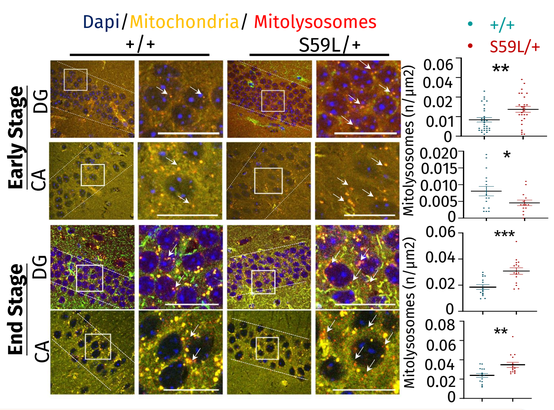
MitoQC

pH Levels
mCherry+GFP
mCherry+GFP

Increased Mitophagy Levels in S59L_MitoQC Skeletal Muscle

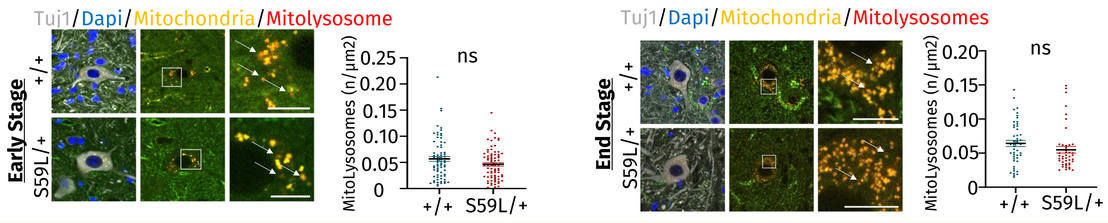


Increased Mitophagy Levels in S59L_MitoQC Hippocampus



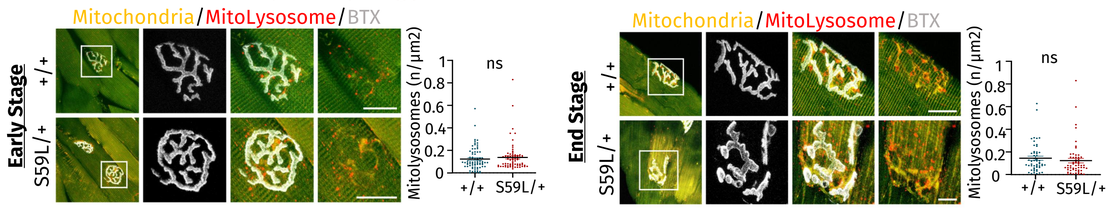
Enhanced mitophagic activity in skeletal muscle and hippocampus of S59L_MitoQC mice at early and end stage. Scale Bar : 30µm
Representative images sections of muscle (left) and hippocampus dentate gyrus (DG) and cornu amonis (CA) (right) from S59L_MitoQC mice at 3 months (early stage) or 12 months (end stage). Mitochondria are yellow and white arrows show the red mitolysosomes following GFP quenching by lysosomal pH. The quantification was assessed by Image J (mQC counter) and shows the number of mitolysosomes per area (µm2).

No Increase of Mitophagy Levels in S59L_MitoQC Spinal Cord MNs



Mitophagy levels are not increased in Spinal Cord MN of S59L_MitoQC mice at early and end stage. Scale Bar : 10µm
Representative images sections of MN (Tuj+) from S59L_MitoQC mice spinal cord at 3 months old (early stage) or 12 months old (end stage). The white arrows show the red mitolysosomes. The quantification was assessed by Image J (mQC counter) and shows the number of mitolysosomes per area (µm2).

No Increase of Mitophagy Levels in S59L_MitoQC Neuromuscular Junctions



Mitophagy levels are not increased in Neuromuscular Junctions (NMJ) of S59L_MitoQC mice at early (left) and end (right) stage. Scale Bar : 20µm
Representative images sections of NMJ (BTX) from S59L_MitoQC mice spinal cord at 3 months old (early stage) or 12 months old (end stage). The quantification was assessed by Image J (mQC counter) and shows the number of mitolysosomes per area (µm2).

Impacts on Mitophagy *In Vitro*

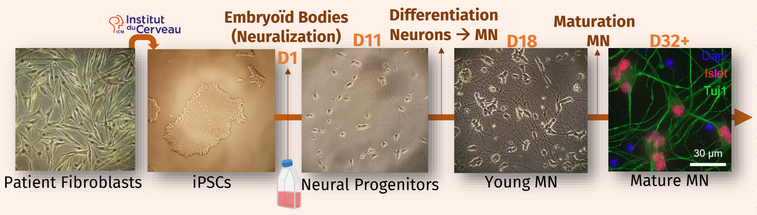
Models : NSC-34 & iPSC-derived MNs

NSC-34

Immortalized **MN** cell line from mice spinal cord

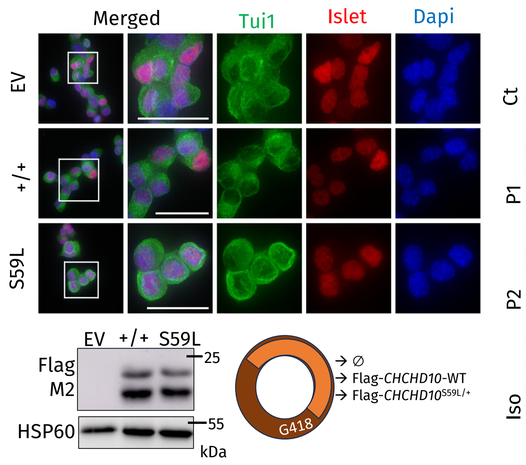
- Empty Vector **(EV)**
- *CHCHD10* Wild Type **(WT)**
- *CHCHD10*$^{S59L/+}$ **(S59L)**

Patient CHCHD10*$^{S59L/+}$ *iPSC-derived MNs

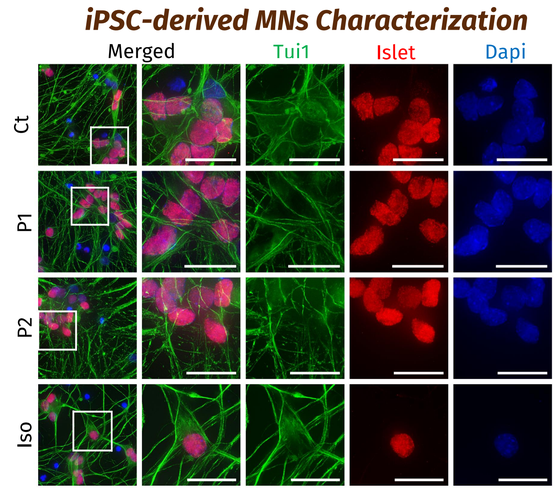


- Ctrl Individual **(69 yo, ♀) « Ct »**
- x2 ALS *CHCHD10*$^{S59L/+}$ Patients : **(58 yo, ♀) « P1 »** **(68 yo, ♀) « P2 »**
- Isogenic Line from P1 **« Iso »**

NSC-34 Characterization

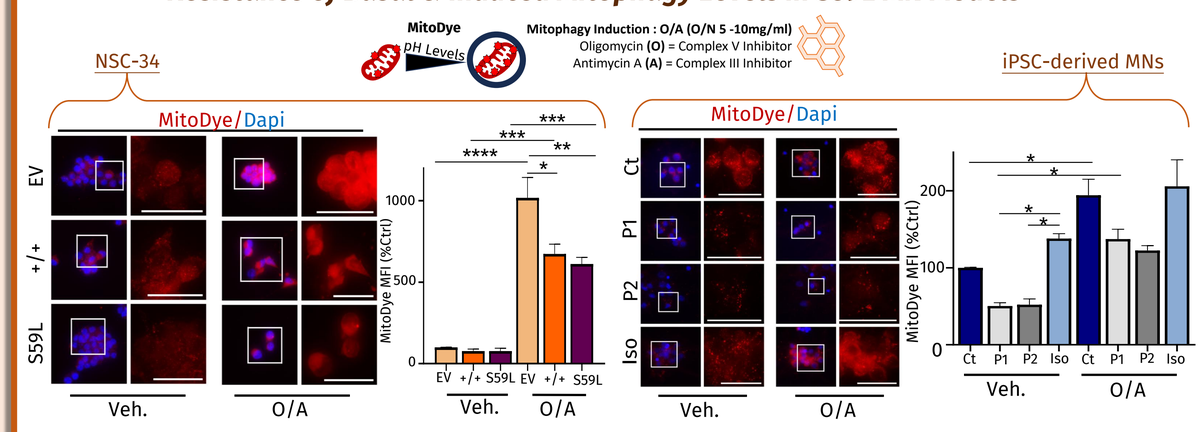


iPSC-derived MNs Characterization



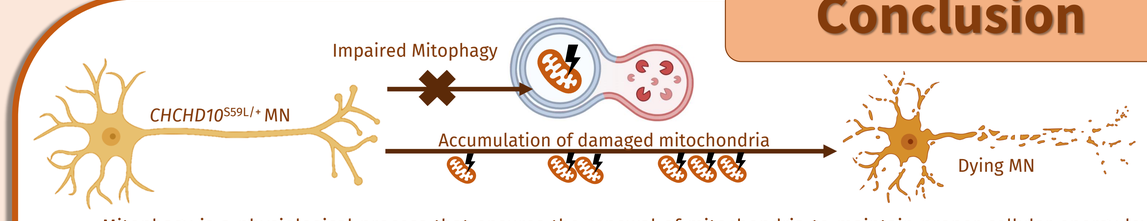
***In vitro* Motor Neuron (MN) models express mature MN markers.** Scale Bar : 30µm
Representative images of mice cell line NSC-34 (Left immunofluorescence panel) stably transfected with Empty Vector (EV), Wild Type *CHCHD10* (+/+), and mutated *CHCHD10* (S59L). MN Markers : Tuj1 (cytoplasmic, green) & Islet (nuclear, red), Nuclear staining : Dapi. A western blot (below left panel) shows the expression of the stably transfected Flag vectors confirming the expression of the protein of interest.
Representative images of iPSCs-derived MNs after 30 days of differentiation (Right immunofluorescence panel) from a control individual (Ct), two patients with *CHCHD10*$^{S59L/+}$ variant (P1 & P2), and an Isogenic line (Iso) coming from P1, genetically modified to rescue the serine in position 59 back to a leucine. MN Markers : Tuj1 (cytoplasmic, green) & Islet (nuclear, red), Nuclear staining : Dapi

Resistance of Basal & Induced Mitophagy Levels in S59L MN Models



Lower basal mitophagy levels and Resistance to induced mitophagy in S59L models. Scale Bar : 30µm
Representative images of NSC-34 (left) or iPSC-induced MNs (rightl) under basal conditions (Veh.), or after mitophagy induction with an overnight (O/N) Oligomycin + Antimycin A treatment (O/A). 10mg/ml for NSC34 and 5µg/ml for iPSC-derived MNs. Mitophagy levels are monitored with MitophagyDye from Donjindo Kit. The dye fixes the mitochondria and becomes brighter when mitophagosomes fused with lysosomes (pH acidification). The shift of fluorescence is monitored by flow cytometry (Cytoflex) and plotted as the Median Fluorescence Intensity (MFI).

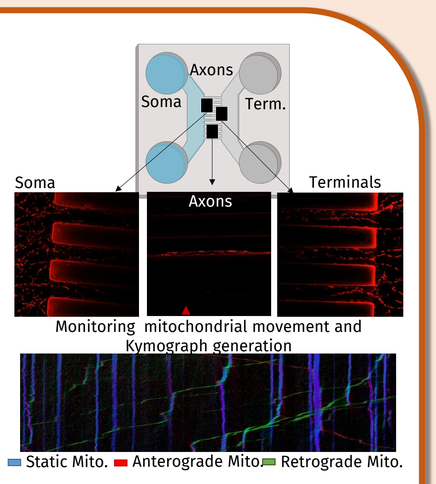
Conclusion



Mitophagy is a physiological process that ensures the renewal of mitochondria to maintain proper cellular energy levels. When mitochondrial stress occurs, depolarization of the mitochondrial membrane impairs ATP production *via* the respiratory chain. These **impaired mitochondria must be recycled; if they accumulate, they can become toxic to the cell**. Membrane damage may lead to the release of mitochondrial DNA (mtDNA), which can be recognized as a damage-associated molecular pattern (DAMP), potentially triggering an **inflammatory response that may become chronic**. Our results suggest that in high energy-demanding organs such as muscle and brain, mitophagy is increased in *CHCHD10*$^{S59L/+}$ models. However, **this increase of mitophagy is not observed in motor neurons or in neuromuscular junctions**, both known to degenerate in ALS and other motor neuron diseases. This lack of mitophagy in target cells was confirmed through *in vitro* further approaches using two MN models. In these models, ***CHCHD10*$^{S59L/+}$ motor neurons displayed low basal levels of mitophagy and were resistant to mitophagy induction** compared to controls. These reduced mitophagy levels may lead to the accumulation of damaged mitochondria, **contributing to motor neuron loss and disease progression**.

Perspectives

First, we aim to identify at **which step mitophagy is impaired in S59L MN** models. In parallel, we are exploring strategies to rescue these dysfunctions. **The renewal of damaged mitochondria *via* mitophagy is highly dependent on mitochondrial transport**. Exhausted mitochondria from the NMJ are actively transported back to the soma for recycling. We are currently **optimizing technics to monitor this transport**, track mitochondrial movement and potentially enhance mitochondrial mobility to promote more effective mitophagy. Using microfluidic devices, we monitor mitochondrial transport in live imaging sessions in iPSC-derived MNs. From the resulting time-lapse movies, we generated kymographs, which represent the displacement of mitochondrial particles over time. **Can enhanced mitochondrial transport reverse impaired mitophagy ? This approach could be the answer**.



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