Investigating Lysosomal Dysfunction and Autophagy Dysregulation in a Novel Patient-Derived Cell Model of Charcot-Marie-Tooth Disease Type 4B3

Malika Sharma[1,2], Sarina X. Mao[3,4], Lan Wang[3,4], Jason P. Chua[3,4,5]

[1]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD; [2]Johns Hopkins Department of Biochemistry and Molecular Biology, Baltimore, MD; [3]Johns Hopkins Institute of Cell Engineering, Baltimore, MD; [4]Johns Hopkins School of Medicine, Baltimore, MD; [5]Johns Hopkins Department of Neurology, Baltimore, MD.

Introduction

- Charcot-Marie-Tooth type 4B3 (CMT4B3) is a severe **inherited peripheral neuropathy**. It can be present in either axonal or dysmyelinating form.
- It is an **autosomal recessive disorder** primarily affecting the peripheral nervous system (**PNS**), leading to muscle weakness, sensory loss, syndactyly, and foot deformities. In severe cases, central nervous system (**CNS**) involvement may also occur, with patients exhibiting features such as "fork and bracket" syndrome, microcephaly, and cognitive impairment.
- CMT4B3 is caused by **loss-of-function mutations** in the ***SBF1* gene**, which encodes for **Myotubularin- related phosphatase 5** (MTMR 5).
- Current mouse and cellular models do not fully recapitulate human disease characteristics, limiting our understanding of CMT4B3 pathology.
- Since MTMR5 functions as an **autophagy suppressor**, we hypothesize that mutations in *SBF1*/MTMR5 associated with CMT4B3 result in **autophagy dysregulation and lysosomal dysfunction**.

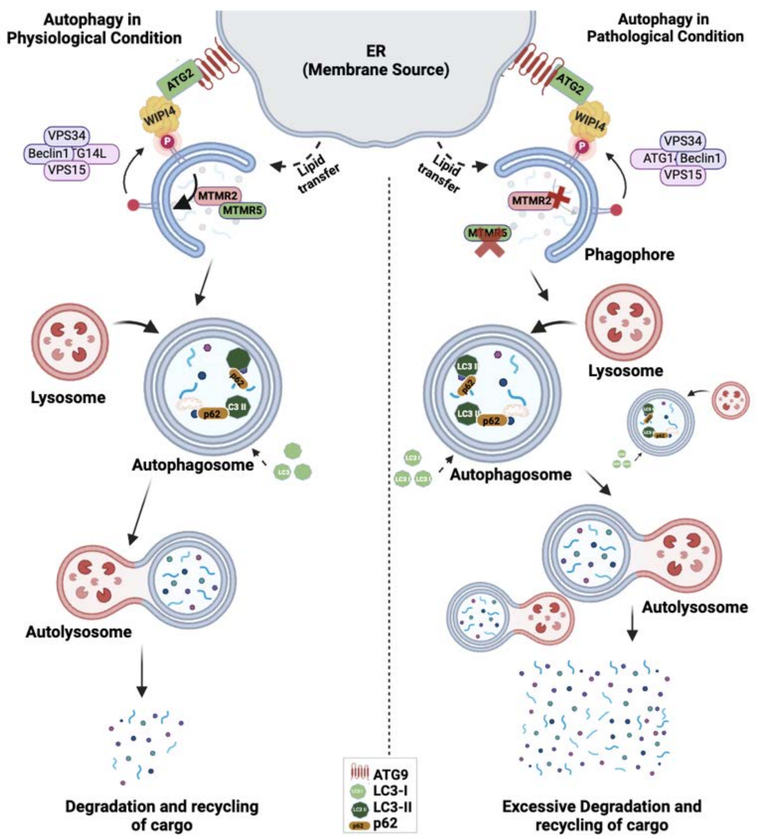


Figure 1: Comparison of the autophagy pathway under physiological conditions and in CMT4B3 pathophysiology*. In CMT4B3, mutations in SBF1/MTMR5 result in upregulated autophagic activity, leading to excessive degradation and contributing to neurodegeneration.*

Results

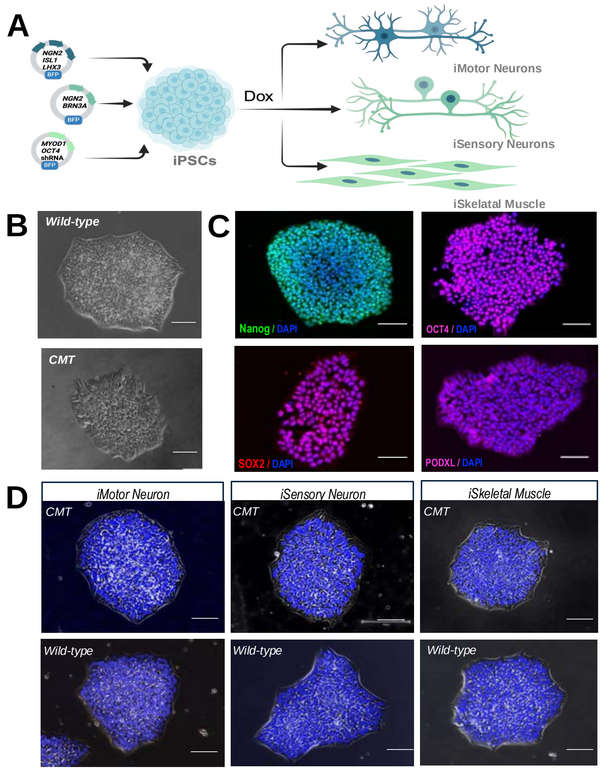


Figure 2: Establishment of patient -derived iPSCs cellular model
2A) Process of differentiating iPSCs into the cells of PNS using PiggyBac/Transposase system used for the stable integration of doxycycline- inducible cassettes. 2B) Wildtype and CMT iPSCs. 2C) Immunocytochemical analysis of pluripotency of iPSCs using Nanog, SOX2, OCT4 and Podocalyxin. 2D) iPSCs expressing BFP confirming the integration of PiggyBac vectors. ***Scale Bar****: 150 µm*

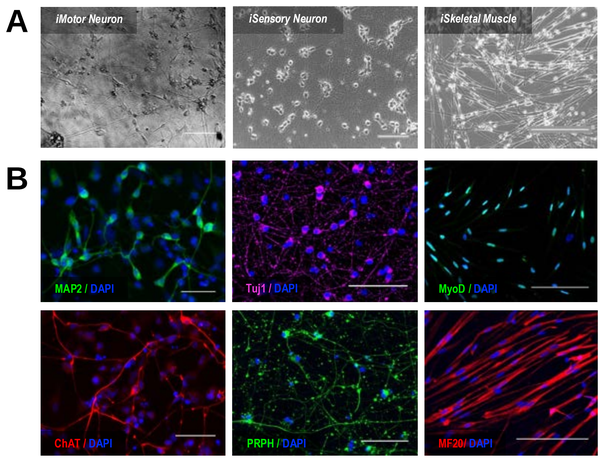


Figure 3: Differentiation of cells of the peripheral nervous system (PNS). *(3A) Cell morphology of mature iMotor neurons (day 14) , iSensory neurons (day 14) and iSkeletal muscle (day 7). 3B) Immunocytochemical analysis of maturity markers showing MAP2 and ChAT expression in iMotor neurons, Tuj1 and PRPH in iSensory neurons and MyoD and MF20 in iSkeletal Muscles.* ***Scale bars****: 300 µm (iMuscle), 150 µm (neurons)*

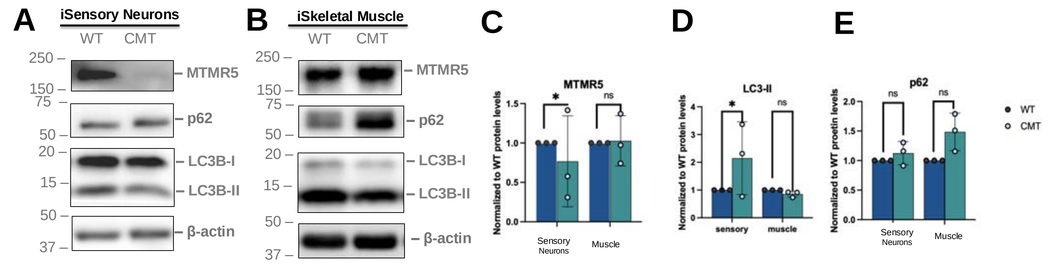


Figure 4. Assessment of autophagy in patient-derived iSensory neurons and iSkeletal muscle.
*(4A, B) Western blot analysis of LC3-II, p62, and MTMR5 in CMT4B3 patient-derived cells compared to wild-type (WT) controls. (4C–E) Quantification of MTMR5, LC3-II, and p62 protein levels. Data are presented as mean ± SEM. Statistical significance: n.s. (not significant), * < 0.1.*

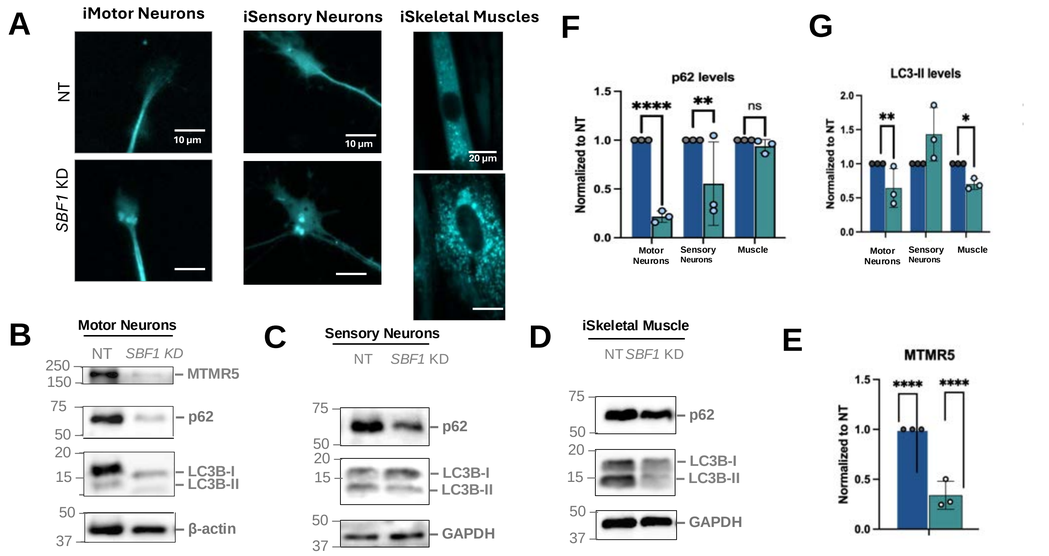


Figure 5: SBF1 knockdown leads to increased autophagy in motor neurons, sensory neurons, and skeletal muscle. (5*A) Live-cell imaging of SBF1 knockdown (KD) motor neurons, sensory neurons, and skeletal muscle expressing mEGFP-LC3B, showing increased LC3-II puncta formation, indicative of enhanced autophagic activity. (5B-D) Western blot analysis confirming SBF1/MTMR5 knockdown and assessing autophagy markers, LC3-II and p62 levels in SBF1 KD cells compared to wild-type controls. (5E-G) Quantification of MTMR5, LC3-II and p62 puncta per cell across different cell types. Data are presented as mean ± SEM. Statistical significance: **** < 0.05,* ** *< 0.01, n.s- non-significant.* ***Scale bar****: 10 µm (neurons), 20um (muscle)*

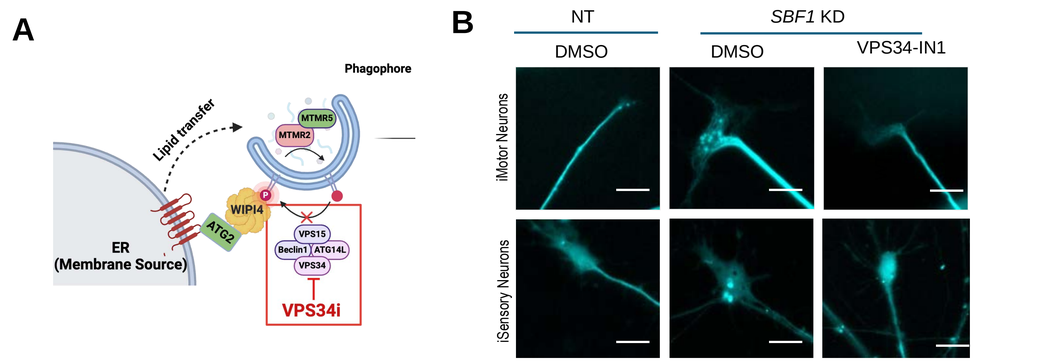


Figure 6: Autophagy disinhibition Recue by VPS34-IN1 in SBF1 knockdown iMotor and iSensory neurons. *(6A) Schematic representation of VPS34-IN1 mechanism of action in modulating heightened autophagy. (6B) Live-cell imaging of SBF1 knockdown (KD) neurons expressing mEGFP-LC3B, showing reduced LC3-II puncta following VPS34-IN1 treatment.* ***Scale bar:*** *10 µm.*

Objectives

- Develop a novel and reliable human iPSC-derived cell model of CMT4B3 using patient-derived cells.
- Differentiate iPSCs into key components of the peripheral nervous system, including motor neurons, sensory neurons, and skeletal muscle.
- Investigate lysosomal dysfunction and autophagy dysregulation in CMT4B3 and their contributions to disease pathogenesis.
- Utilize mEGFP-LC3B-tagged motor neurons, sensory neurons, and skeletal muscle to visualize autophagy in real- time, achieved through *SBF1*/MTMR5 knockdown via shRNA.
- Evaluate potential therapeutic strategies by testing candidate rescue approaches with high translational value.

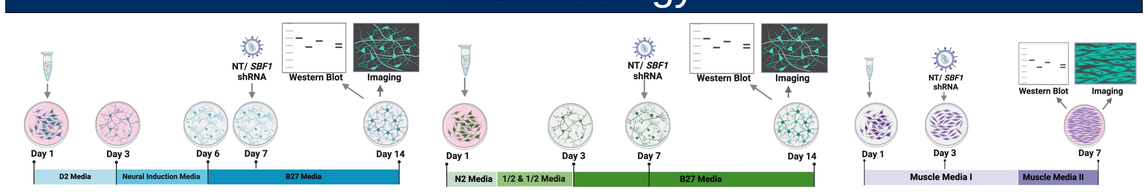
Future Directions

- Assess autophagy in patient-derived iMotor neurons.
- Use CRISPR-corrected iPSCs as isogenic controls.
- Differentiate iPSCs into Schwann cells to expand PNS cell models.
- Analyze cell viability and dependence on hyperactive autophagy in CMT4B3.
- Study the cell or non-cell autonomous effect of *SBF1* mutations.
- Investigate mitophagy and mitochondrial health in CMT4B3 cells.
- Test therapeutic candidates and motor neuron autophagy modulators.
- Use proteomics to study MTMR5 interactors and their functions in neurons.

Conclusion

- This novel human-iPSC-based model system for CMT4B3 and provides a rapid (<14d) and robust (>99% pure) differentiation protocol for generating motor neurons, sensory neurons and skeletal muscle cells.
- Our initial studies indicate that the loss of MTMR5 protein is associated with inappropriate enhancement of autophagy, highlighting its significant role across different cell types.
- This human iPSC-derived model serves as a strong platform for detailed analysis of CMT4B3 mechanisms, paving the way for discovering new therapeutic strategies tailored specifically to patients based on their unique mutation profiles.

Methodology



Resources

Methods

References

Lab Website

Acknowledgement

- Members of the Chua lab
- The Shultz family
- CMT4B3 Research Foundation
- Institute of Cell Engineering, Johns Hopkins University
- Johns Hopkins School of Public Health