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# Poster P32

## Investigating Axonal Transport In Charcot-Marie-Tooth Disease Type 2A Using A Pluripotent Stem Cell-based Model

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Charcot-Marie-Tooth (CMT) disease is one of the most common forms of inherited peripheral neuropathy and has many different subtypes. One such subtype is sensory and motor neuropathy CMT Type 2A (CMT2A), for which no treatments currently exist. CMT2A is caused by mutations in Mitofusin 2, and it is unknown how these mutations drive disease. Hence, we set out to create the first human embryonic stem cell (hESC) model of CMT2A to investigate the impact of a CMT2A-causing mutation in a disease-relevant cell type.

We generated a panel of hESC clones by introducing the disease-causing heterozygous R94Q mutation into Mitofusin 2 via CRISPR-Cas9 editing. The clone panel was subsequently differentiated into limb-innervating motor neurons and used for live trafficking assays. Limb-innervating motor neurons containing the disease-causing mutation displayed a mitochondrial trafficking defect that could be rescued via HDAC6 inhibition. We further tested the same HDAC6 inhibitor in a zebrafish model of CMT2A, where chronic dosing rescued motor deficits in treated zebrafish. Furthermore, to obtain a mechanistic insight into CMT2A pathogenesis we show that Mitofusin 2 containing the R94Q mutation interacts more strongly with the trafficking adapter Trak1, leading to Trak1 having a reduced interaction with the axonal motor protein kinesin.

We have successfully created hESCs containing a CMT2A-causing mutation that can be differentiated into limb-innervating neurons, providing a new in vitro platform for CMT2A research. Furthermore, results here contribute to evidence that axonal transport deficits are a common CMT2 hallmark. This work provides a new hypothesis for CMT2A pathophysiology and is a foundation for the further study of axonal transport machinery and its functionality in CMT2A.