Human Induced Pluripotent Stem Cells as a Model for Charcot-Marie-Tooth Disease type 2A.

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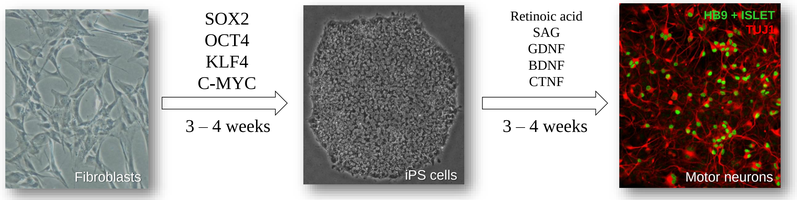
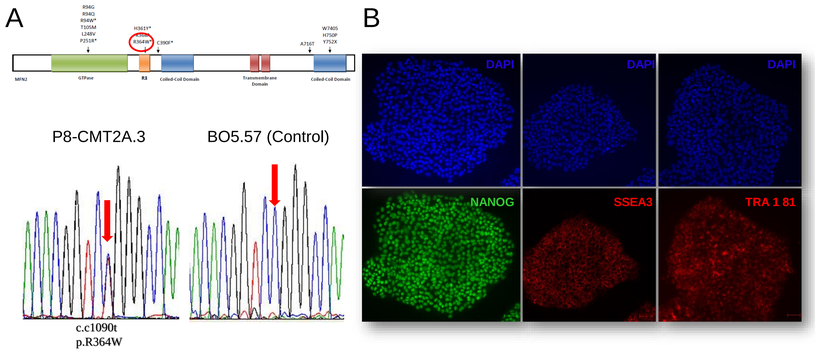
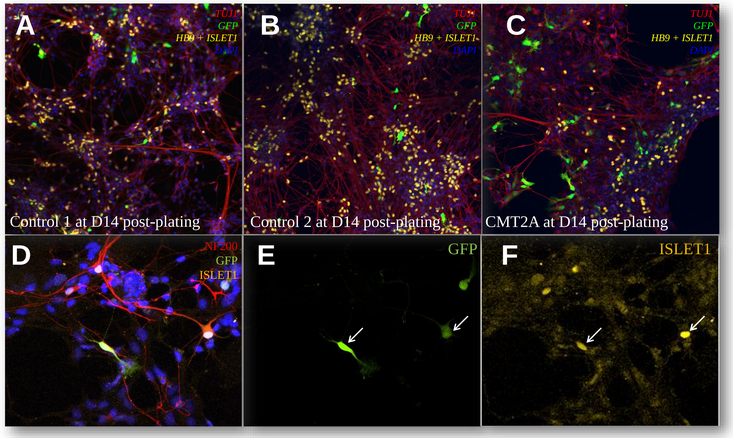
Charcot-Marie-Tooth disease type 2A is caused by heterozygous point mutations in the MITOFUSIN2 (MFN2) gene, which encodes a GTPase associated with mitochondrial fusion. Although overexpression of mutant Mfn2 in rat and mouse neuronal cell culture results in significantly impaired mitochondrial transport along axons, it has not yet been established whether neurons derived from patients with CMT2A carrying only a single copy of the mutated gene and a normal allele, would show a similar impairment.



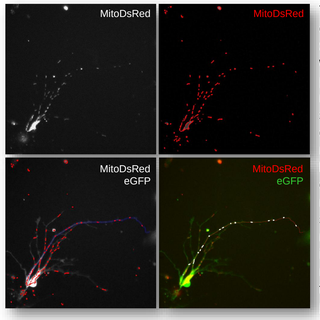
Induced pluripotent stem cells (iPS) are derived from somatic cells after the forced expression of defined transcription factors, and are capable of being differentiated into cell types from the three embryonic germ layers, including spinal cord motor neurons.



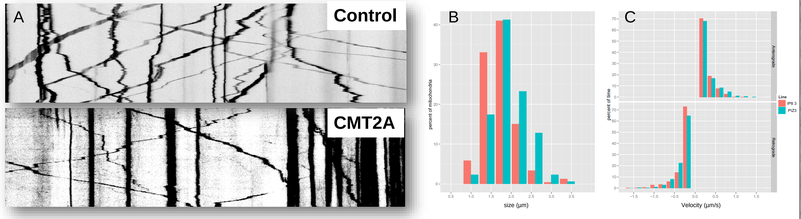
In this study, we developed iPS cell lines from skin fibroblasts of a patient with a MFN2 point mutation by transduction with retroviral constructs encoding OCT4, SOX2, KLF4 and C-MYC. iPS cells from the patient carry the disease causing MFN2 mutation, a C – T transition at position 1090 (causing a R364W amino acid substitution) (A). CMT2A iPS cells exhibit markers of pluripotency, including NANOG, SSEA3 and TRA 1 81 (B).



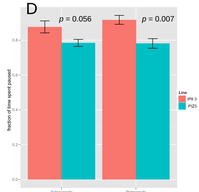
IPS cells from the CMT2A patient and two non-affected controls were differentiated following a dual SMAD inhibition protocol as monolayer adherent cultures. Patterning for spinal cord motor neurons was achieved by addition of Retinoic Acid, Sonic Hedgehog Agonists (SAG) and neuronal growth factors (BDNF, GDNF and CTNF) and yielded neuronal cultures consisting of at least 20% ISLET1/HB9 immunoreactive cells (A, B and C). In order to study mitochondrial axonal transport, these neuronal cultures were transfected at D25 of differentiation with two vectors: a MitoDsRed construct (ClonTech, Mountain View, Ca) and a eGFP construct, each one driven by a Human Ubiquitin promoter. eGFP positive neurons which were also immunoreactive to ISLET1 could be identified in the culture (D, E, and F).

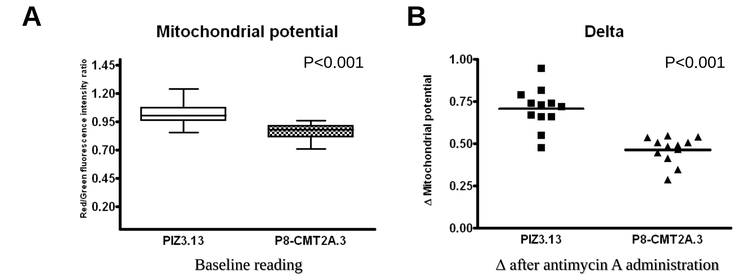


Transfected cultures were live imaged using an environment-controlled chamber at 37ºC and 5% CO_2 (Pathology Devices, Westminster, MD). Time lapse images were acquired at D28 to D31 of differentiation (D10 to D13 post-dissociation) at 40x magnification every 1 s for 5 minutes. Kymographs were prepared using NIS Elements software (Nikon), by tracking mitochondria moving along eGFP-positive axons. Quantification of mitochondrial movement was performed using a custom software written in MatLab® (Mathworks). For each frame, the software enhances the image to enable location of neurites, allows for manual tagging of the axon, identifies mitochondria on the selected axon and quantifies various properties of mitochondria (positions, size, mean intensity). Data from individual frames is then compiled into trajectories for each mitochondria, enabling further analysis of the dynamics of their motion, including velocity, asymmetry of motion and presence of long excursions.



Preliminary analysis of mitochondrial dynamics (n = 5 axons per group) revealed subtle differences between CMT2A and control lines. Mitochondria were smaller in the CMT2A neurons, compared to controls (A) and stayed paused longer (D). However, contrary to what has been shown in rodent DRG neuronal cultures, the CMT2A mitochondria had similar velocity distribution as normal controls (C), retaining the fast component of mitochondrial axonal transport (A). Further studies are ongoing.





Preliminary analysis of mitochondrial membrane potential using the cationic dye JC1 suggests a reduction (depolarization) of mitochondrial potential in CMT2A neuronal cultures (p<0.001) (A). This was further confirmed by measuring the variation (delta) of membrane potential in response to Antimycin A, a strong depolarizing agent. As demonstrated in B, CMT2A neuronal cultures responded less to Antimycin A when compared to a control cell line (PIZ3.13), suggesting that mitochondrial membranes are already depolarized in the CMT2A cultures at baseline.

This study demonstrates the potential of iPS cells to model neurogenetic diseases and to offer a human platform for the study of Charcot-Marie-Tooth disease, specially axonal forms where neuronal dysfunction are believed to play a central role.

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