Mitochondrial dysfunction links mutations in TDP-43 and C9orf72 iPS-derived motor neurons from ALS patients

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Introduction
Hexanucleotide expansions in the C9orf72 are the most frequent cause of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD), accounting for up to 50% of familial ALS cases. While mutations in TARDBP are a rare cause of ALS, the deposition of TDP-43 positive cytoplasmic inclusions remains a common neuropathology for approximately 97% of ALS cases, including C9orf72 cases. Identifying common pathways between C9orf72 and TDP-43 would significantly contribute to our understanding of the disease mechanism.

Objective
The aim of this study is to identify if C9orf72 and TDP-43 mutations affect mitochondrial function using iPS-derived MNs from patients and isogenic controls.

Methods
In this study, we differentiated patient motor neurons derived from induced pluripotent stem cells (iPSCs) carrying hexanucleotide expansions in the C9orf72 gene or mutations in TDP-43 (M337V and I383T). We generated isogenic iPSC lines where the expansions were successfully removed by CRISPR/Cas9 in C9orf72 iPSCs. Seahorse XFe was used to assess mitochondrial respiration, ATP production and spare respiratory capacity and live calcium imaging was used to determine mitochondrial calcium buffering. Neurons were grown on microfluidic chambers for studying axonal transport and MitoTracker movement was quantified during live imaging in the microgrooves.

Results
We found both C9orf72 and TDP-43 (M337V and I383T) MNs show reduced mitochondrial basal respiration at baseline and reduced spare respiratory capacity when ER stress was induced by thapsigargin. Mitochondrial potential was also reduced in C9orf72 MNs, while the TDP-43M337V and TDP-43I383T MNs did not show differences when compared to healthy controls. When stimulated with 100 µM glutamate during live calcium imaging, we found reduced mitochondrial uptake of calcium from the cytosol in C9orf72 and TDP-43 MNs compared to healthy and isogenic controls. Imaging of axonal transport revealed reduced speed of retrograde mitochondrial transport in TDP-43M337V and TDP-43I383T, which correlated with downregulation of the molecular motor adaptor dynactin-1. We also detect significantly reduced mitochondrial length and surface area in patient iPS-MNs, indicating increased fragmentation.

Conclusions
This study shows that ALS iPS-derived MNs with mutations in C9orf72 and TDP-43 have deficiencies in essential mitochondrial functions, such as respiration, calcium buffering and mitochondrial dynamics.