Investigating the role of calpain cleavage as an early pathogenic mechanism in mouse models of Machado Joseph disease and Motor Neuron Disease

Katherine J Robinson, Amanda Wright, Annica Roberts, Stuart Plenderleith, Maxinne Watchon, Angela S Laird

Centre for Motor Neuron Disease Research, Department of Biomedical Sciences, Faculty of Medicine, Health and Human Sciences, Macquarie University, Sydney, Australia

MACQUARIE University

Australian Government National Health and Medical Research Council

MJD FOUNDATION

Anindilyakwa Land Council

THE SNOW FOUNDATION

Abstract

Protein aggregate pathology is a disease phenotype common to many neurodegenerative diseases. Post-mortem examination of brain tissue from patients with Machado Joseph disease (MJD, also known as spinocerebellar ataxia-3) has revealed the presence of protein aggregates containing both full-length and shorter protein fragments. Similarly, TDP-43 fragments have been detected in spinal cord tissue from sporadic motor neuron disease (MND) patients.

In this study, we investigated the pathogenic contribution of calpains (calcium-activated proteases) in two transgenic mouse models; CMVMJD135 mice modelling MJD and inducible hTDP-43ΔNLS mice, modelling MND. We detected the presence of cleaved proteins (α-spectrin and TDP-43) in plasma and/or affected tissues in both MJD and MND mice. Our findings suggest that calpains may become hyperactive early in the disease process (at symptom onset) and may drive protein aggregation and apoptosis in MJD and MND mice. Inhibition of calpain proteases warrants further investigation as a potential therapeutic for both MJD and MND.

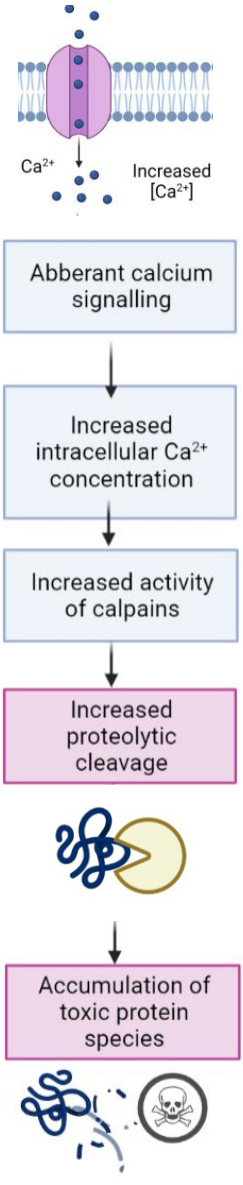
Background

- Fragmentation of proteins, via proteolytic cleavage, is known to alter protein function and contribute to proteinopathy in neurodegenerative diseases such as Machado Joseph disease (MJD) and motor neuron disease (MND).

- Aberrant cleavage of proteins into smaller fragments can lead to formation and accumulation of protein aggregates.

- Calpains (calcium-activated proteases) cleave proteins such as TDP-43 and ataxin-3, perturbing protein function and increasing the likelihood that protein fragments may accumulate into protein aggregates.

- We have previously found that treatment of MJD zebrafish with calpain inhibitor compounds can reduce the presence of toxic ataxin-3 protein species and improve zebrafish swimming.



- We hypothesise that calpain overactivity is an early disease phenotype that contributes to disease progression in models of MJD and MND.

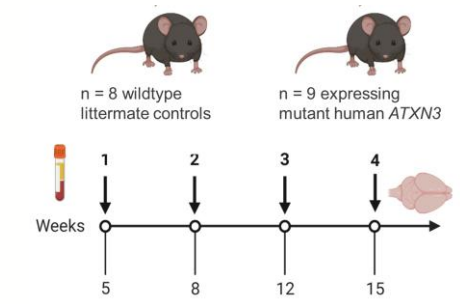
Aims

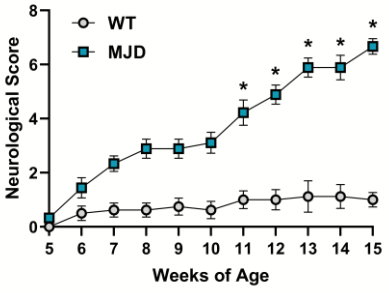
1. To determine if cleaved α-spectrin (a marker of calpain activity) could be detected in samples obtained from mouse models of MJD and MND.
2. To determine whether calpain activity was an early or late disease phenotype.

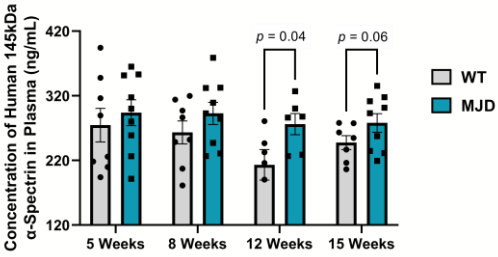
Methods

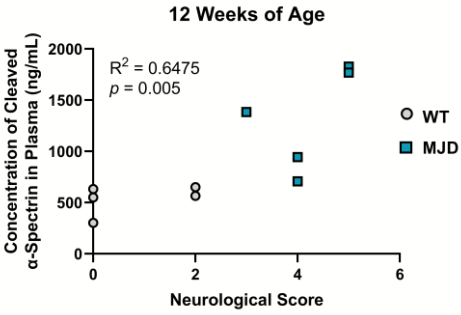
- Male CMVMJD135 mice expressing mutant human ataxin-3 (130-143Q) were compared to wild type littermates.
- Inducible NEFH-tTA/tetO-hTDP-43ΔNLS bigenic male mice were compared to non-transgenic littermates.
- In absence of doxycycline, bigenic mice express mutant human TDP-43 (ΔNLS8) and develop human MND-like phenotypes.
- Blood was taken from the saphenous vein and tissue (cerebellum or spinal cord) extracted for cleaved α-spectrin via ELISA and western blotting

Plasma levels of cleaved α-spectrin are increased in 12-week MJD mice

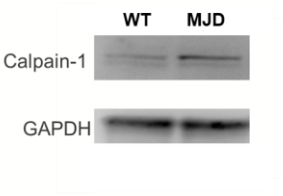


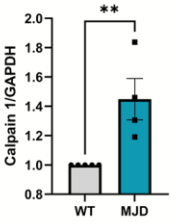


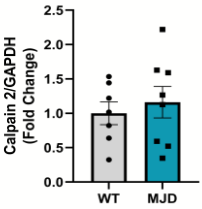


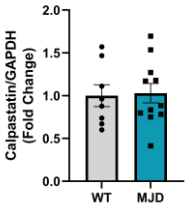


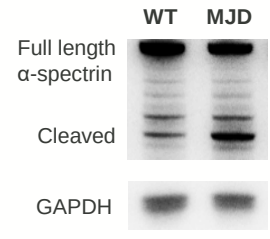
Calpain 1 and cleaved α-spectrin are elevated in the MJD cerebellum

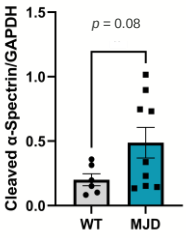


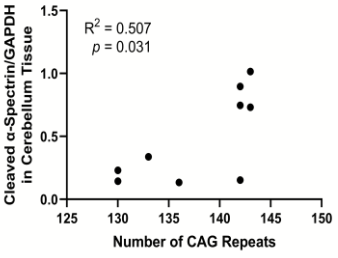






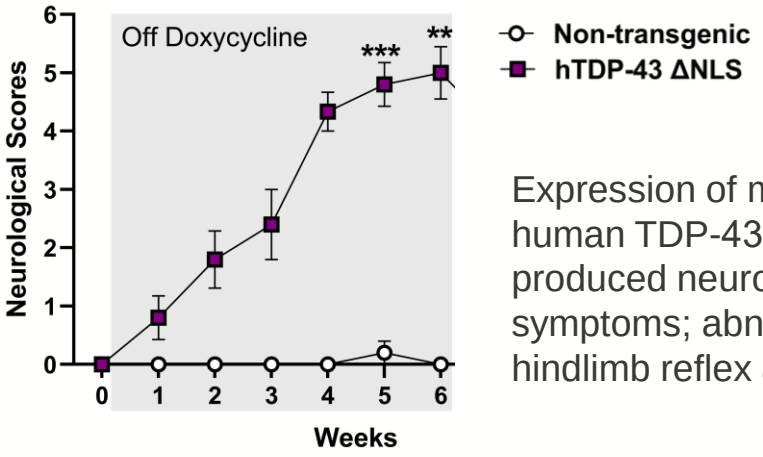




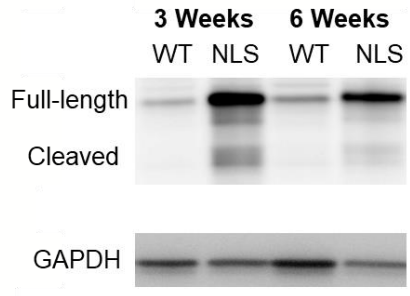


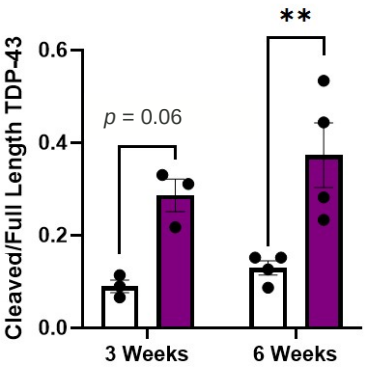
- Levels of cleaved α-spectrin were found to be increased but did not reach statistical significance in 15 week MJD mice, aligning with findings from plasma.
- Statistically significant correlation between inherited CAG length and cleaved α-spectrin

Findings from inducible NEFH-tTA/tetO-hTDP-43ΔNLS mice

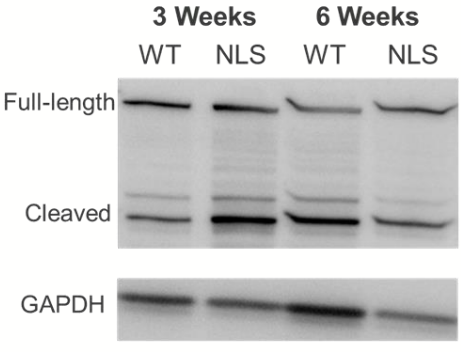


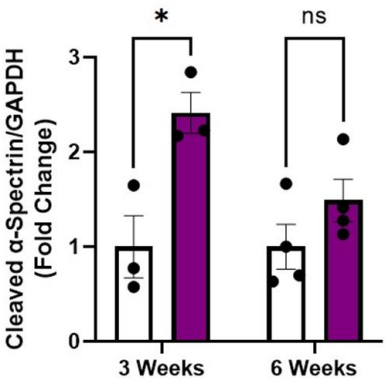
Expression of mutant human TDP-43 (ΔNLS) produced neurological symptoms; abnormal hindlimb reflex and tremor





Analysis of lumbar spinal cord tissue revealed full length and cleaved TDP-43 species (~33kDa and ~22kDa), with increased levels of cleaved TDP-43 at 6 weeks off doxycycline.



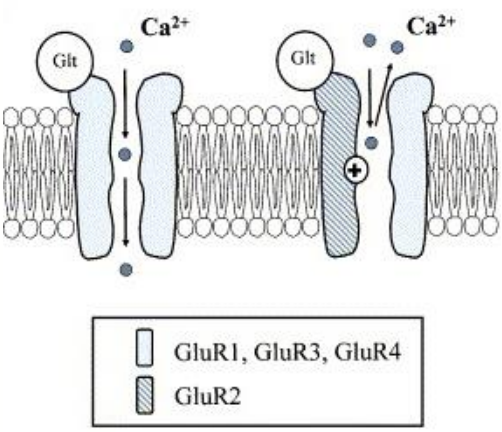


Increased cleaved α-spectrin in the lumbar spinal cord at 3 weeks, with relatively less detected at 6 weeks.

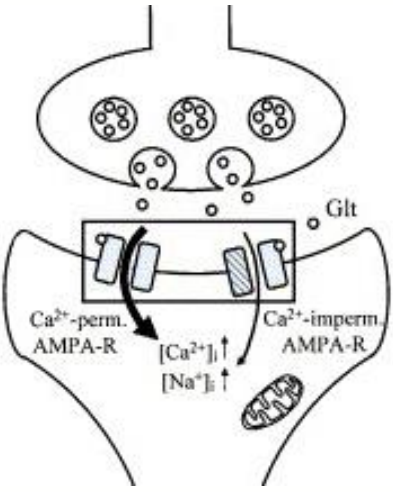
Possible mechanisms leading to hyperactivity of calpain proteases

What is causing elevated calcium concentrations and downstream calpain hyperactivity?

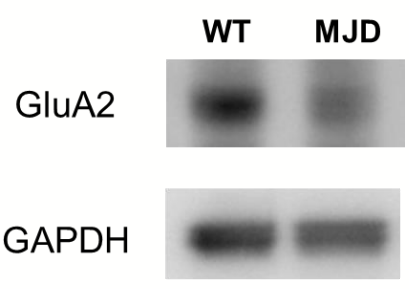
- Ionotropic glutamate receptors (AMPA and NMDA) permeable to calcium ions
- Permeability of AMPARs to calcium regulated by GluA2 subunit
- Dysregulation of GluA2 has been linked with MND; less GluA2 = more calcium entry into neurons
- Reduced GluA2 levels previously characterised in inducible NEFH-tTA/tetO-hTDP-43ΔNLS mice (Wright et al., 2021)

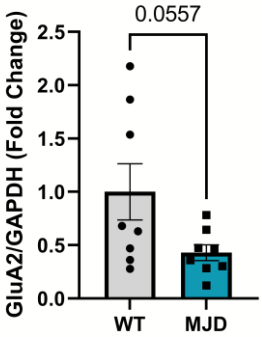


Van Den Bosch et al., 2006



- Novel finding of decreased levels of GluA2 detected in cerebellum tissue from MJD mice.
- Loss of GluA2 could lead to elevated calcium concentrations, causing chronic activation of calpains





Summary

Aims	MJD	MND
Are cleaved protein fragments detected in post mortem tissues from patients?	Yes	Yes
Increased cleaved α-spectrin detected in plasma samples from mice?	Yes	In progress
Increased presence of shorter protein fragments in affected tissues?	Yes	Yes
Alterations in calpain 1, calpain 2 or calpastatin?	Calpain 1	In progress
Downregulation of calcium-impermeable GluA2 subunit of AMPA receptor?	Yes	Yes Wright et al 2021

- Increased levels of 145kDa cleavage product of α-spectrin detected in the early stages of disease, less present at later stages (145kDa product is likely further cleaved by caspases).
- Plasma could be used as a non-invasive biomarker of calpain activity.
- Compounds that inhibit calpain proteases should be explored as a potential therapeutic for MJD and MND.
- Our preliminary evidence suggests that calpain inhibitor treatments can decrease neurological symptoms in MJD mice.