The neuromuscular junction - the hidden player in MND: studies from MND model mice and MND patients

P.G. Noakes,[1] S.T. Ngo,[2,4,5,6] K.M. Lee,[1] K. Chand,[1,5] N.A. Lavidis,[1] K. Kesavan,[1] J. Kumar,[1] E. Wimberger,[1,2] T. Tracey,[1,2] A. Russell,[3] E. Wolvetang,[2] P. McCombe,[4,5] R. Henderson[4] and L. Jeffree,[4] [1]School of Biomedical Sciences, The University of Queensland, St Lucia, QLD 4072, Australia., [2]Australian Institute for Bioengineering and Nanotechnology, The University of Queensland, St Lucia, QLD 4072, Australia. , [3]School of Exercise and Nutrition Sciences, Institute for Physical Activity and Nutrition (I-PAN), Deakin University, Geelong, VIC 3220, Australia., [4]Royal Brisbane & Women's Hospital, Herston, QLD 4029, Australia., [5]Centre for Clinical Research, The University of Queensland, Herston, QLD 4029, Australia. and [6] Queensland Brain Institute, The University of Queensland, St Lucia, QLD 4072, Australia .

Background: Motor neuron disease is a neurodegenerative disease characterized by the death of upper and lower motor neurons. Individuals affected by MND experience severe muscle weakness and atrophy, ultimately resulting in muscle paralysis and death. A central event in all cases of MND is the withdrawal of the motor nerve terminal from its target muscle cells (Maloney, de Winter & Verhaagenet, 2014). This decline in the integrity of neuromuscular connections leads to progressive muscle paralysis and death. Our goal is to understand the molecular and cellular mechanisms that contribute to the loss of neuromuscular connections in MND.

Methods: Muscles from MND model mice (SOD1G93A and TDP43Q331K) and their respective aged matched controls were obtained from euthanised mice. These muscles were collected at pre-symptomatic and disease onset stages. We also collected human muscle biopsies from early diagnosed MND patients and Non-MND donors. Both mouse and human muscles were immuno-stained for neuromuscular connections, synaptic laminins and muscle specific tyrosine kinase (MuSK) (Lee *et al.*, 2017). We also processed human muscle samples for electron microscopy to examine the ultrastructure of their neuromuscular connections. Human muscle biopsies were also used to isolate muscle stem cells. Muscle stem cells were differentiated into multinucleated muscle cells. These muscle cells were then assessed for their ability to cluster post-synaptic acetylcholine receptors (AChRs) in response to recombinant agrin treatment (Ngo *et al.*, 2012). Agrin is a motor neuron secreted molecule that binds to its receptor Muscle specific kinase (MuSK) to induce the formation of post-synaptic AChRs in muscle (Ghazanfari *et al.*, 2014).

Results: In MND model mice, we have observed declines in synaptic laminins -α4, -α5 and -β2, which are adhesion molecules located between motor neurons and muscle (Lee *et al.*, 2017). We have also observed reduced expression in MuSK, which is needed to stabilize postsynaptic specialisations at NMJs, in response to the motor neuron factor agrin (Ghazanfari *et al.*, 2014). Importantly, these changes coincide with altered synaptic transmission and disassembly of the neuromuscular junction (NMJ), which we and others have reported to all occur before the loss of upper and lower motor neurons (Chand *et al.*, 2018). Our human studies have also revealed a similar loss of synaptic laminins and MuSK from NMJs of muscles from early-diagnosed MND patients. The down regulation of synaptic laminins could explain the changes at MND-NMJs that we have observed including: misalignment of active zones, encroachment of Schwann cells into the synaptic cleft, and motor terminal withdrawal from muscle. The down regulation of MuSK expression could contribute to the dispersal of postsynaptic acetylcholine receptor clusters (AChRs) from NMJs in the muscle of MND patients. This down regulation of MuSK at the NMJs from MND patients also supports our *in vitro* findings, which show that muscle from MND patients appear not to respond to agrin, suggesting a fault in the agrin-MuSK signalling pathway.

Conclusion: Collectively, these data add support the idea that alterations of NMJ adhesion and NMJ-muscle signalling are early peripheral contributions to MND.

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