Overview of TDP43 in MND

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In humans, our genes influence everything from our eye and hair colour to our temperament and the likelihood of us developing disease. Our genes are made from our DNA which instructs our billions of cells to create proteins that are the building blocks of all life and our bodily functions. In disease, either caused by genetic mutations or unknown environmental factors, our proteins can become abnormally aggregated within cells and tissues, disrupting the function of the proteins and affecting the balance of the body's systems. A particular protein implicated in Motor Neuron Disease (MND) is called TDP43 (scientifically; TAR DNA binding protein 43). A gene called TARDBP is responsible for providing instructions for making this protein. Within the TDP43 protein, there are several parts (regions) that perform different roles contributing to the function of the protein. TDP43 has an N terminal, two RNA/DNA binding domains (RRM1, RRM2), a nuclear localisation signal (NLS) and a C-terminal (Figure 1).

What is the function of TDP43 within the brain and spinal cord?

The TDP43 protein is expressed throughout the human body, however in the context of MND, we focus on its expression within the brain and spinal cord (regions predominantly affected by MND). The complete function of TDP43 is yet to be fully determined, however, to date, it has been identified to have important roles in the processing and maintenance of RNA (scientifically, ribonucleic acid). RNA is crucial for the transfer of genetic information from our DNA to proteins. In addition, TDP43 is involved in many important steps of protein production and is therefore able to influence various functions of a cell. The presence of TDP43 within our cells is therefore very important for normal functioning.

Within the brain and spinal cord, the TDP43 protein is predominantly located within the centre of cells (scientifically termed the 'nucleus'). Structurally, TDP43 has a region called the NLS (nuclear localisation signal) that allows TDP43 to shuttle in and out of the nucleus to the outside of cells (scientifically termed the 'cytoplasm'), conducting its important cellular functions. Another important aspect of the TDP43 protein is that it self-regulates its own production. It is important that future research uncovers more about the complex functions of the TDP43 protein and its interactions within the brain and spinal cord.

Better understanding of TDP43's 'normal' function in healthy cells will assist researchers in understanding why things go wrong with the TDP43 protein in diseases such as MND and how best to overcome, prevent and treat these abnormalities.

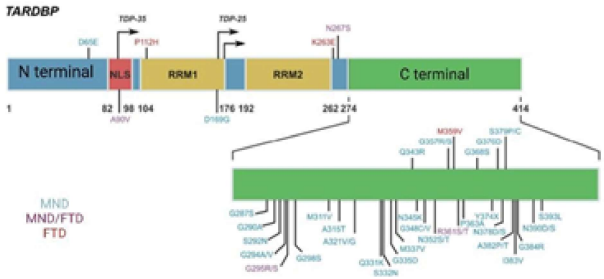

Figure 1. Adapted from Bright et al 2021, The structure of TDP43 protein: TDP43 is encoded by the TARDBP gene. Structurally TDP43 contains an N terminal (blue), two RNA/DNA binding domains (yellow-RRM1/2), a nuclear localisation signal (red-NLS) and C terminal (green). Small, coloured text refers to the location of various genetic mutations identified in the TARDBP gene linked to MND and FTD.

What goes wrong with TDP43 in the Central Nervous System (CNS) in MND?

In the early 2000s, researchers discovered that abnormal misfolding and aggregation of TDP43 proteins were present in the cytoplasm of motor neurons within the brain and spinal cord in MND patients. These abnormal aggregations of TDP43 were identified to have significant alterations in their structure and function (scientifically, the abnormal TDP43 proteins were hyperphosphorylated, ubiquitinated and cleaved into fragments). Essentially, in MND, TDP43 loses its structure and function and is abnormally redistributed from the nucleus to the cytoplasm of cells (Figure 2), where it abnormally accumulates and causes the cell to die. Pathological studies of brain and spinal cord tissue from MND patients have enabled researchers to determine that abnormal TDP43 is present within the brain and spinal cord of 95% of MND patients. Further studies by researchers have also determined that abnormal TDP43 in MND can, in some cases, be caused by known genetic mutations in certain genes linked to MND (e.g. TARDBP, C9orf72). Within the 'C terminal' of the TDP43 protein a number of genetic mutations have been identified to date, many of which are linked to MND (Figure 1).

The main genetic cause of TDP43 pathology has been identified to be mutations in a gene called C9orf72 (scientifically chromosome 9 open reading frame 72). While these known genetic mutations causing TDP43 pathology have enabled more research into the genetic causes of MND, unfortunately these known genetic mutations account for only a small percentage of MND patients. Therefore, the majority of MND patients who do not have a known genetic mutation causing TDP43 pathology have what is termed 'sporadic' TDP43 pathology. This means that there is no known cause and the TDP43 pathology could be linked to undetermined environmental factors.

The precise mechanisms that underlie the abnormal changes to the TDP43 protein within the CNS in MND remain unknown. Research that aims to understand this is critical for the development of better tools to diagnose disease, measure progression and responses to treatments, and ultimately for finding treatments and a cure for MND. To begin addressing these unknowns, research must focus on answering the following:

- How abnormal aggregates of TDP43 can be cleared from affected cells within the brain and spinal cord while maintaining unaffected cells
- How to prevent or reverse the misfolding and abnormal aggregation of TDP43 within cells in the brain and spinal cord
- How to interfere with TDP43's self-regulation to disrupt the imbalance of the protein's steady-state within the brain and spinal cord, in order to understand how and why the transport of TDP43 between the nucleus and cytoplasm is disrupted in MND

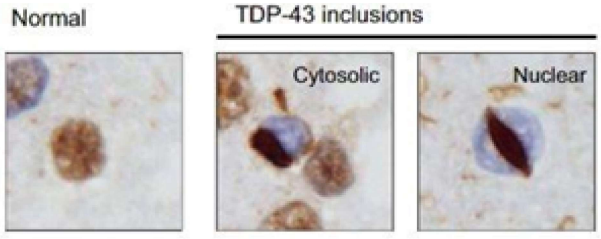

Figure 2. Adapted from Ederle & Dormann 2017 FEBS, The abnormal mislocalization of TDP43 in MND pathology. Within the brain and spinal cord. Normally TDP43 is localised to the nucleus and is able to shuttle in and out of the cell to perform its functions in processing and maintaining RNA. However in MND, TDP43 becomes lost to the cytoplasm where it abnormally aggregates. This abnormal aggregation can also be seen in the nucleus.

How my research is tackling the 'unknowns' about TDP43 pathology in MND

Supported by the MND Research Australia Bill Gole MND Postdoctoral Fellowship, my research aims to investigate a specific region of the TDP43 protein that is yet to have been fully investigated. As mentioned previously, the focus of research into the TDP43 protein has been on the 'C terminal' which is where the majority of genetic mutations have been identified. However, the 'N terminal' of the protein contains some important structures (NLS, RRM1/2) (Figure 3) that are critical for TDP43's transport between the nucleus and cytoplasm of cells.

Unfortunately, there is very little research to date that focuses on the N terminal of TDP43 and there is significantly more to be learned about the movement of TDP43 between the nucleus and cytoplasm in both healthy and diseased states. It is likely that there are regulators of TDP43 transport that involve interaction with the N-terminus specifically that are yet to be discovered. Therefore, the overall objective of my research is to address the significant gap in our knowledge regarding the understudied N terminal of TDP43 and to discover novel regions and molecular pathways of this region of TDP43. I aim to determine the precise mechanisms underlying TDP43's movement between the nucleus and cytoplasm and how this becomes disrupted in MND.

We believe this research holds the key to understanding what goes wrong during MND pathogenesis and the 'how and why' of TDP43 mislocalisation and aggregation in cells within the brain and spinal cord. Ultimately, if we can define and uncover unexplored regions, molecular pathways and novel regulators of the TDP43 protein, we are in a prime position to utilise this information to determine the mechanisms underlying the cause of TDP43 pathology in MND. This will enable us to pave the way for future research towards manipulation of these mechanisms in order to sooner and better diagnose MND, develop targeted treatments for TDP43 pathology specifically and ultimately find a cure for MND.

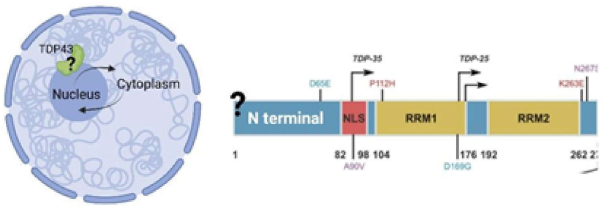

Figure 3. Schematic drawing of the N terminal region of TDP43 that has had limited research to date. It is likely there are unknown regions located in the N terminal or interactions partners with this region of the TDP43 protein that could play critical roles in the regulation of TDP43's transport in and out of cells and its disrupted transport in MND.