MND Australia
International Research Update

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Outcomes from the MND/ALS Australian National Research Conference

This article comes after the first in-person Australian/NZ MND/ALS conference since the beginning of the COVID-19 pandemic. It was wonderful to see all of the researchers present their work and how, despite the pandemic, researchers were continuing to make strides to better understand MND, provide better care and potentially treat it.

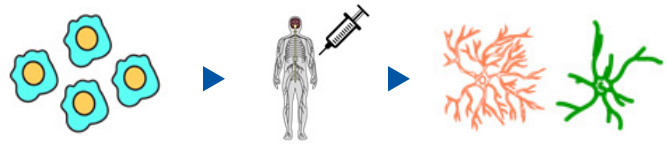
This issue of the MND International Research Update demonstrates the breadth of MND research, containing studies of why di-peptide repeats that are characteristic of the C9ORF72 mutation may be toxic, how these repeat proteins might act as biomarkers for C9orf72-associated ALS, a finding that TDP-43 accumulates in nerve axons, and a clinical trial of stem cell transplantation for ALS patients.

Hematopoietic stem cells for amyotrophic lateral sclerosis treatment

In ALS one of the major observations made is that there is excessive and widespread activation of central nervous system immune cells (microglia and astrocytes). Therefore, it is reasonable to think that deactivating or suppressing the activation of these cells may be beneficial for patients suffering from ALS. A team led by Dr Claudia Caponnetto from Genoa, Italy recently worked to mitigate the excessive activation of these immune cells in ALS patients via injection of hematopoietic stem cells into patients.

Hematopoietic stem cells?

Hematopoietic stem cells are blood stem cells capable of becoming all types of blood cells including red blood cells, platelets and white blood cells, including astrocytes and microglia. These cells are capable of being harvested from a patient, proliferated, and then reinjected into the same patient. Surprisingly, hematopoietic stem cells are capable of crossing the blood-brain barrier and also exert a suppressive effect on inflammation in degenerating central nervous system models.

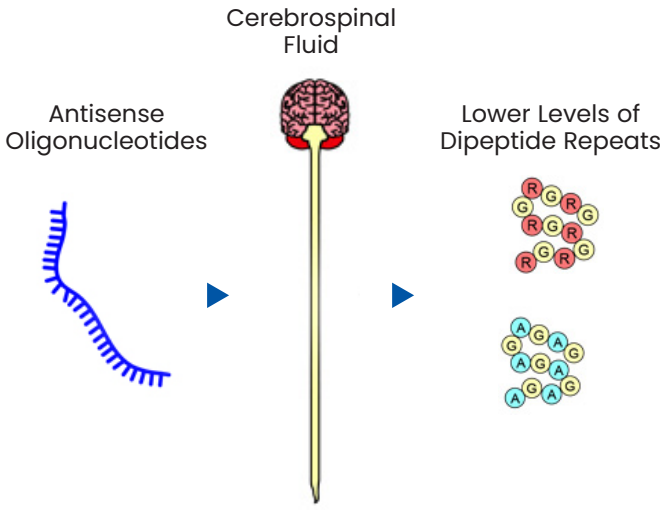


What were the outcomes of this trial?

The trial being discussed here was a phase I/IIa trial, meaning that the main outcome was treatment tolerance and safety for patients. The trial was run on a small number of patients, with only 8 of eleven enrolled patients receiving the treatment. The treatment was well tolerated and patients did not have any unexpected side effects. Unfortunately, the study was not powered (not enough patients) to adequately measure if the treatment had any beneficial effects on disease course. This being said, the major finding was that the treatment appears safe, so future studies can likely increase the treatment regime, increase patient numbers, and investigate effectiveness of the treatment. Safety in potential treatments for diseases is of the utmost importance for future success in trials.

Poly-GR and Poly-GA in cerebrospinal fluid as a biomarker for C9orf72-ALS

An ongoing problem in MND research has been the lack of useful biomarkers for diagnosis of the disease, tracking of disease progression and of the potential effect of clinical trials. Researchers from the University of Massachusetts, led by Dr Fen-Biao Gao, have recently shown that, at least in C9orf72-associated ALS cases, they can successfully use detection of poly-GR and Poly-GA dipeptide repeats as a biomarker in clinical trials for suppressing these toxic polypeptides.



Poly-GR and Poly-GA

In C9orf72-associated ALS, hexanucleotide repeat RNA is translated into repetitive polypeptides (very small proteins) composed of either glycine-alanine (GA), glycine-arginine (GR), glycine-proline (GP), proline-arginine (PR), or proline-alanine (PA). These dipeptide repeats are highly toxic to cells through several mechanisms and are unique to C9orf72 cases of ALS or frontotemporal dementia. As such, they are a potential biomarker for disease onset or progression.

Poly-GA and Poly GR as markers in cases of C9orf72 suppression

As these dipeptide repeats are unique to C9orf72 disease, they are also a potential target for therapeutic intervention. In this research, the authors used spinal fluid from patients treated with an antisense oligonucleotide (ASO) designed to prevent dipeptide repeats from being synthesised in cells. The researchers found that in cases where patients were treated with the ASO, they were capable of tracking the decrease in dipeptide repeats, especially for Poly-GA and Poly-GR. This is important because it means that for larger clinical trials of ASOs for C9orf72-ALS, this method could be used to measure ASO effectiveness alongside other measures of patient disease progression.

Pathological TDP-43 in axon nerve bundles

Most of us are aware that TDP-43 is the major pathological marker of ALS. Specifically, phosphorylated and mislocalised TDP-43 is strongly linked with an ALS diagnosis. Researchers from Hiroshima University, led by Dr Hirofumi Maruyama, recently published a report of their findings in post-mortem tissues and patient biopsies from ALS and control patients. This team was investigating if the presence of pathological TDP-43 in axon nerve bundles is a marker exclusive to ALS.

TDP-43 and its role in axons

Axons are the long extension of a nerve cell that leads from the cell body to the axon terminal, where axon terminals are the parts of motor neurons that connect to and direct signals into muscles. In some cases, the distance from the cell body to the axon terminal can exceed one metre, which on a scale for proteins is an extremely large distance to travel. TDP-43 is a protein that helps carry important RNAs to the axon terminals. If TDP-43 gets stuck in axons or the axon terminal, nerve cells die due to the lack of important molecules making it to where they need to be.

Post-mortem vs pre-mortem

Much of our understanding of ALS comes from post-mortem examination. This is due to the central nervous system being difficult to interrogate in living people and animals. The researchers here asked an important question as to whether they could measure pathological TDP-43 pre-mortem. This is possible due to axon terminals being found in connection with muscles, which are far more amenable to biopsy than the spinal cord.

Findings in this work

Most interestingly, the research team found that they were capable of detecting pathological TDP-43 in axon-muscle biopsies from living people. Some of these patients were not currently diagnosed with ALS via normal criteria. These patients were later diagnosed with ALS, whereas patients that showed no pathological TDP-43 in their axons were not diagnosed with ALS. This makes this method a potentially useful marker for diagnosing and following ALS progression.

Back to the dipeptide repeats and why they are toxic

It has become apparent that not all dipeptide repeats in C9orf72-ALS are equally toxic to cells. Poly-GR and Poly-PR peptides are the only dipeptide repeats that significantly decrease protein synthesis in cells. Researchers, led by Andrei Korostelev at the University of Massachusetts, have shown that poly-GR and poly-PR are capable of getting stuck in ribosomes and stopping them from working properly.

Ribosomes are the site of protein translation

The central dogma of biology is that DNA is transcribed into RNA which is followed by the translation of RNA into proteins. A key component of this in cells is the ribosome. Ribosomes are large structures composed of several different proteins which function to read RNA and facilitate the synthesis of proteins. If ribosomes stop working properly, then cells do not function properly because they can no longer make new proteins.

How do poly-PR and poly-GR affect other proteins

You might ask why only these two dipeptide repeats seem to affect ribosomes. The answer is found in their sequence. The arginine (R) amino acid in poly-PR and poly-GR is the answer. Arginine is a large and positively charged amino acid, making the poly-GR and poly-PR peptides extremely positively charged. This high level of positive charge means they are likely to interact with negatively charged proteins.

Cryo-electron microscopy shows poly-GR poly-PR in ribosomes

The authors of this study used cryo-electron microscopy (a very high-powered form of microscopy) to directly observe that the poly-PR and poly-GR peptides were strongly binding to areas on ribosomes that were negatively charged, thus blocking ribosomes from working properly. More interestingly, the authors found that erythromycin was capable of stopping the poly-PR and poly-GR from binding and restored the ribosomal function.

MND Research Shorts

Chaperone proteins exist to help cells deal with misfolded and aggregated proteins. In most cases, chaperone proteins appear to interact with specific types of misfolded or aggregated proteins. Work by Dr Iris Lindberg from the USA has shown that a chaperone called proSAAS can sequester toxic TDP-43 fragments away from the rest of the cell and is potentially protective in models of TDP-43 aggregation.

Hyperexcitability of motor neurons is one of the key markers of ALS. Hyperexcitability can occur due to influx of positive ions into neurons. Dr Richard Wade-Martins and his team from the UK have recently shown that in very young iPSC (Induced Pluripotent Stem Cells) derived from C9orf72 motor neurons, hyperexcitability is driven by calcium influx. This is important because we may be able to block calcium ion-channels selectively to alleviate disease.

Mutations in the SOD1 protein prevent correct folding of the protein and lead to its accumulation and aggregation in ALS. Dr Kay Double and her team from Australia published a very interesting article that reports misfolded SOD1 in sporadic ALS cases. They suggest that a common biochemical pathway that destabilises SOD1 may be occurring in most ALS cases.

MND patients suffer with breathing as the disease progressively worsens. Detecting problems with patient respiration earlier would lead to better outcomes for non-invasive ventilation. Dr Nathan Staff and his team carried out a test to compare methods to measure respiration to see which test could earliest detect alterations to respiration. They found that measuring overnight oximetry to detect small changes in oxygen levels was the most sensitive manner of determining early respiration issues in ALS patients.

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