# MND AUSTRALIA INTERNATIONAL RESEARCH UPDATE

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## New insight into early stages of MND, environmental toxins and more

In the first quarter of 2019 we gain crucial insight into aspects of motor neurone disease (MND) that had previously eluded some of the best minds in the world of MND research. There have been advances in our understanding of the molecular changes that occur very early in the development of MND and in strategies to make the diagnosis in these early stages.

There has also been development in our knowledge of an environmental toxin linked to MND and the microorganisms that generate it in our waterways.

Read on to find out about these and other new discoveries.

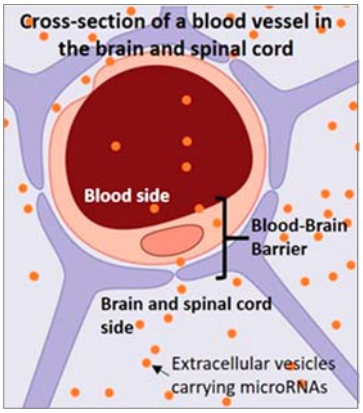
## Molecular signatures in our blood can point to MND in its early stages

The ability to diagnose a person with MND in its early stages has remained challenging for clinicians and researchers. Many of the first symptoms of MND are also seen in other conditions, resulting in high rates of mis-diagnosis until later in disease. However, advances in understanding of MND open the potential to identify molecular signatures unique to MND in easily accessible clinical specimens such as blood samples. These molecular signatures, which comprise specific amounts of naturally occurring molecules in the body, are termed biomarkers (see box below).

A class of molecules that are showing promise as clinically relevant biomarkers are microRNAs (miRNAs). These are small molecules that can regulate how much certain genes produce the proteins they code for. Daniel Saucier and co-workers in Brunswick, Canada have discovered a unique miRNA signature in the blood of people with MND. This is particularly intriguing because the identified miRNAs originated in the brain and spinal cord but entered the circulatory blood system, two systems that are usually kept separated from each other by layers of tissue that form the Blood-Brain Barrier (BBB).

Daniel and his team figured out that the miRNAs were transported across the BBB via tiny packages called extracellular vesicles. Importantly, a few of the altered miRNAs in people with MND correlated with their disease severity. Daniel's team went on to examine the target genes of the altered miRNAs and found that they are involved in functions that are defective in diseased motor neurones.

Not only has this discovery shed light on the diagnostic potential of miRNA signatures in blood, but it has also highlighted that miRNAs should be further investigated to better understand the molecular changes involved in MND.



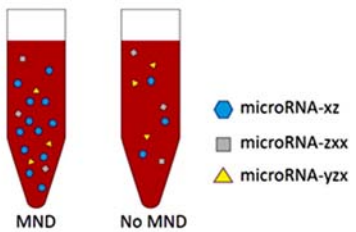

## Biomarkers: molecules in the body that signify dysfunction and disease

In different physiological states, including during disease, specific genes and molecules in the body can have a unique signature that clinicians are able to detect in samples of bodily fluids or tissues from patients. Such signatures are called biomarkers. Aside from genes, classes of molecules that have been investigated as biomarkers include ribonucleic acids (RNAs) such as microRNAs (miRNAs) and messenger RNAs (mRNAs), different proteins including enzymes and antibodies, growth factors and receptors on cells.

The discovery of biomarkers is extremely important as they provide an accurate way for clinicians to diagnose diseases even at earlier stages than the first appearance of physical symptoms. Biomarkers also shed light on the molecular mechanisms involved in the disease process and can pave the way to identifying therapeutic targets.

**Biomarkers: hypothetical example**

If several studies have shown that the levels of a biological molecule, e.g. a type of microRNA (microRNA-xz in this example) in the blood are much higher in people with MND compared to people without MND, it can potentially be used as a biomarker.




Blood samples from 2 individuals; 1 with MND, 1 without MND.

## *MND Research Shorts*

- Inflammation of motor neurones (MNs) and their surrounding support cells is commonly seen in MND. Immune system molecules called cytokines influence signals that can change inflammatory processes in the nervous system. Researchers in Finland and Belgium used a mouse model of *SOD1* -linked MND to investigate a cytokine called interleukin-33. Long-term treatment with interleukin-33 reduced MN inflammation, revealing a potential strategy to protect motor neurones in MND.
- Clumps of sticky proteins, called inclusions, are seen in all affected motor neurones in people with MND. Commonly detected in these inclusions are intermediate filaments, key structural components of the network of molecules that form the 'skeleton' of cells – the cytoskeleton. A study carried out in Ontario, Canada discovered changes in 2 miRNAs (see box) that ultimately caused dysregulation of the intermediate filaments, their aggregation into inclusions and other problems with the cytoskeleton of MNs.
- Signalling molecules called growth factors are critical for neurone development. However, several studies have now implicated a role for certain growth factors in MND. One of them, FGF-2, gained interest from researchers in Germany, Spain and the USA for its potential duality in motor neurone health. Gene activity patterns in a cellular model of *SOD1*-linked MND revealed that before there were any signs of disease, there were FGF-2- and SOD1-dependent changes in other growth factors known to be important for normal MN function. This study highlights that the roles of FGF-2 and other growth factors are likely to be important early in MND.
- Motor neurones of over 95% of people with MND contain clumps of TDP-43 and other proteins. Also unique to the MNs that are most vulnerable to MND is the production of an enzyme called MMP-9. Researchers in the USA tested whether reducing the levels of MMP-9 in the MNs in a mouse model of TDP-43-linked MND could be protective. This strategy reduced TDP-43 toxicity. However the technique they used to target MMP-9 caused premature death in some mice. Thus further refinement of the targeting strategy is needed to eliminate any harmful side effects.

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## Environmental toxins and MND

It is now widely understood that a combination of ageing, lifestyle and environmental influences, in addition to genetics, contributes to the risk of developing MND. Cyanobacteria, commonly known as blue-green algae, have gained attention as a potentially significant environmental risk factor for MND. They comprise a large class of bacteria that produce a range of toxins, 'cyanotoxins', that affect different types of cells in the body. One of the cyanotoxins, BMAA (β-methyl-N-amino-L-alanine), affects neurones and was originally implicated in neurological dysfunction on the island of Guam in the 1950s. On Guam it was identified to cause a condition called amyotrophic lateral sclerosis-Parkinsonism-Dementia (ALS-PDC) that affected the indigenous Chamorro people at an incidence of about 50-100 times more frequently than these diseases occur anywhere else in the world. Researchers investigating this phenomenon discovered that the lifestyle of the Chamorro people involved eating fruit bats and cycad seeds in which BMAA had accumulated. In the years since, studies in cellular and animal models have strengthened the link between BMAA and neurological dysfunction. However, it has remained unknown which species of cyanobacteria generate BMAA and its related neurotoxins, 2,4-DAB and AEG. Jake Violi and his colleagues at the University of Technology, Sydney sought to identify the species of cyanobacteria that produce these neurotoxins in 11 freshwater sites in eastern Australia. They isolated 19 species and revealed that 2,4-DAB was produced by all of them, AEG by 18 of them, and BMAA by 17 of them. This study highlights how important it will be to determine the full impact of these cyanotoxins on neurological health, and that there is a critical need to examine what environmental factors drive the growth of these cyanobacteria species and their production of BMAA, 2,4-DAB and AEG.

## The puzzling complexity of *C9ORF72* and dipeptide repeat proteins

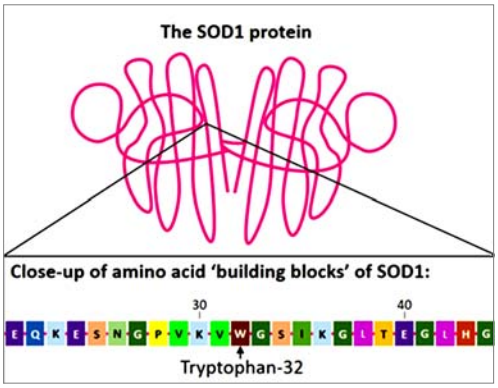
An abnormality in the *C9ORF72* gene is responsible for a major proportion of inherited cases of MND. This genetic defect causes the aberrant production of six different small molecules called dipeptide repeat proteins (DPRs). DPRs are sticky little molecules that have a strong tendency to aggregate together and form clumps, called inclusions, in cells.

There are biochemical differences between the six DPRs that can result in a diverse set of toxic outcomes for cells. Two of the DPRs, poly(GR)s and poly(PR)s, have been shown to be the most toxic types in different animal and cellular models, whereas poly(GA)s are able to form highly toxic, fibrous 'amyloid' inclusions that can spread between cells. Recent studies in cellular models have shown that more than one type of DPR can be generated within the same cell.

April Darling and colleagues in Florida, USA collaborated with researchers in Moscow, Russia, to investigate the interplay between the different DPRs. They used cultures of motor neurones modelling *C9ORF72*--linked MND and demonstrated that different combinations of each type of DPR in cells resulted in distinct changes in the structure of each DPR, where in the cell the DPRs located to, and the toxicity they caused compared to cells that only produced one type of DPR. These findings highlight that the effects of distinct combinations of the six C9ORF72 DPRs on the disease state are significant and need to be further investigated so we can better understand *C9ORF72*--linked MND.

## Key contributor to *SOD1*-linked MND identified

SOD1 is one of the most well-studied proteins associated with MND. In MND the SOD1 protein becomes misshapen, acquires toxic functions, and spreads between motor neurones. Despite the tireless research of MND scientists, the exact nature of SOD1's toxicity has been challenging to figure out. SOD1 is encoded in the genome and produced in cells across most eukaryotic organisms (eukaryotes include all animals and plants, as well as single-celled fungi such as yeast). Between different species, some of the building blocks (small molecular units called amino acids; AAs) of SOD1 and other proteins differ. This sometimes results in big differences between the same proteins produced by different species. Fascinatingly, a few years ago, researchers discovered that the 32$^{nd}$ AA of the full AA chain that makes up human SOD1, an AA called tryptophan (tryptophan-32), is unique to human SOD1 and to SOD1 in other primates. Michèle DuVal and co-workers in Edmonton and Vancouver, Canada hypothesised that tryptophan-32 is key in SOD1's acquisition of toxicity. They tested this using a zebrafish model of MN dysfunction and degeneration. They tested the effect of drugs that interfere with tryptophan-32, and the effect of substituting it with a different AA, and found that these changes reduced the toxicity of SOD1 and improved the health of the MNs. This study provides new insight into how SOD1 causes toxicity in MNs and poses a new avenue for researchers to go down in trying to target tryptophan-32.




## A new trio of molecules implicated in sporadic MND

Most people with MND do not have a family history of the disease. This form of MND is termed 'sporadic' MND (sMND). A few years ago, Grazia Maugeri and her fellow researchers in Catania and Rome, Italy classified two subgroups of people with sMND (sMND1 and sMND2) based on the activity of their genes. The activity of a person's genes can be measured by examining how much mRNA their cells produce (see box on page 1). These measurements generate a 'transcriptional profile'. Differences in transcriptional profiles between people diagnosed with a disease and people without the disease can highlight genes, proteins and molecular mechanisms that can potentially be targeted with drugs.

Grazia compared the transcriptional profiles of the people with sMND2 to that of mice modelling *SOD1*-linked familial (inherited) MND. She found 16 genes in common between people with sMND2 and *SOD1*-MND mice that exhibited changed activity compared to people and mice without MND. Grazia further studied three of these genes, *PACAP*, *EGFR* and *MMP-2*, and found that the proteins encoded by the genes interacted with each other in an intricate pathway that affected the health of model MNs.

The work of these researchers suggests that further investigation of the interaction between the PACAP, EGFR and MMP-2 proteins, and how they affect health of motor neurones, is warranted to reveal potential new drug targets for MND.

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