MND AUSTRALIA INTERNATIONAL RESEARCH UPDATE

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New models and gene therapy approaches unleashed for MND

New and improved laboratory models of MND and genetic therapies in development have made a splash this winter. The generation of human and animal models relevant to common forms of MND offer the promise of "gold standard" tools to understand the mysteries of MND and accurately predict therapeutic agents, while gene therapy is no longer an experimental approach, but is nearing clinical reality.

The age of gene therapy for MND

Hot on the heals of the recent clinical approvals of Spinraza® for childhood spinal muscular atrophy and Exondys 51™ for Duchenne muscular dystrophy, gene therapies are under intense experimental and clinical development for MND.

Three recent studies illustrate the exciting potential of different gene therapy approaches for MND (see box below). Firstly, David Schaffer and co-workers from the USA exploited "CRISPR", a powerful gene editing tool, to precisely target and disable the mutant *SOD1* gene in MND mice which conferred striking improvements in motor neurone survival and lifespan of mice. Remarkably, this was the first demonstration of CRISPR gene therapy applied to a whole animal model of neurodegenerative disease, paving the way for this technology to treat other forms of MND.

Next, Timothy Miller and colleagues from the USA have been pursuing designer DNA drugs called "antisense" for years to shut down the offending *SOD1* gene in MND. After proving this approach was safe and well tolerated in people with MND, the group recently returned to the lab bench and devised a second-generation version of antisense drug with increased potency. Not only was their new antisense drug superior when administered to animal models of MND, but this gene therapy rapidly restored muscle function and reversed blood levels of a promising biomarker for MND called "pNFH", providing compelling evidence that their antisense therapy is ready for clinical trial, slated for early next year.

Lastly, an international team, headed by Mimoun Azzouz in the UK who pioneered gene therapy approaches for MND, recently refined his viral gene therapy to exquisitely silence the *SOD1* gene using a more effective delivery mode in MND mice using clinical grade virus in readiness for potential clinical studies.

Thus, CRISPR, antisense and viral gene therapy approaches under development and nearing clinical trials in MND suggest the age of gene therapies may be near for MND.

Gene therapy 101

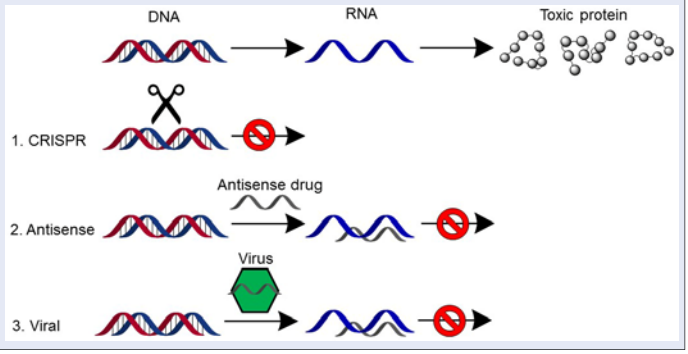
In all cells, genes made up of DNA encode a genetic message called RNA, which provides the instructions to assemble proteins which are the building blocks of life. Most mutant MND genes produce toxic proteins that are harmful to motor neurones and cause disease. Gene therapy involves manipulating DNA or RNA to shut down toxic protein production to treat or even prevent disease.

There are three main forms of gene therapy:

Genome editing which uses a breakthrough technology called "CRISPR" to specifically cut out and inactivate harmful genes.

Antisense technology which uses delivery of designer DNA drugs to "turn off" harmful genes.

Viral gene therapy which uses modified viruses as vehicles to deliver genetic material to "turn off" harmful genes.



MND Research Shorts

- *Project MinE is a large scale collaboration between 16 different countries, including Australia, that seeks to analyse the DNA profiles of at least 15,000 MND patients and 7,500 healthy controls to discover new risk factor genes for MND. Researchers from the Netherlands provided an update on Project MinE which is nearing 50% completion and has yielded new risk genes using this ground breaking international collaboration and data sharing to unravel the genetic basis of MND.*
- *Researchers in Japan have developed an innovative approach to purge toxic TDP-43 protein clumps from motor neurones. By engineering new-generation molecules called "intrabodies" which seek out toxic TDP-43 and package it for destruction within cells, they successfully eliminated TDP-43 from animal models. This evidence suggests a promising strategy for attacking TDP-43 from within motor neurones in MND.*
- *A comprehensive study from the USA provides evidence for a common set of 100 proteins which accumulate and deposit in affected tissues from animal models of MND, Alzheimer's and Parkinson's disease. This protein signature points to a common failure of protein handling and housekeeping networks within nerve cells that may point to early markers of MND.*
- *Abnormal expansions in genetic repeats, or genetic stutter, are increasingly implicated in MND. A group in Japan recently reported a potential association between abnormal genetic stutter in the ATXN80S gene, also linked to the degenerative brain disease spinocerebellar ataxia 8, and MND, increasing the spectrum of genetic repeats and MND.*

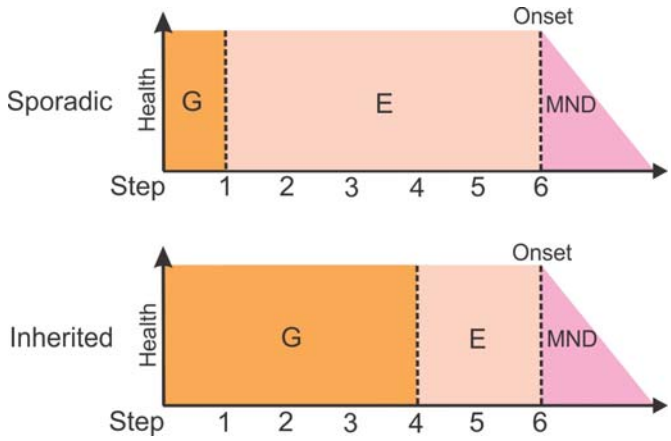
mnd Australia

Revisiting the multi-step hypothesis of MND

MND most likely results from a conspiracy of genetic, environmental, lifestyle and ageing factors. Using statistical modelling based on cancer incidence research, Ammar Al-Chalabi from the UK and his colleagues previously estimated that MND results from an average of 6 molecular steps inside motor neurones.

In a recent study, this team refined their model by focussing on families with inherited MND and determined that gene mutations reduce the number of steps required to trigger MND. Some inherited gene mutations already accounted for 4 steps needed for MND, leaving only 2 steps most likely attributed to environmental risk factors.

These results suggest that patients with inherited MND should be the focus for discovery of environmental risk factors and, encouragingly, the relatively fewer number of steps leading to MND, compared to other complex brain and psychiatric disorders, raises hope for development of effective therapies for all forms of MND.



G = genes, E = environment.

Image adapted from Al-Chalabi and Hardiman (2013) Nat Rev Neurol 9:617-628.

A GReat model of ALS/FTD

Mutations in the *C9orf72* gene are the most common genetic cause of MND and associated frontotemporal dementia (FTD). Genetic defects in *C9orf72* are proposed to result in production of different toxic proteins called dipeptide repeats (DPRs) which clump together and poison motor neurones.

Genetically engineered mice carrying human *C9orf72* mutations have been created for research, however it remains unclear which DPR is important to trigger MND.

To single out the culprit DPR, Leonard Putrucelli and colleagues from the USA recently generated new mice carrying a DPR called "polyGR". These polyGR mice developed motor deficits, memory problems and brain degeneration, closely mimicking MND/FTD. In addition, these mice showed evidence for accumulation of toxic polyGR with protein synthesis machinery in nerve cells, strengthening evidence that defective *C9orf72* disrupts protein synthesis, leading to motor neurone demise in MND.

These mice represent an important leap in the field, implicating toxic polyGR specifically in MND/FTD, while highlighting protein synthesis as a potential therapeutic target for these disorders.

A human model for sporadic MND, finally!

The majority of people with MND have no family history of the disease which strikes "out of the blue". This so-called sporadic MND has no defined causes, therefore it has been challenging to reproduce in the laboratory. Researchers have instead relied on models of inherited MND which have delivered no effective treatments for patients to date, in the absence of relevant sporadic MND models.

Hideyuki Okano and co-workers in Japan have taken up this challenge and used advanced stem cell technology to generate motor neurones "in the dish" from dozens of patients with sporadic MND. Remarkably, these motor neurones recapitulated key disease features and signatures of MND, including accumulation of abnormal protein deposits, pruning of nerve cell connections and cell death.

This study is the largest scale effort to model and reproduce sporadic MND pathology using a human system in the laboratory to date.

Further studies using larger and more diverse cohorts of sporadic MND patients are eagerly anticipated.

Turning up T-cells to turn down MND

Inflammation in the nervous system is a prominent feature of MND and significantly contributes to motor neurone damage and disease progression. It is well known that the circulating immune system, particularly types of white blood cells, or T-cells, can invade the nervous system and influence inflammation.

An Australian team led by Brad Turner and Steve Vucic has exploited this natural process by chronically treating a mouse model of MND with a drug called "IL-2". IL-2 treatment significantly ramped up circulating T-cells which flooded into the spinal cord and dampened harmful inflammation from "microglia", cells which damage motor neurons. Importantly, boosting T-cells protected motor neurones and prolonged lifespan in MND mice. In parallel, T-cell numbers studied in MND patients correlated with better prognosis, strengthening evidence for a protective role of T-cells in MND.

Excitingly, these studies form a strong basis for two current clinical trials in MND testing the effects of IL-2 in the European MIROCALS trial, and Tecfidera in the Australian TEALS trial, both aimed at boosting and harnessing protective immune cells to slow the progression of MND.

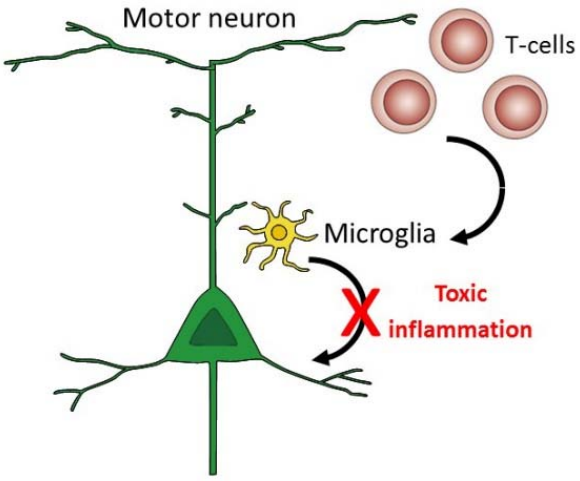


Image adapted from Dr Rosie Clark, University of Tasmania

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