Environmental factors and motor neurone disease

A/Prof Ken Rodgers
School of Life Sciences
The University of Technology Sydney, Australia
ALS/MND was recognised as a medical condition almost 150 years ago. What have we done in 150 years?

- We know that 10% of MND cases are familial and have discovered around 30 mutations in 10 genes

- We know that 90% of MND cases are sporadic and the causes are unknown

- Environmental risk factors are still largely a mystery
Environmental factors and MND

- Why do we know so little about them?
  - It’s complicated
    - A huge number of factors could be involved
    - There could be a critical time of exposure
    - Exposure to multiple factors could be required
  - It’s not treated as a research priority

Examples include: Endogenous viruses, diet, smoking, drinking, stress, fatigue, mobile phones, military service, welding, agricultural activity, flying aircraft, playing soccer, air pollution, electrical trauma, head injury, exposure to: electromagnetic fields, pesticides, herbicides, lead, mercury, arsenic, aluminium and algal blooms.
Focus of MND research

Environmental causes are being ignored at major conferences like this and research in this area is not being funded properly even although they could be responsible for causing the disease in 90% of MND cases!

The 27th International Symposium on ALS/MND will focus on cutting-edge research aimed at identifying and describing ALS/MND associated genes and gene networks, their functional relevance and the translation of this knowledge for the development of novel therapeutics.” Jochen Prehn
How can we identify risk factors?

- Compare the incidence of MND in two populations

- Observational studies examining specific environmental factors are usually small often show trends but can be inconclusive or sometimes contradictory

- Meta-analysis can be carried out by combining data from many smaller observational studies to increase the power of the data and provide more reliable answers
Exposure to heavy metals

Wang et al., Identification of risk factors associated with onset and progression of amyotrophic lateral sclerosis using systematic review and meta-analysis. Neurotoxicology 2016 (available online)
Exposure to pesticides and agricultural chemicals

Wang et al. Identification of risk factors associated with onset and progression of amyotrophic lateral sclerosis using systematic review and meta-analysis. Neurotoxicology 2016 (available online)
Interaction between susceptibility and environmental factors

- Even although the incidence was increased not everyone who was exposed to organophosphate pesticides developed MND.

- ALS/MND mortality in a pesticide factory however was reported to be 3 fold that of the general population (Burns, 2001).

- There was a higher incidence of mutations in the PON1 gene in Italian ALS patients than the general population (Ticozzi, 2010).

- Everyone might be exposed to a risk factor but only some people are susceptible due to their genetic background.
Environmental factors and MND

MND is multifactorial

Environmental factors are the thing that you can change - we could avoid them if only we knew what they were!

modifiable environmental factors + unmodifiable genetic factors
A study is now underway (with Prof Gilles Guillemin and Prof Dominic Rowe) in which we will measure levels of PEPs in blood samples from MND patients and controls (MQ Biobank).

A recently published study found a significant correlation between blood levels of certain PEPs and MND in Michigan (Su, 2016).
Insecticides and Mitocides in NSW

**Insecticides:**
Methomyl: A carbamate \((E,Z)\)-methyl \(N\)-\{[(methylamino)carbonyl]oxy\}ethanimidothioate
Phosphine (phosphane) \(PH_3\) \((P_2H_4)\). Carbaryl (1-naphthyl methylcarbamate
Organophosphate insecticides
Omethoate: Organophosphate 2-\{[(Dimethoxyphosphoryl)sulfanyl]-\(N\)-methyl-acetamide
Phorate: Organophosphate pesticide (o,o-Diethyl s-ethyamino) methyl phosphorodithioate
Dimethoate: Organophosphate. O,O-dimethyl \(S\)-[2-(methylamino)-2-oxoethyl] dithiophosphate
Chlorpyriphos: (O,O-diethyl O-3,5,6-trichloropyridin-2-yl phosphorothioate) Organophosphate.
Pyrethroid insecticides
Fipronil: A phenylpyrazole CNS toxin. WHO class II moderately hazardous.
Bifenthrin: A pyrethroid CNS toxin.
Cypermethrin: A pyrethroid CNS toxin.
Deltamethrin: A pyrethroid CNS toxin
Permethrin: A pyrethroid \((\pm)\)-3-Phenoxybenzyl 3-(2,2-dichlorovinyl)-2,2-.

**Mitocides:**
Omethoate: An organophosphate anticholinesterase
\((O,O\text{-dimethyl}\ S\text{-methylcarbamoylmethyl phosphorothioate})
Bifenthrin: A pyrethroid CNS toxin.
Abamectin:
# Herbicides and Fungicides in NSW

## Herbicides:
- Monosodium methyl arsenate (MSMA)
- Paraquat
- Organochlorides (for control of broadleaf weeds)
- MCPA (2-methyl-4-chlorophenoxyacetic acid)
- 2,4-D: (2,4-Dichlorophenoxyacetic acid).
- Dicamba: is 3, 6-dichloro-2-methoxybenzoic acid

## Fungicides:
- Tebuconazole: A triazole fungicide (4-Chlorophenyl)- 4,4-dimethyl-3-(1H, 1,2,4-triazol-1-ylmethyl)pentan- 3-ol)
- Iporodione: An organochloride fungicide (3-(3,5-Dichlorophenyl)-N-isopropyl-2,4-dioxoimidazolidine-1-carboxamide)
- Metalaxyl-M: An acylamino acid/anilide (methyl N-(methoxyacetyl)-N-2,6-xylyl-D-alaninate)
- Propiconazole: A triazole (1-[2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl]methyl]-1,2,4-triazole
- Captan: A phthalimide (3aR,7aS)-2-[(trichloromethyl)sulfanyl]-3a,4,7,7a-tetrahydro-1H-isoindole-1,3(2H)-dione
- Metal containing fungicides
  - Maneb: polymeric complex of manganese
  - Zineb: polymeric complex of zinc
  - Mancozeb: combination of Maneb and Zineb
  - Copper: 500g/kg present as cupric hydroxide
  - Copper: Present as oxychloride
  - Copper: Present as oxide
- Ziram: Zinc; N,N-dimethylcarbamodithioate

We are constantly being exposed to a lot of chemicals. In many cases their toxicity has not been well studied.
Cyanobacteria (blue-green algae) and MND

The story of an unusual disorder on the Pacific island of Guam
Discovery of Guam by Magellan

1521 Ferdinand Magellan

A high incidence of amyotrophic lateral sclerosis Parkinson’s dementia complex (ALS-PDC) was reported by US naval doctors. It was 100x higher than anywhere in the world!
ALS-PDC on Guam – 100 fold increase

The disease complex was related to lifestyle not confined to genetic background

Environmental factors were involved

Cyanobacteria are often in the algal blooms that you see on lakes and rivers and are even in the ocean

BMAA

β-methylamino-L-alanine
Evidence that BMAA is linked to MND

- Two primate studies (Cox, vervet 2015 and Spencer macaque 1987) show behavioral or histological changes consistent with a neurodegeneration

- Hot-spots (clusters) of MND were linked to exposure to BMAA in France (mussels), USA (lakes)

- Potential mechanisms identified (Acute vs Chronic)
  - Protein misfolding and ER stress (Rodgers, Okle)
  - Excitotoxicity (Weiss, Lobner)
  - Metal binding (Nunn, Weiss)
Detecting BMAA in Australia

- LC-MS/MS using an Agilent 6490 QQQ with UPLC
  - Retention time
  - Parent ion mass
  - Product ion mass & ratio

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<th>Isomer</th>
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<th>Product Mass</th>
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Four strains of lab cultured Australian cyanobacteria tested

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<tr>
<th>Species</th>
<th>Location of isolation</th>
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<td><em>Microcystis aeruginosa</em></td>
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<td>Yes</td>
<td>Yes</td>
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BMAA and DAB are present in Australian cyanobacteria and in cycad seed from Central Australia.
Human neuroblastoma cells (SH-SY5Y) were treated with sub-lethal doses BMAA alone (500µM), or BMAA plus DAB (500µM + 500µM) for 48 hours.
We show for the first time in these studies using human neuron-like cells that many pathways triggered by the toxins produced by blue-green algae (BMAA and DAB in combination) can be linked to MND/ALS.
The gene-time-environment (GTE) hypothesis for MND

- Genetic susceptibility, age and multiple environmental exposures, each contribute to risk and increase the burden of ‘disease causing factors’ that can precipitate disease once a threshold level is reached.

The gene-time-environment (GTE) hypothesis for MND

- Applying a multistep model to five ALS population registers in Europe the data supported the hypothesis that a process requiring six distinct steps leads to the onset of amyotrophic lateral sclerosis.

- ‘A linear relationship between the log incidence and log age of onset is consistent with a multistage model of disease. The slope estimate suggests a six-step process’

Take home message: An understanding of the environmental contribution to MND is essential since it is the only easily modifiable component of the overall risk.

You can donate directly to this research at [https://www.giving.uts.edu.au/donate](https://www.giving.uts.edu.au/donate) look for MND/algae on the pull down menu (red arrow below). We pledge that 100% of the donation goes to research (there are no administration costs).
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- Dr Lee Bowling (UTS)
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