KING'S HEALTH PARTNERS

An Academic Health Sciences Centre for London

Pioneering better health for all

Whither to The Creeping Paralysis?

Progress on the road to curing motor neuron disease

Christopher Shaw

Overview of presentation

Making the diagnosis of MND
- Who gets MND?
- What are the symptoms and signs?
- What treatments can we give?

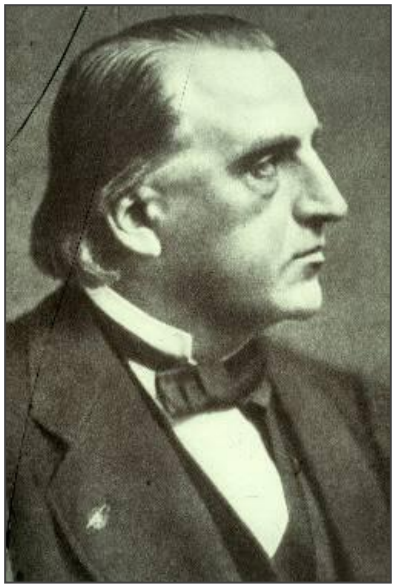
The pathology of MND
- Loss of motor neurons
- Accumulation of toxic proteins

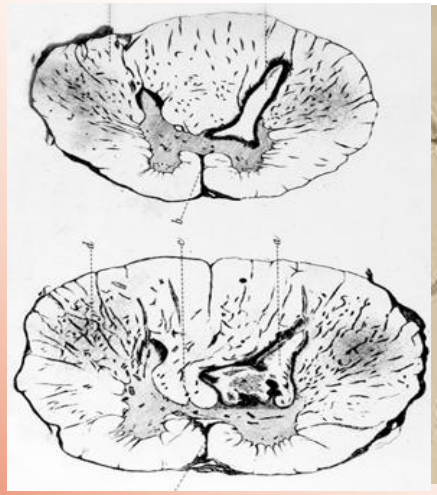
The genetics of MND
- Impact of recent genetic discoveries
- TAR DNA Binding protein (TDP-43)
- Fused in Sarcoma (FUS)

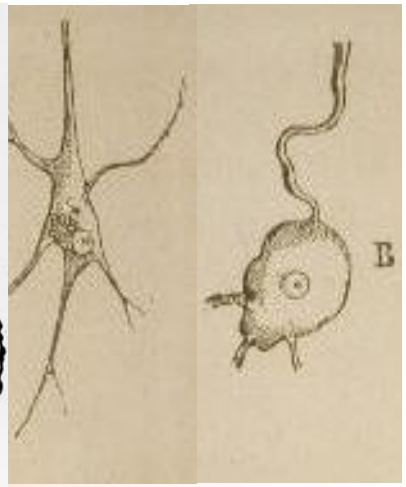
Using genetics to model MND
- Cellular models
- Animal models

Progress towards finding a cure
- A personal message

What is in a name: motor neuron disease







Jean-Martin Charcot 1869
First to identify MND as a distinct disease

Demonstrated upper and lower motor neuron degeneration

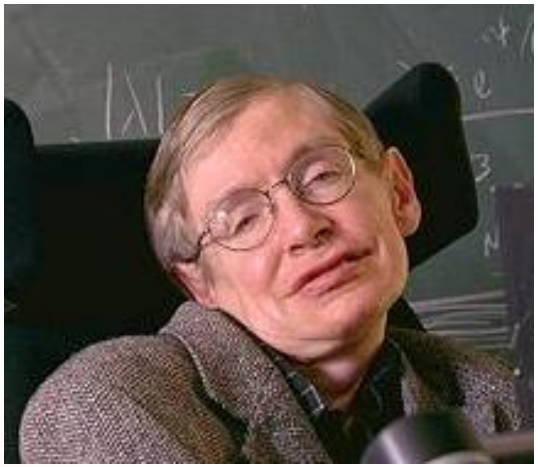
Named “Amyotrophic Lateral Sclerosis”

“Motor Neuron Disease” by Lord Brain

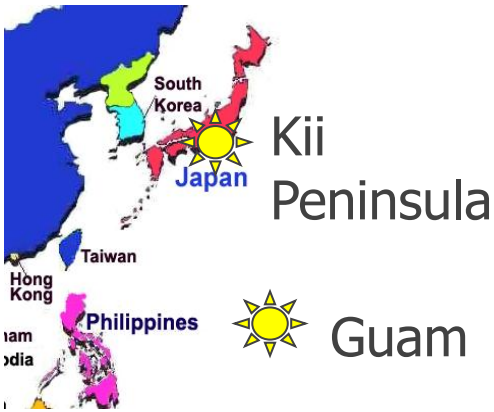
“Lou Gehrig’s disease” in USA

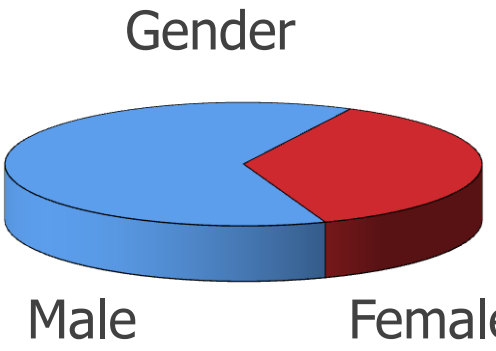
“The creeping paralysis” colloquial term

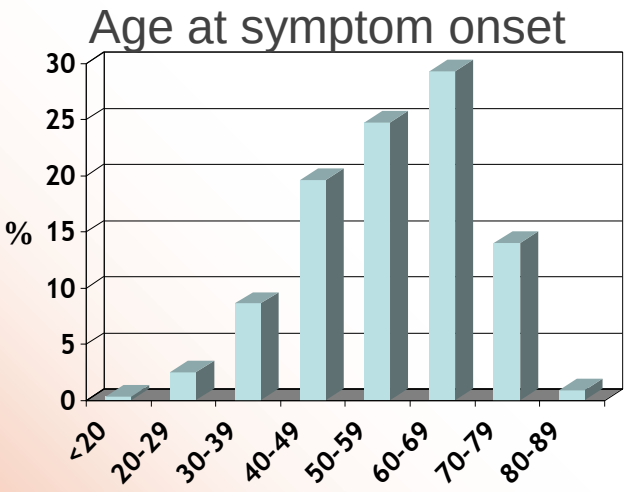
Famous faces linked to Motor Neuron Disease

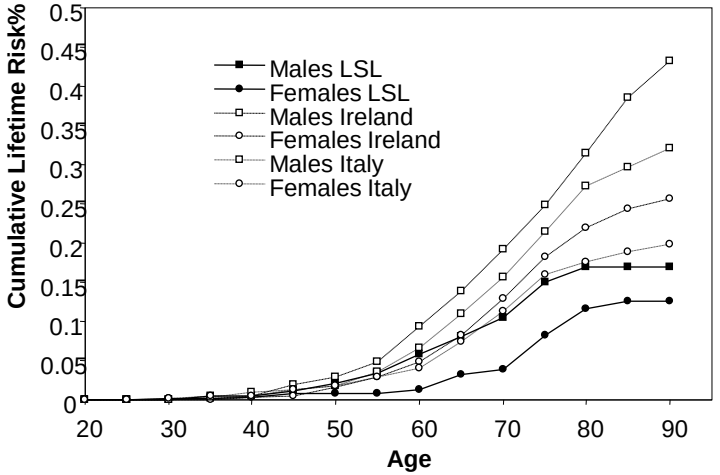


Motor neuron disease is a global problem









5,000 in the UK and 300,000 people world-wide live with MND

Clinical symptoms of MND

Onset

Limbs	weakness of grip, decreased dexterity foot drop, leg stiffness and tripping
Throat	slurred speech, difficulty chewing or swallowing

Progression

Limbs	unable to hold objects, write, feed, or toilet unable to walk, stand, or turn over in bed
Throat	unable to speak, swallow food or saliva
Breathing	breathless with exertion or lying flat
Cognition	dementia is rare but subtle deficits are common

Relentlessly progressive, accumulation of disability, next month worse than the last

Death due to respiratory failure within an average of 22 months from diagnosis

MND/ALS, although rare is the most common reason people seek euthanasia

Clinical signs of MND

Symptoms of progressive motor neuron dysfunction in limb's, throat

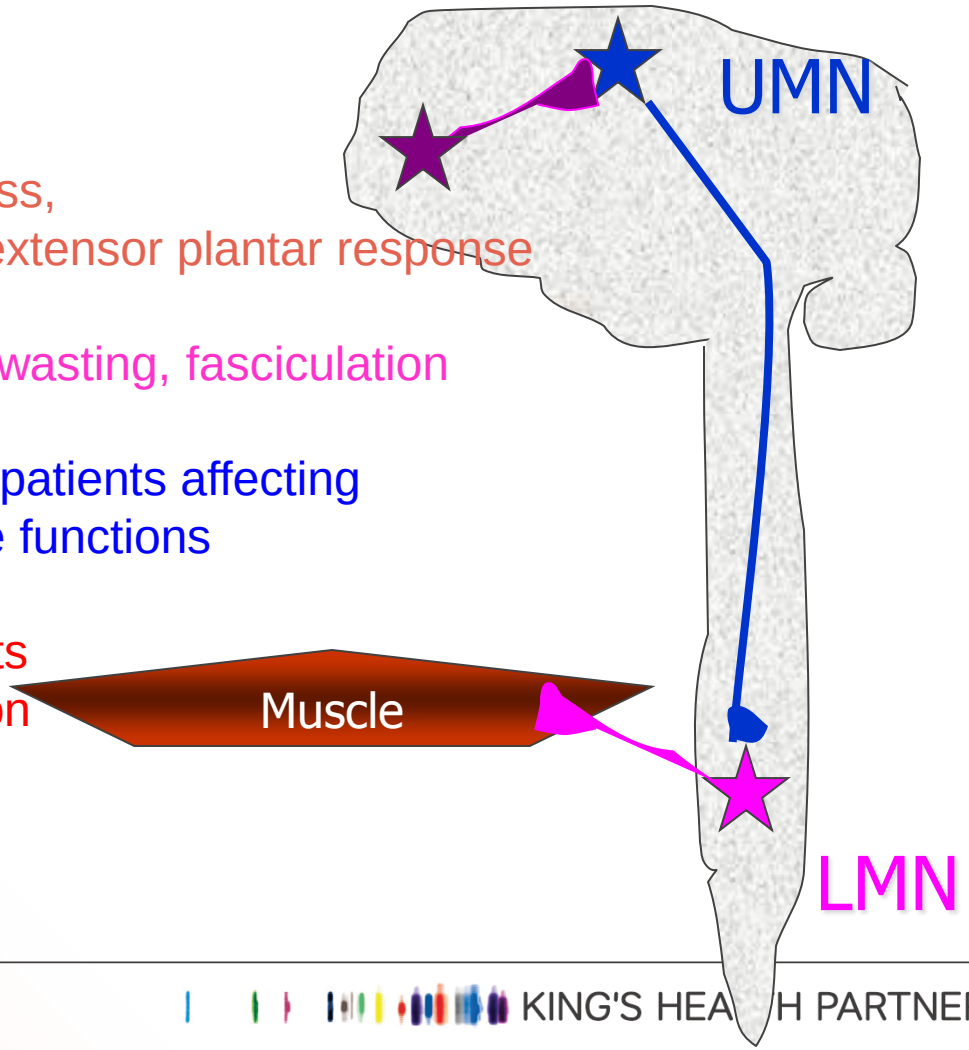
Pre-Motor — Planning and initiation of movements are difficult

UMN — modest weakness, stiffness, spasticity, hyperreflexia, extensor plantar response

LMN — major weakness, muscle wasting, fasciculation

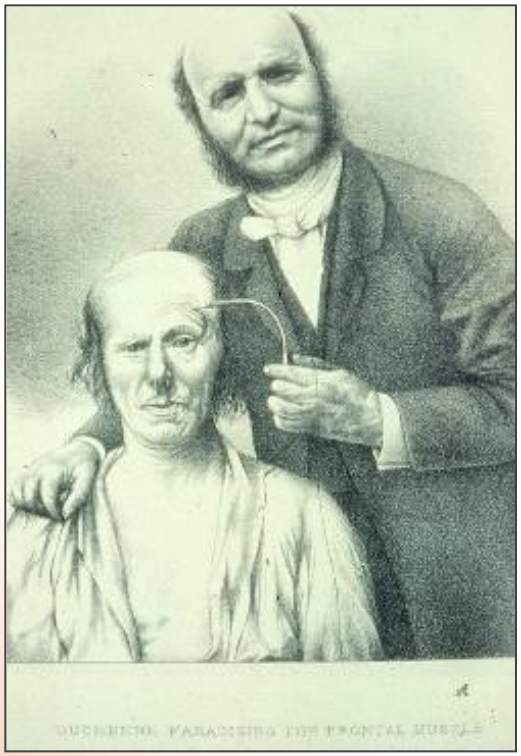
Cognition — subtle deficits in 30% of patients affecting verbal fluency, executive functions

Spares — sensation, eye movements bladder and bowel function

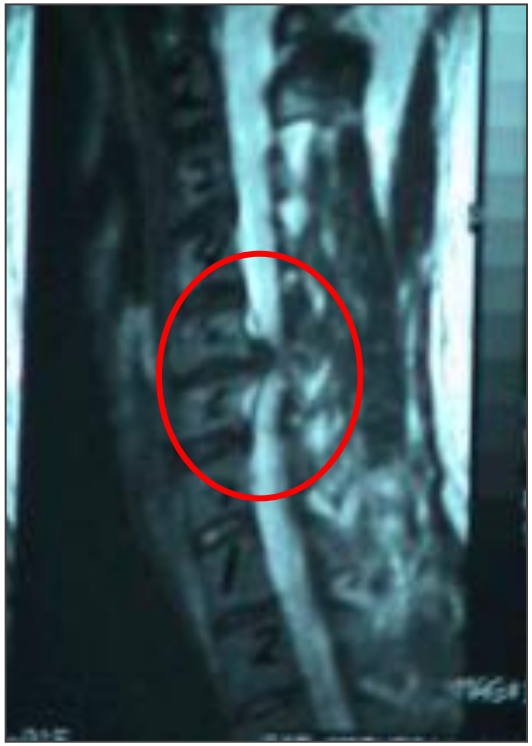


Diagnostic tests are to exclude MND mimics

Neurophysiology



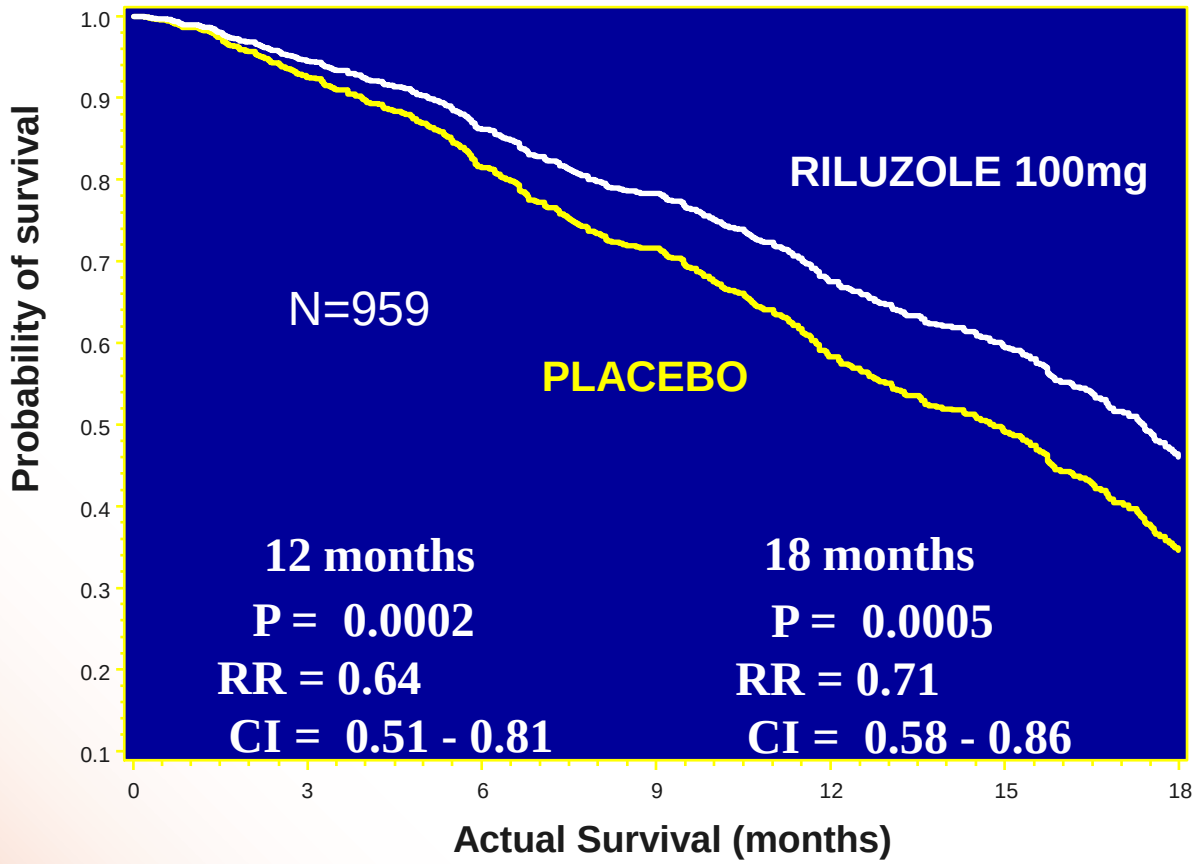
Neuroimaging



Blood Tests

There is no single or simple diagnostic test for MND. The diagnosis is based on the exclusion of other conditions that can mimic MND and good clinical judgement. The diagnosis is made on average 12 months after symptom onset

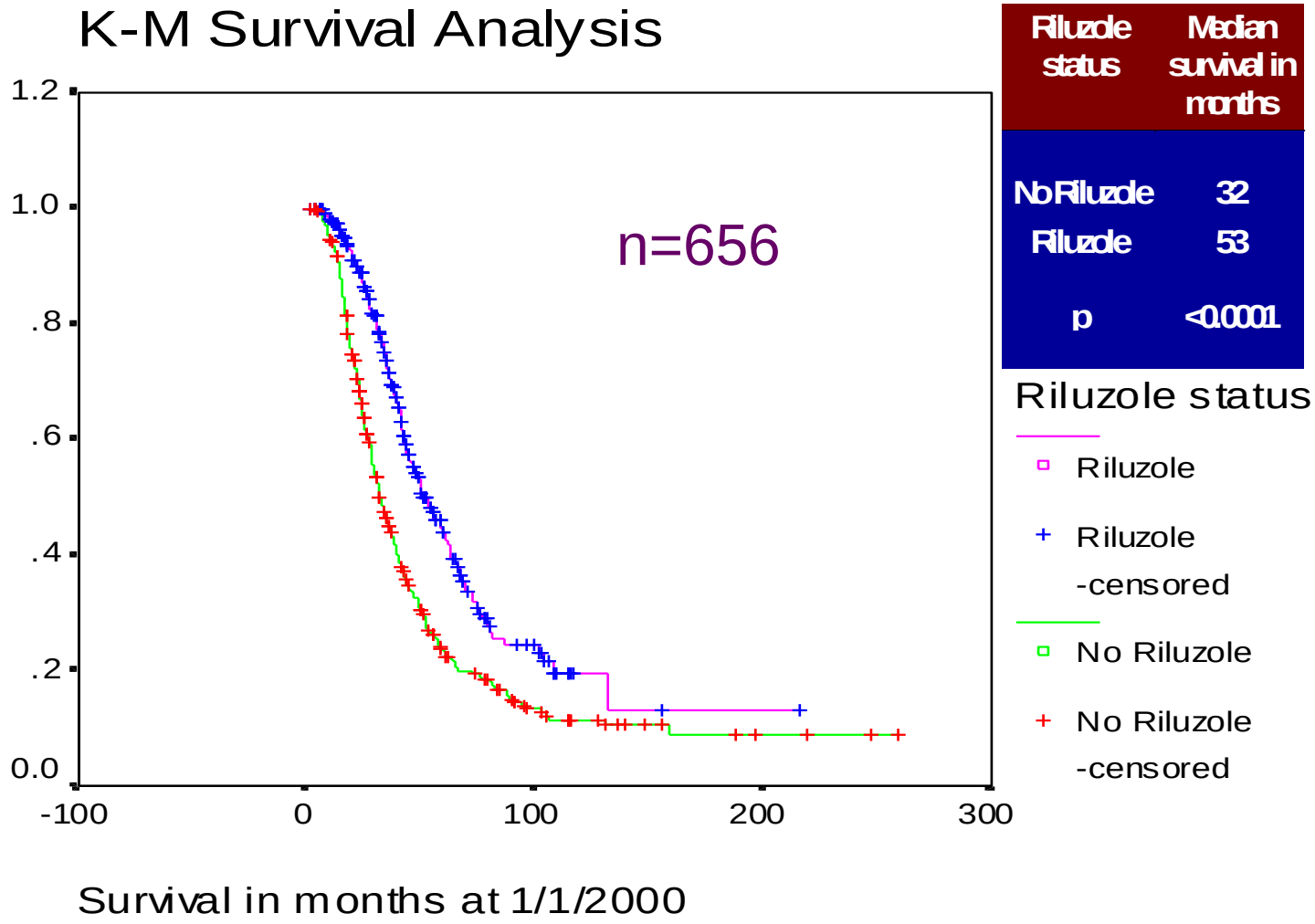
>100 drugs tested, only one works, riluzole



Riluzole increased life expectancy by 3 months over an 18month trial

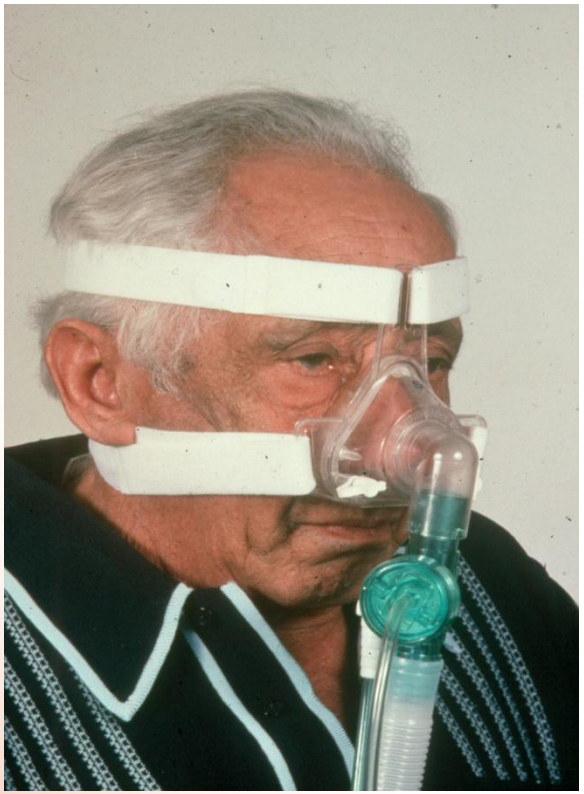
G. Bensimon, L. Lacomblez, N. Leigh, V. Meininger

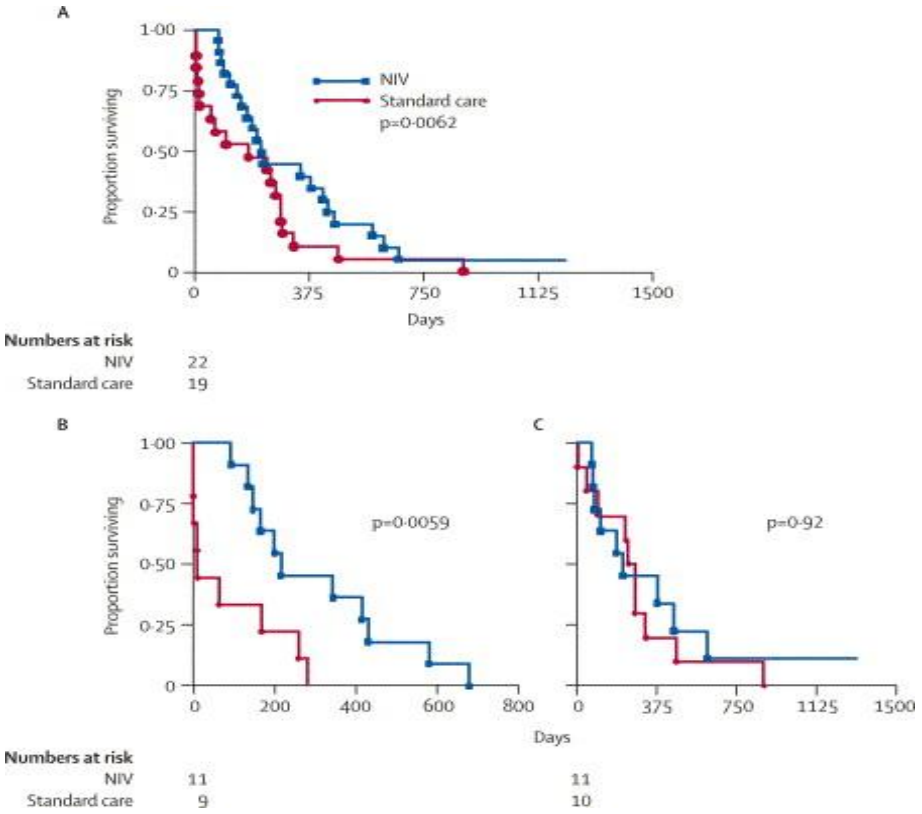
Riluzole improves survival in a clinic population



Riluzole status	Median survival in months
No Riluzole	32
Riluzole	53
p	<0.0001

Non-invasive ventilation improves survival

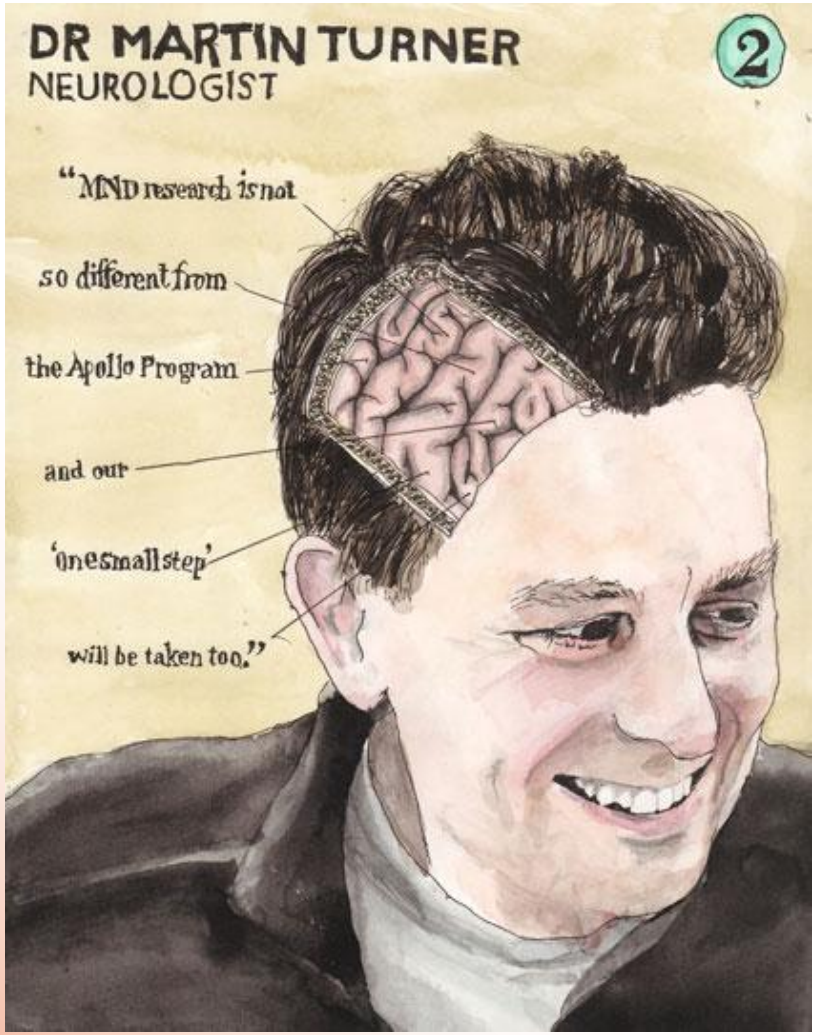


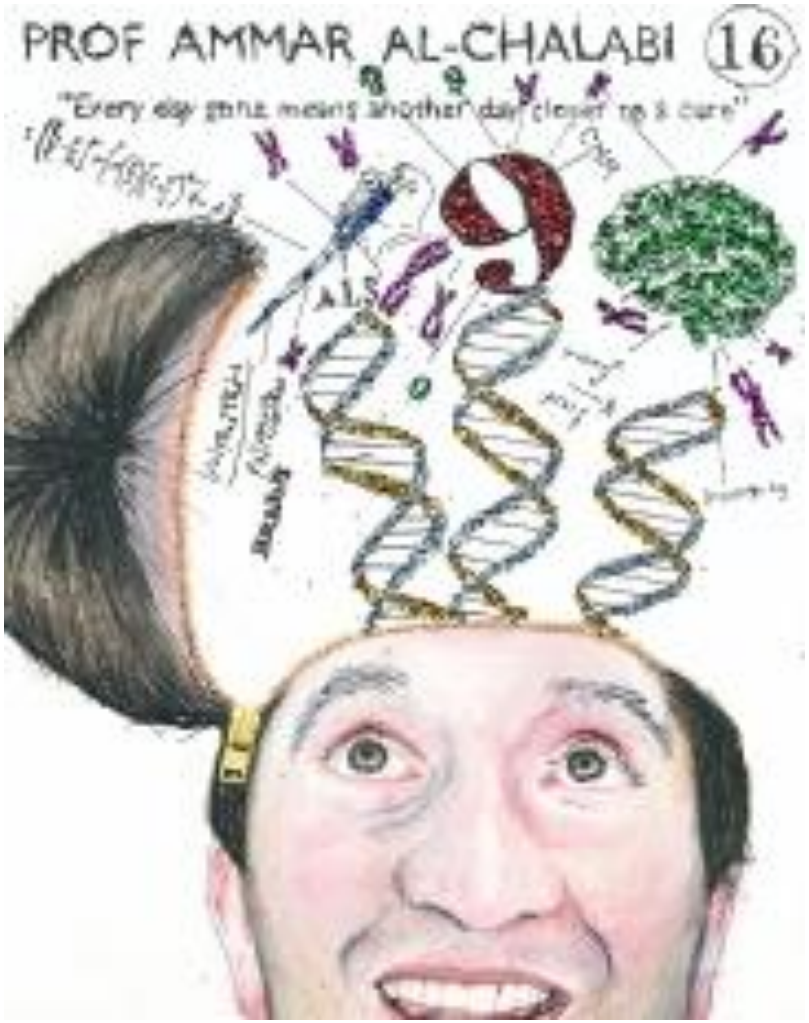


Non-invasive ventilation improves survival, particularly if MND starts in the limbs

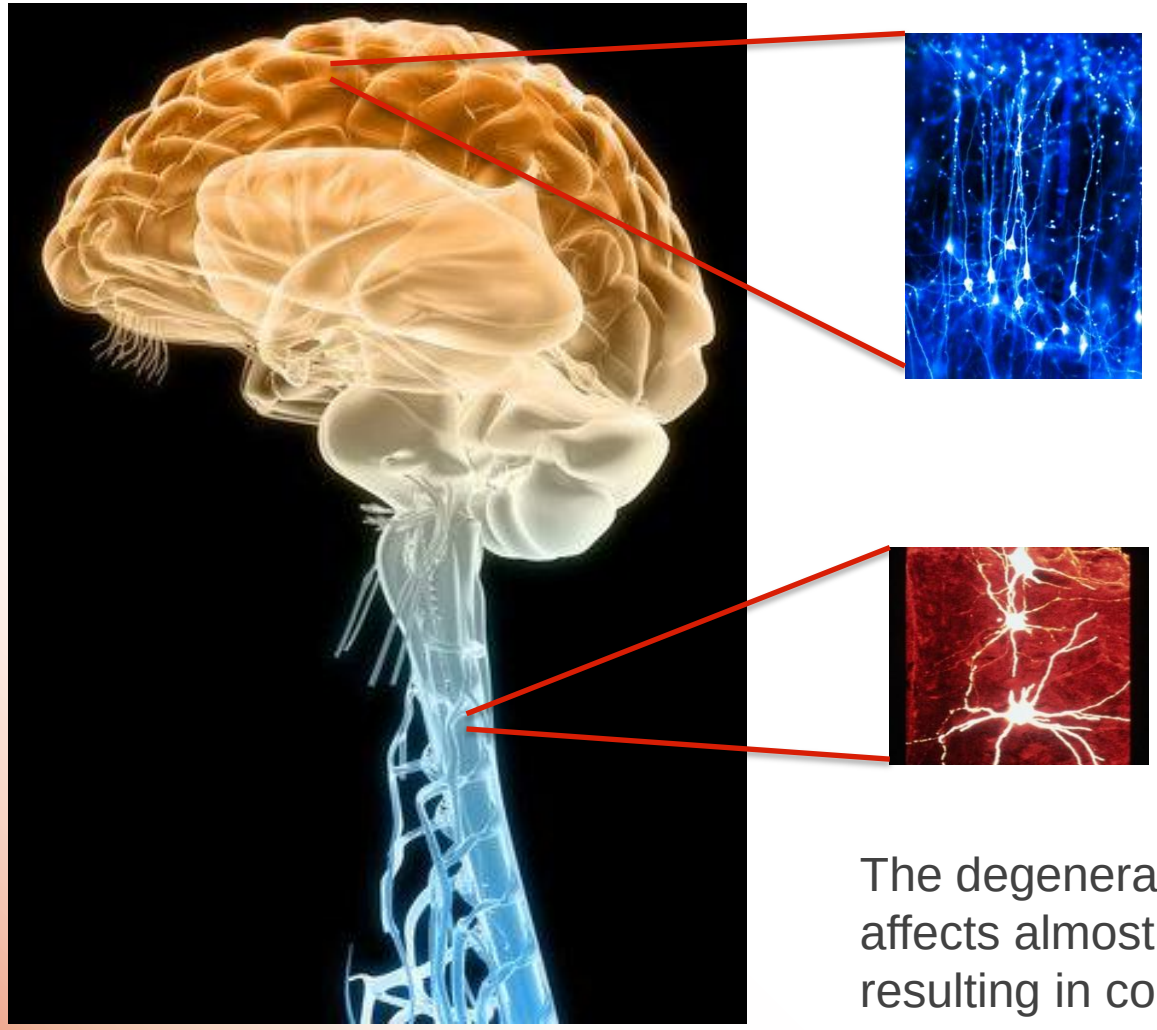
More importantly it improves quality of life by reducing symptoms of CO_2 retention

What is going on in the brain that causes MND?





Why do people with MND develop paralysis?

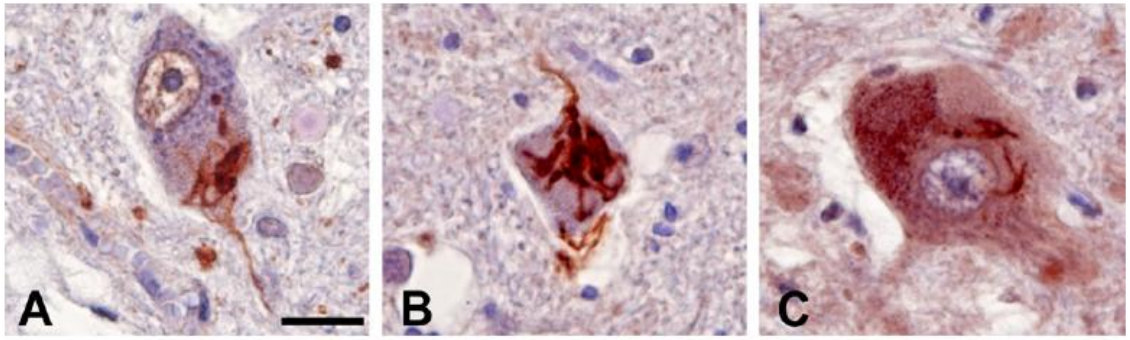


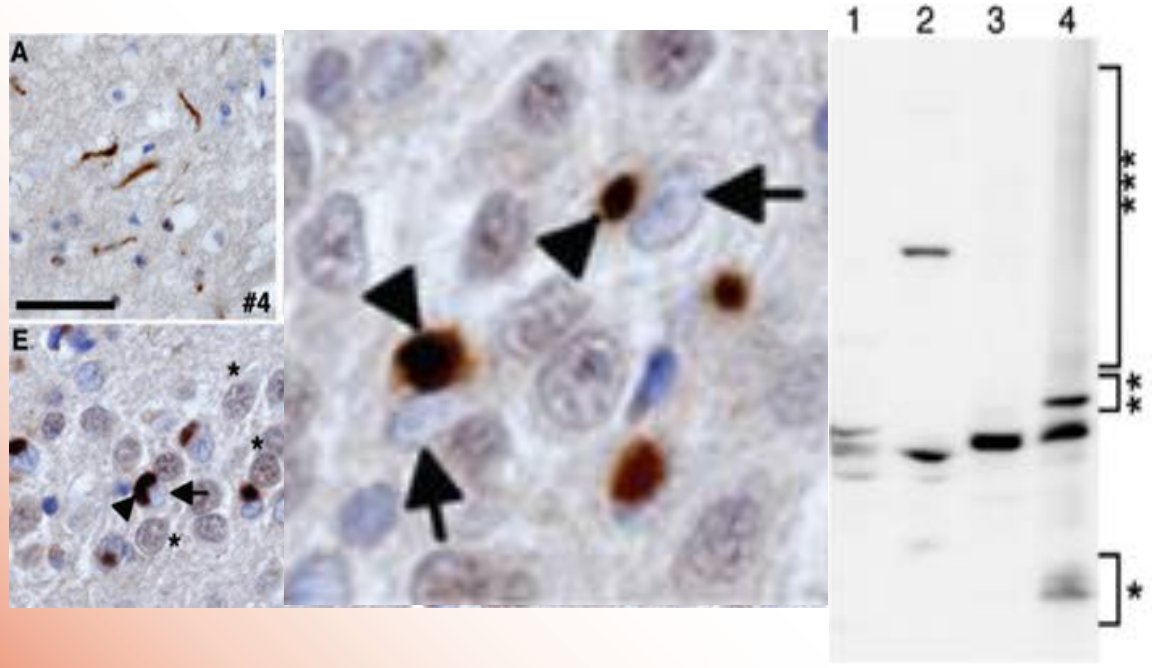
Pyramidal motor neurons in the frontal lobe degenerate and die causing severe spasticity and mild weakness of muscle groups

Motor neurons in the spinal cord degenerate and die causing wasting and major weakness of muscle groups

The degenerative process spreads until it affects almost all motor neurons eventually resulting in complete muscular paralysis

Why do motor neurons degenerate?





TDP-43 protein accumulates in the cell body of motor neurons in 95% of all MND cases.

Normally TDP-43 resides in the nucleus where it processes gene transcripts

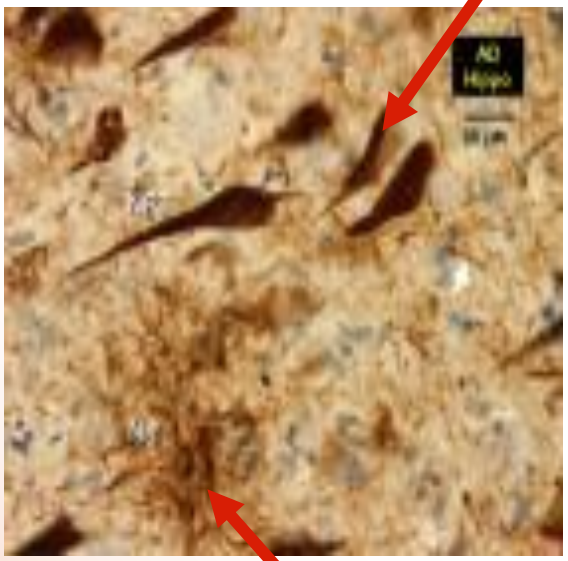
TDP-43 protein also accumulates in cortical neurons in 60% of people with fronto-temporal dementia.

TDP-43 protein is sticky and it fragments to form detergent resistant aggregates

MND similar to other neurodegenerative diseases

Alzheimer's Disease

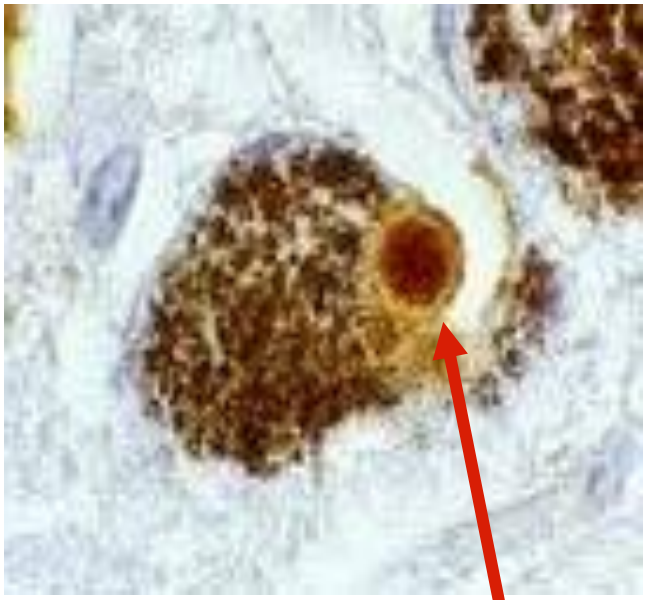
Tau tangles



Amyloid plaque

Cortical neurons in the brain

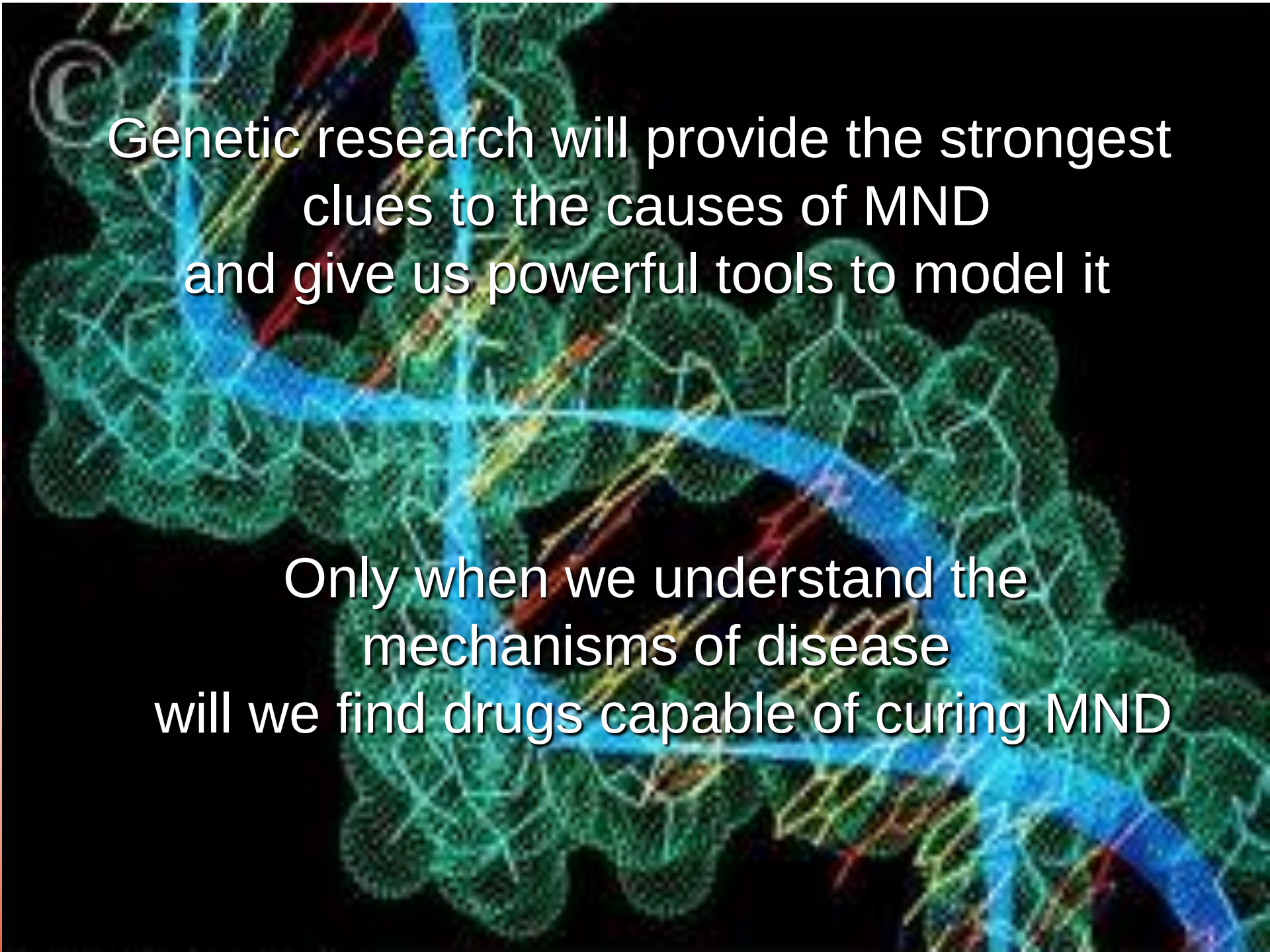
Parkinson's Disease



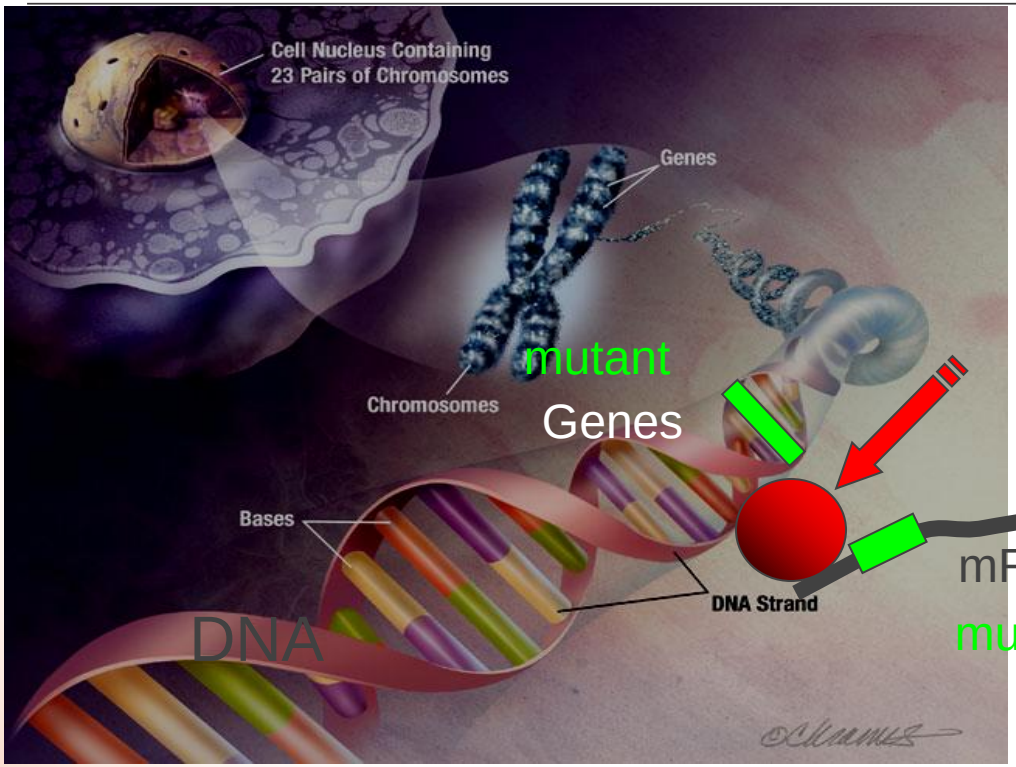
Lewy body

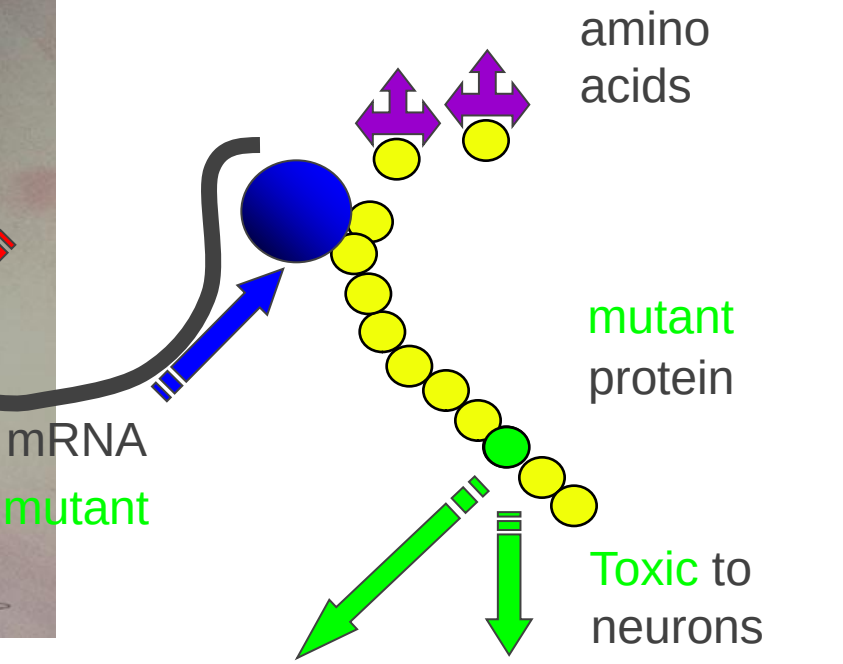
Pigmented neurons in the brainstem

Protein aggregation is common to all neurodegenerative disorders

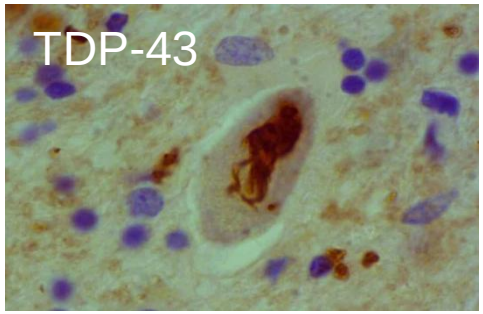

Genetic research will provide the strongest
clues to the causes of MND
and give us powerful tools to model it
Only when we understand the
mechanisms of disease
will we find drugs capable of curing MND

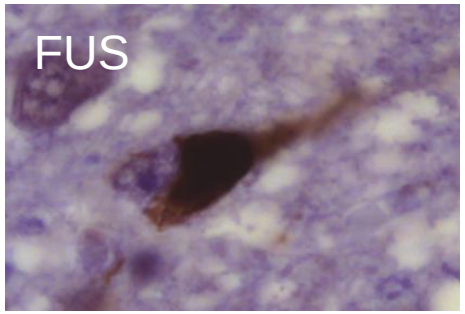
Chromosomes, genes, RNA, proteins and MND



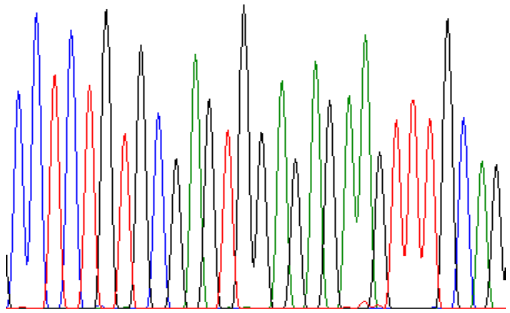


Mutant or damaged proteins accumulate inside motor neurons initiating their degeneration

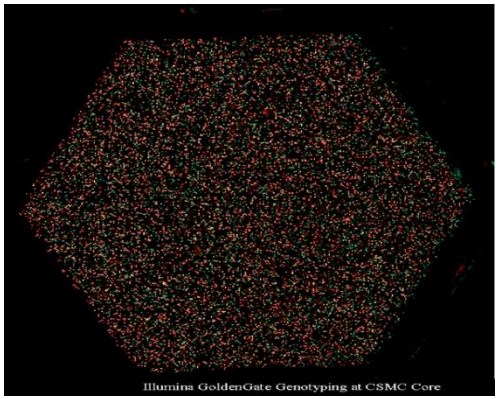




A revolution in DNA sequencing will transform genetics



2000
Sanger sequencing
ABI 3100
1 persons genome
15 years
£200 Million

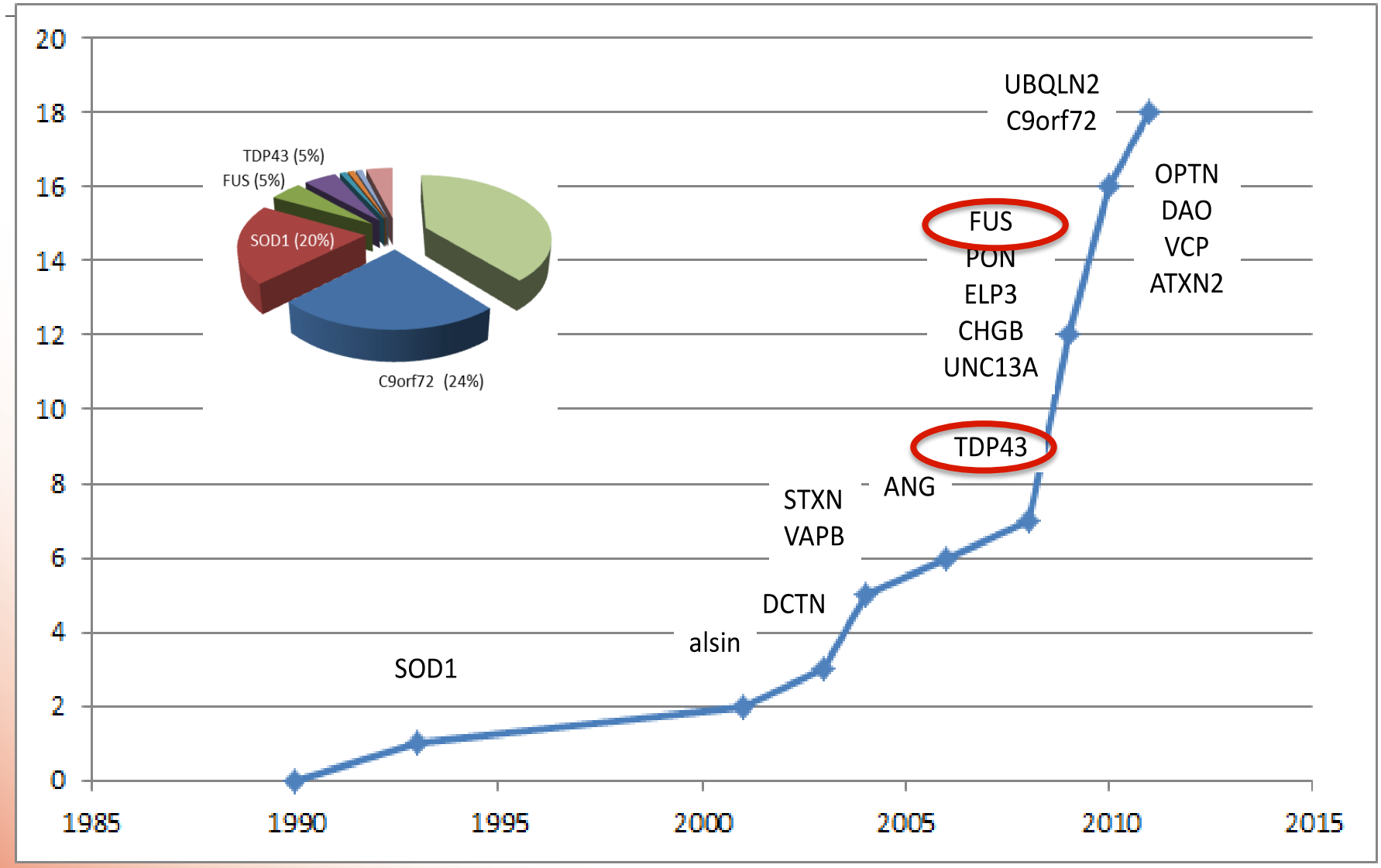


2012
Sequencing by synthesis
Illumina HiSeq 2000
1 persons genome
7 days
£2,000

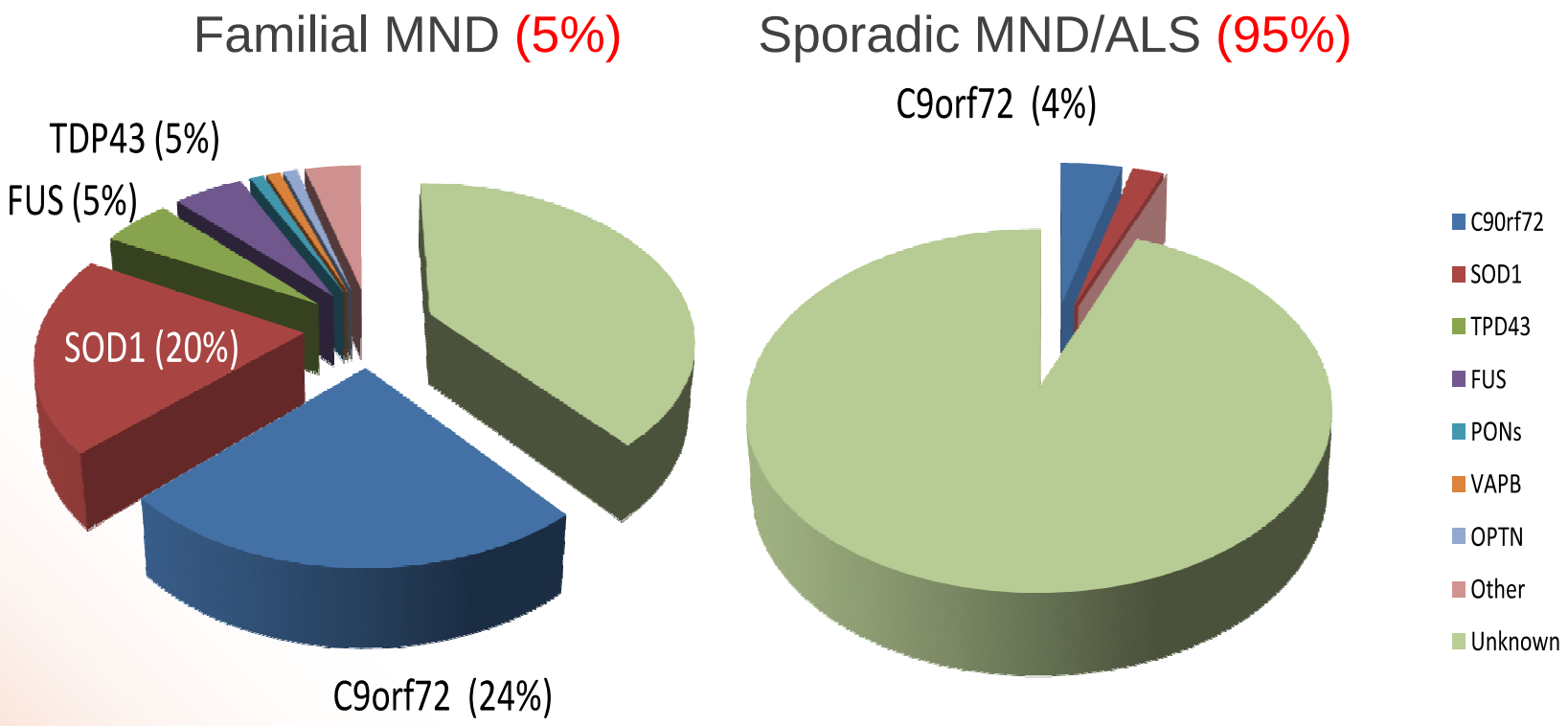
Sequencing DNA is now 1,000 times faster and 100,000 times cheaper

New sequencing technologies have the power to identify all MND genes

The rate of MND/ALS gene discovery is increasing



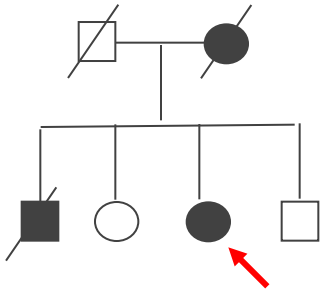
Proportion of MND patients with known gene defects

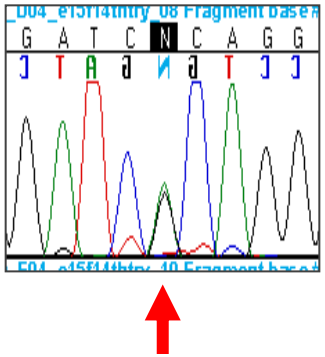


The genes for 50% of familial and 5% of sporadic MND/ALS are known and can be offered for diagnostic and predictive testing in patients

How does gene testing help MND patients?

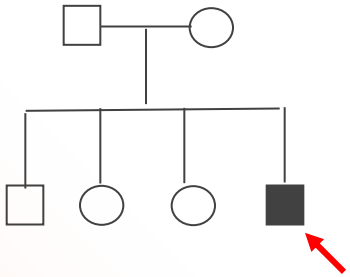
Familial MND

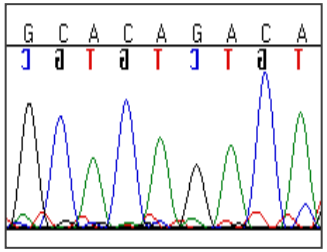




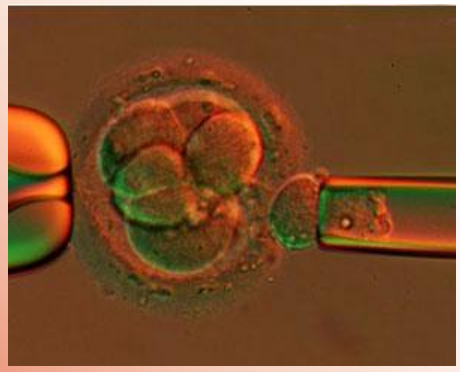
Gene testing can go some way to answering why the disease occurred

Sporadic MND





Excluding the presence of gene mutations can be greatly reassuring

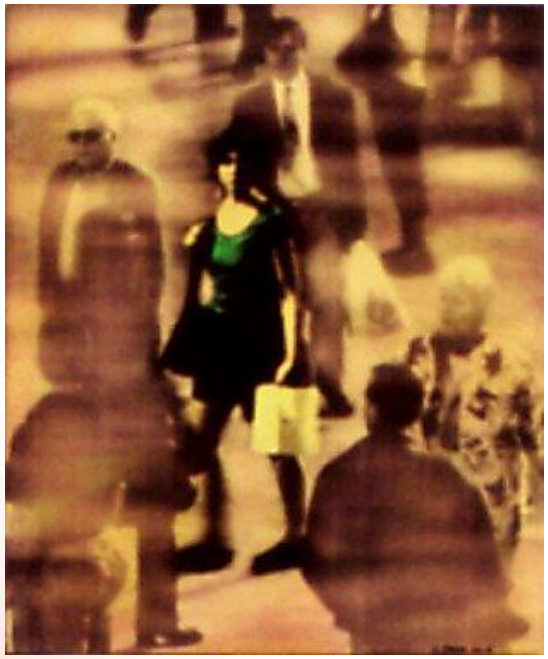


IVF and gene testing

Defective genes can be prevented from recurring in future generations

Why do we focus on familial MND genes?

Population effect of FMND is small



Only one in ten people with MND have a family history of MND

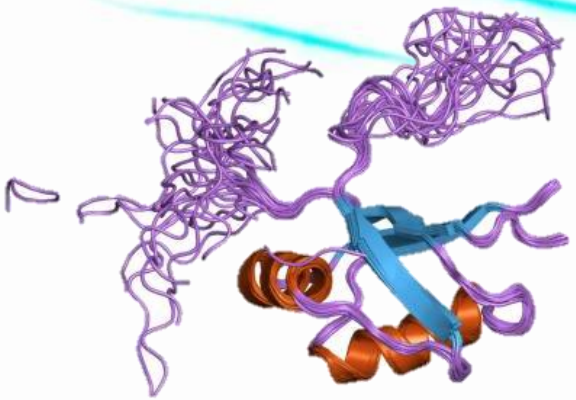
Almost everyone is fearful of passing it on to their children

Biological impact of FMND gene is huge

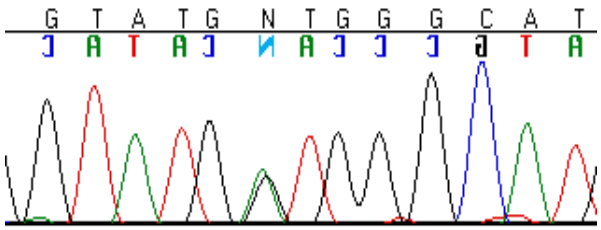
MND-causing gene mutations can be used to model disease in cells, animals

This allows us to study disease mechanisms and develop therapies

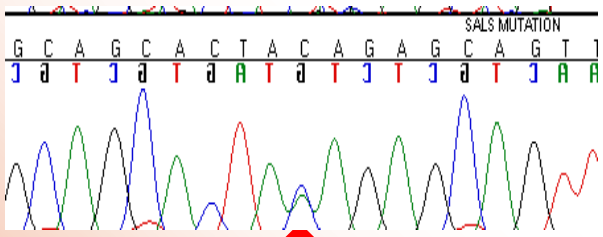
TAR DNA Binding Protein
(TDP-43)



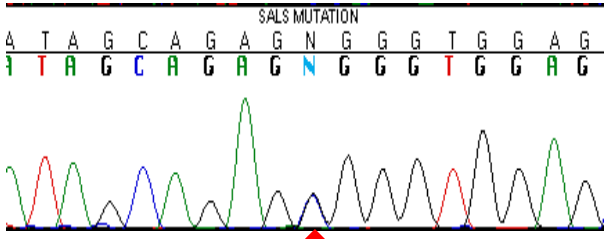
Mutations in *TARDBP* segregate with MND



Familial
M377V



Sporadic ALS
C>A Q331K



Sporadic ALS
G>C G294A

Sreedharan Science 2008

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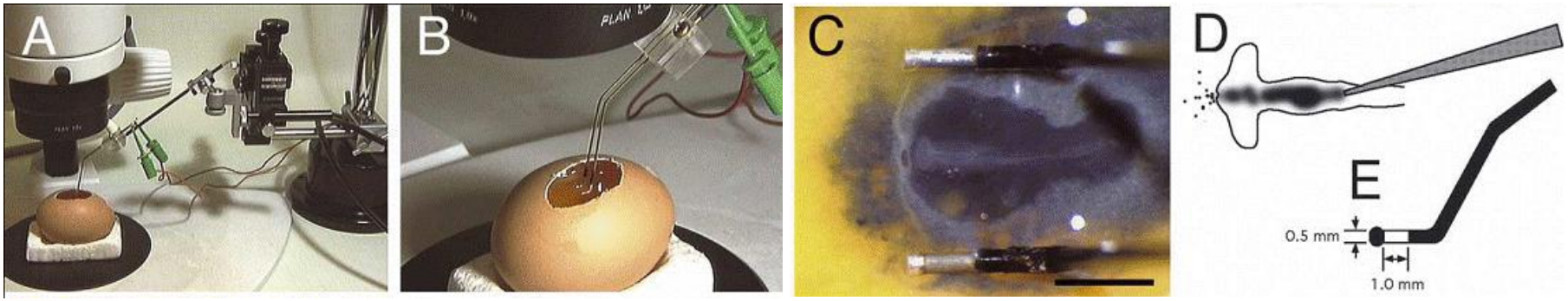
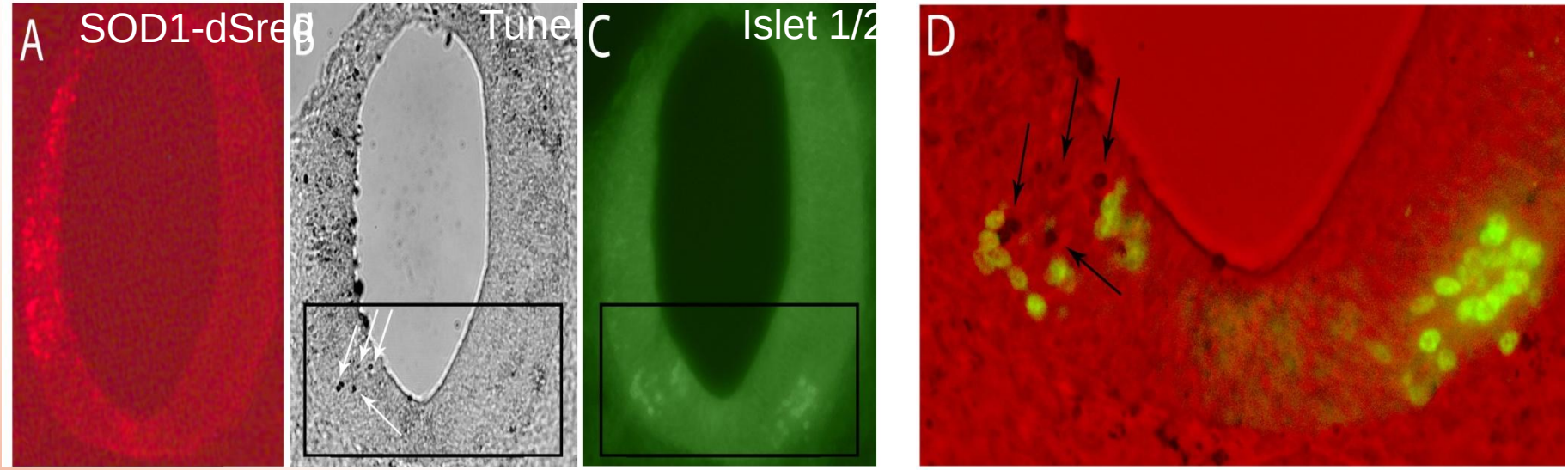
Chick spinal neurons to model TDP-43 toxicity





Electroporation of chick spinal cord with mutant SOD1 causes motor neuron death

Tripathi et al *submitted* 2012

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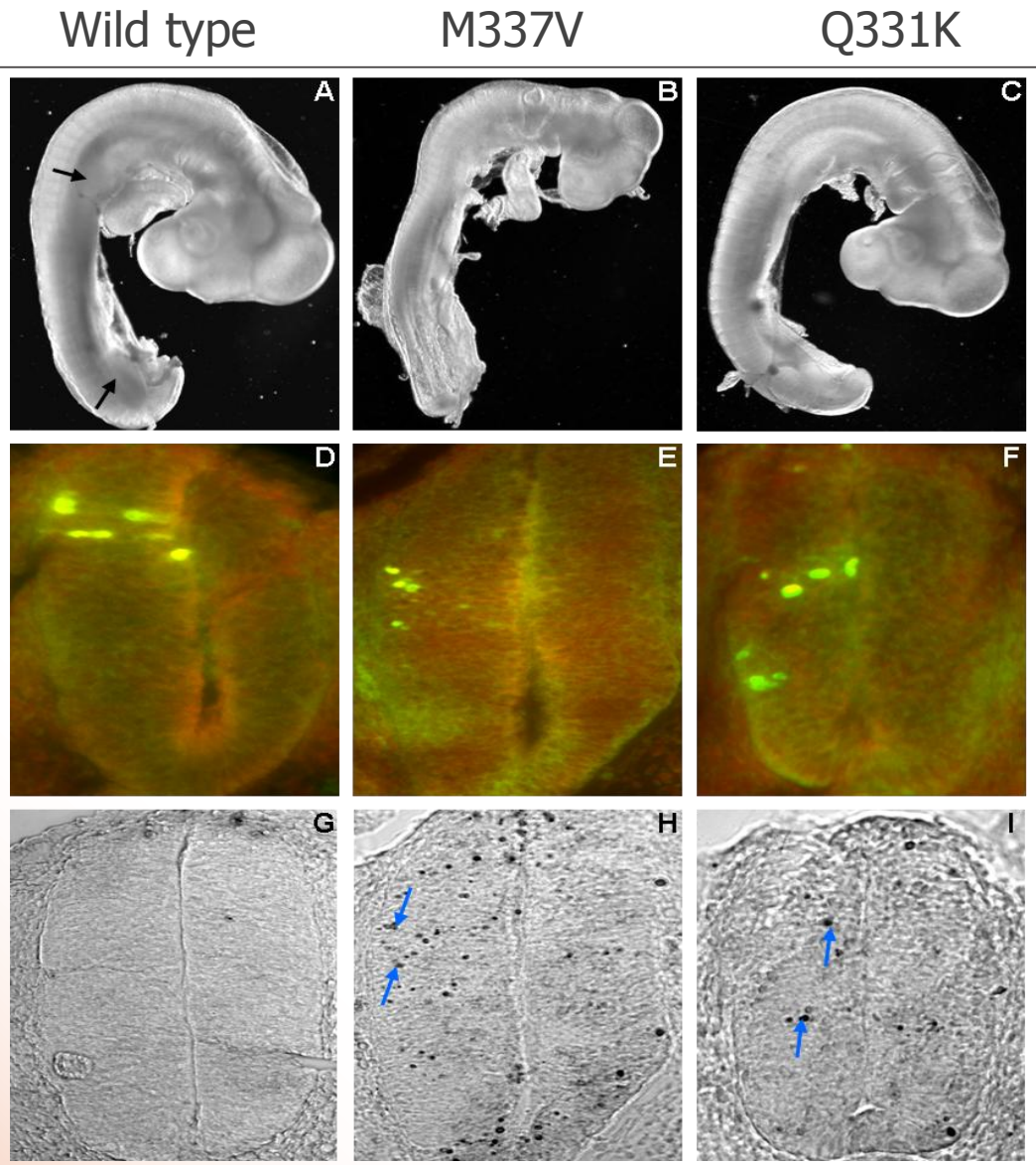
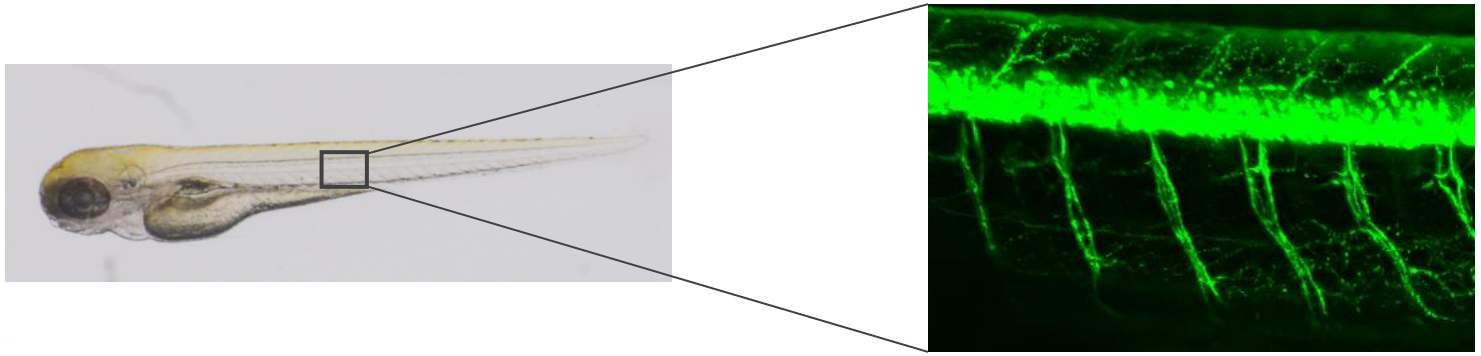
TDP-43 is toxic to spinal cord neurons



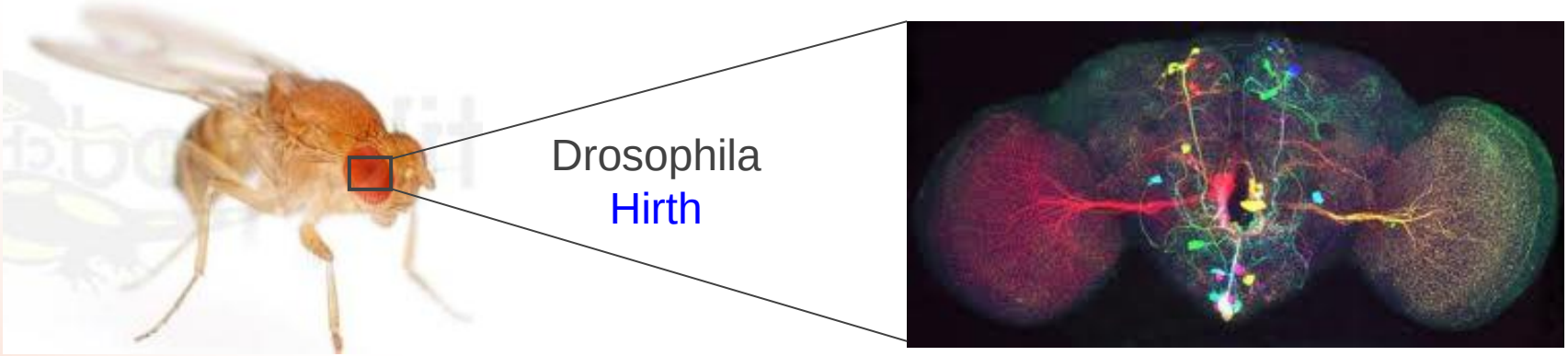
Sreedharan et al. *Science* 2008

Animal TDP-43 models to study disease mechanisms

Zebrafish
Houart

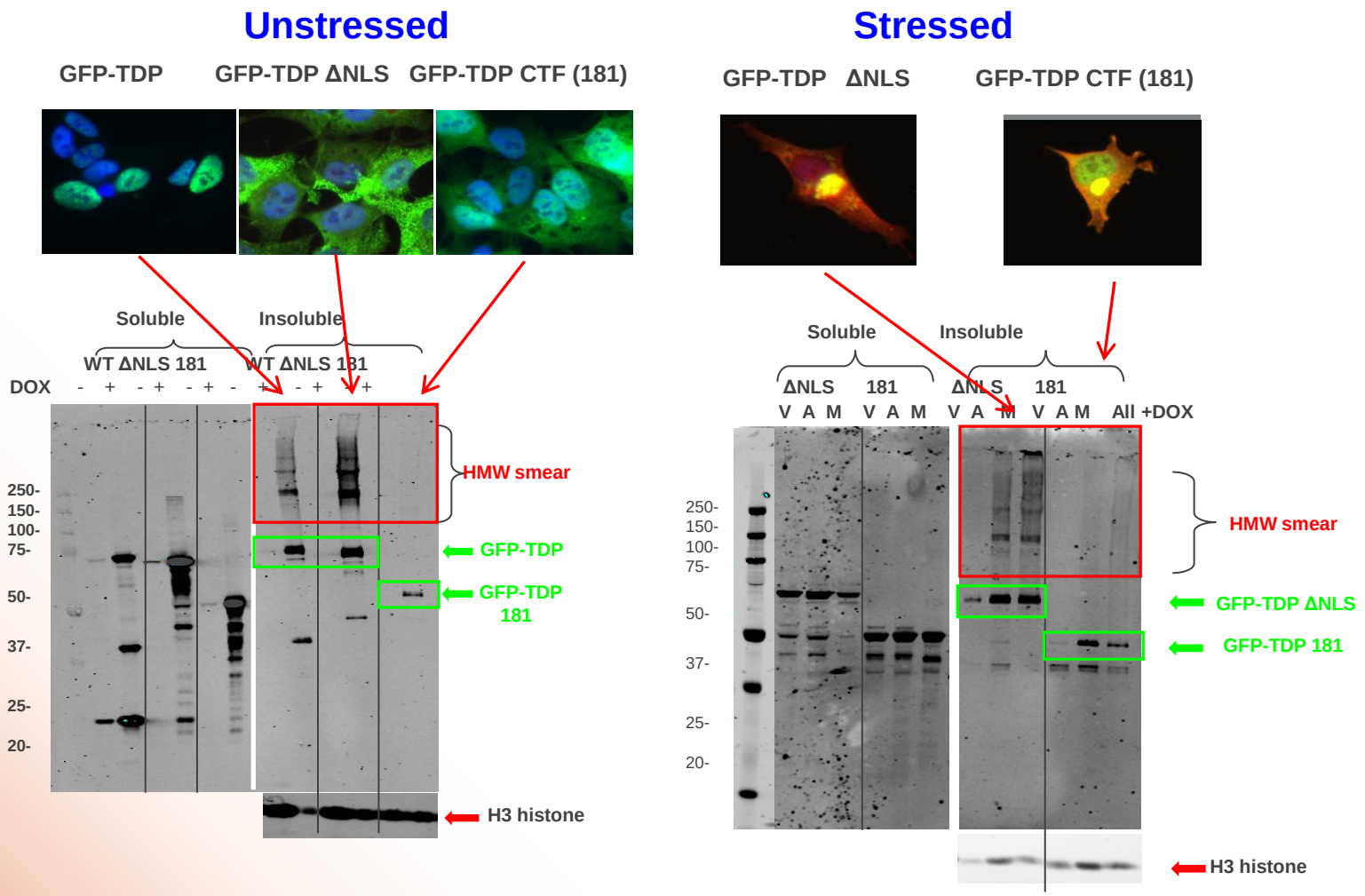


Drosophila
Hirth



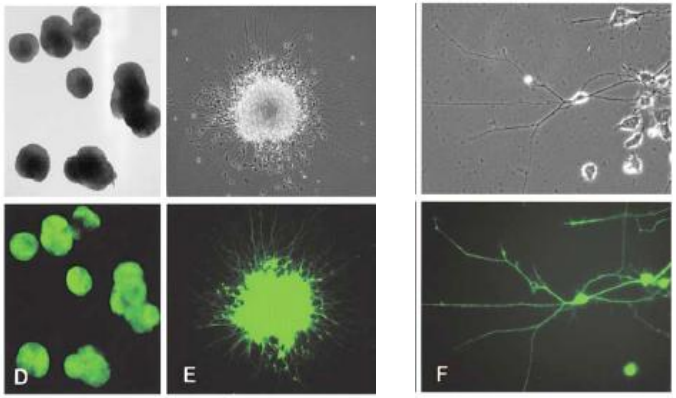
Mouse
Cleveland

Cellular TDP-43 aggregation for drug discovery



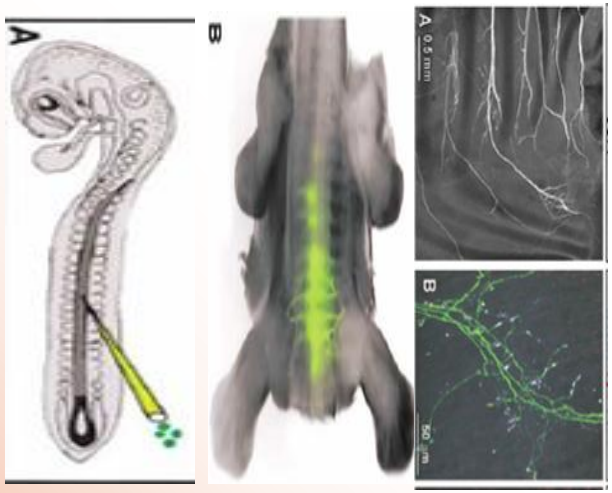

Using stem cells to model MND

Motor neurons from embryonic stem cells



Mouse embryonic stem cells were treated with a chemical coctail (retinoic acid and sonic hedgehog)

After two weeks they took the shape of motor neurons and became dependant on the same growth factors as normal neurons



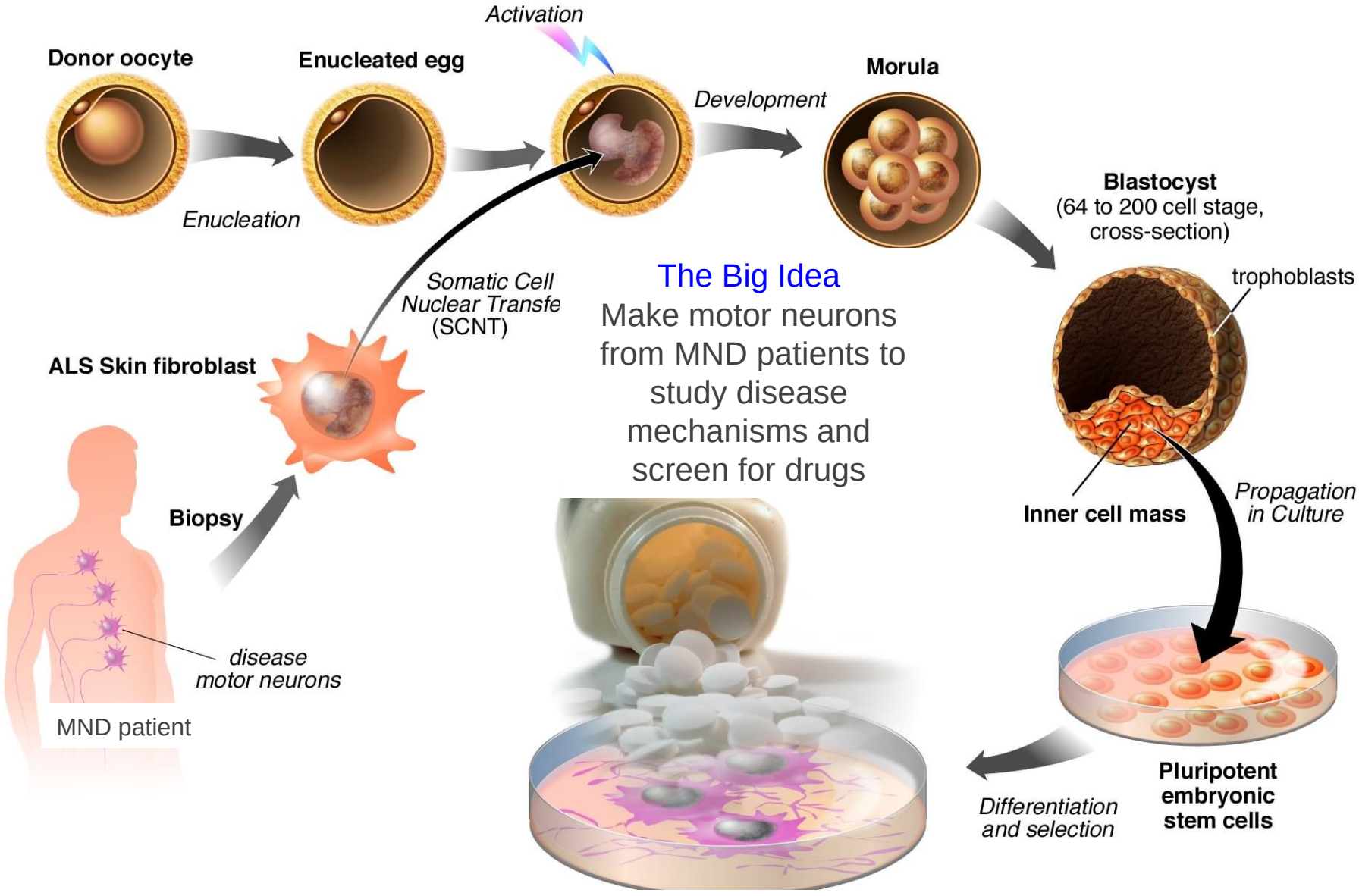
When transplanted into the chicken embryo spinal cord they migrated to the anterior horn where motor neurons reside

Transplanted motor neuron axons made functional contact with muscle cells

Embryonic stem cells can generate motor neurons in the laboratory

Wichterle *Science* 2003

Cloning to make human MND motor neurons



The Big Idea

Make motor neurons from MND patients to study disease mechanisms and screen for drugs

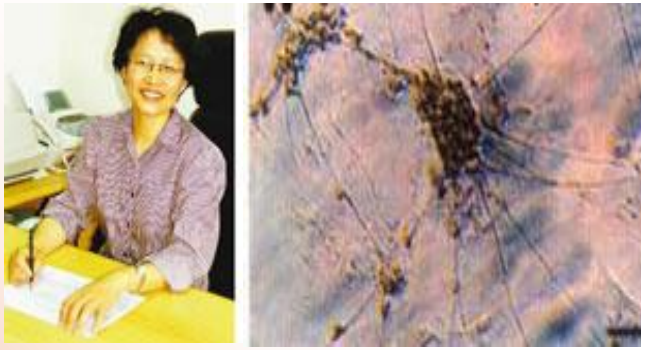
Motor Neurons carrying MND-causing gene defects

J.Simon 2014

Clones, Chimeras and Controversy

Ian Wilmut and Dolly

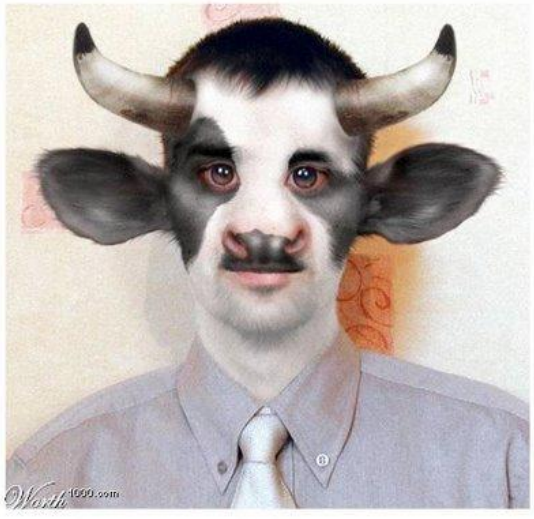
Woo-Suk Hwang and Snuppy



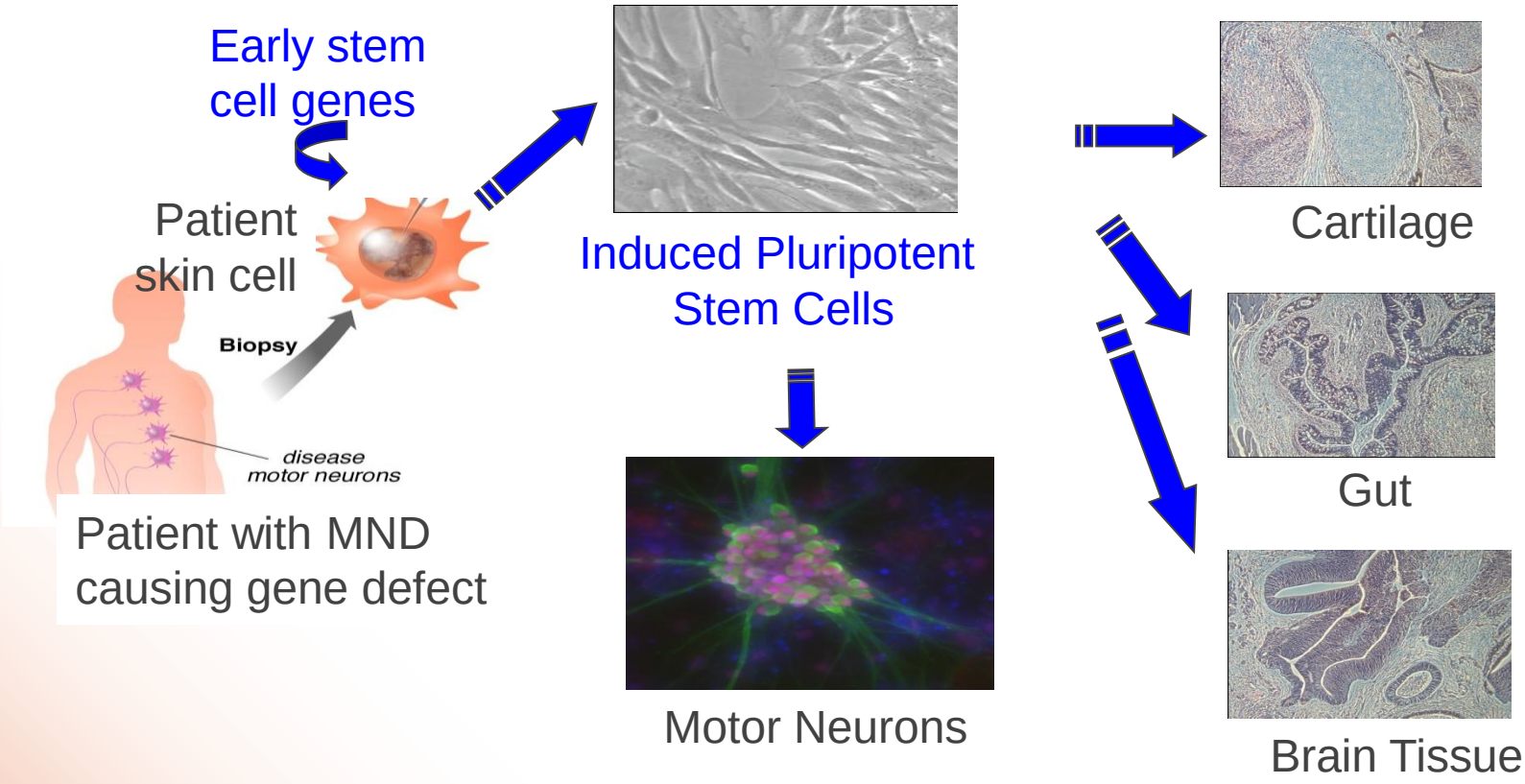
Huizhang Sheng: Cloning human cells into rabbit and cow eggs

Human-animal hybrid embryos
“Warmly received” by the press

Animal-human hybrids from the internet



An alternative means of generating human stem cells

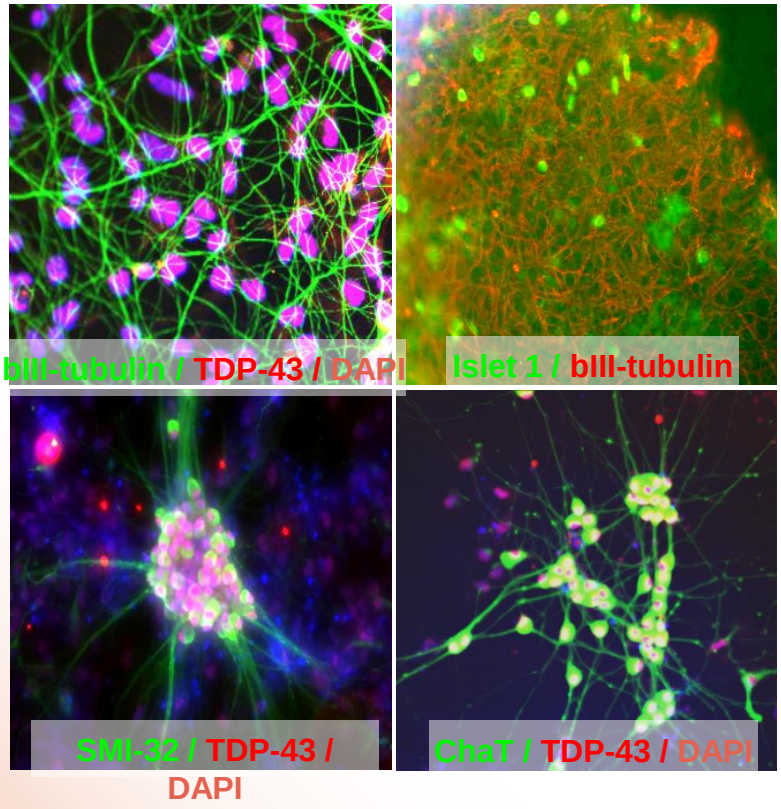


"Disease in a dish" model to: study mechanisms and conduct drug screening

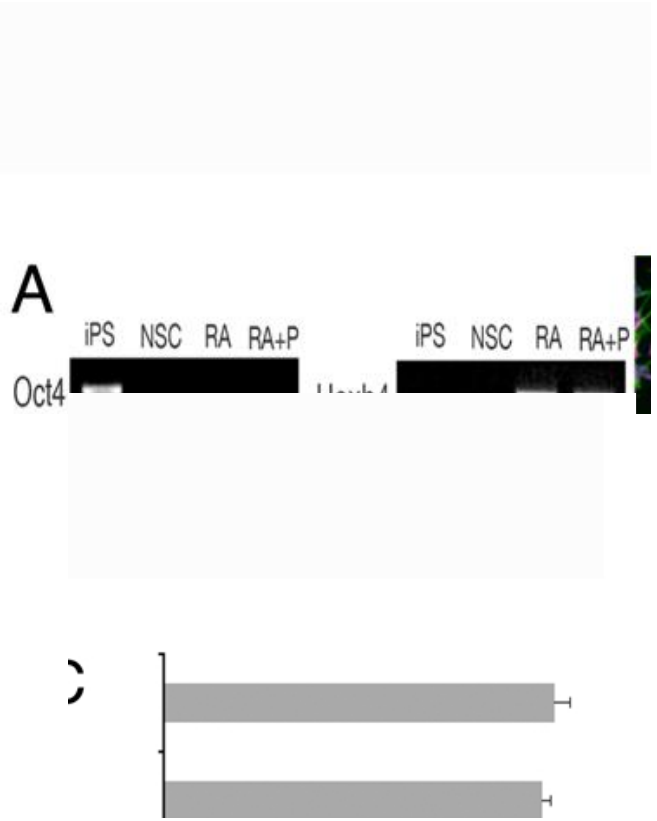
Cell therapy: individualised neuronal cell replacement therapies

Shinya Yamanaka et al. *Science* 2007

Patient-derived stem cells can make motor neurons



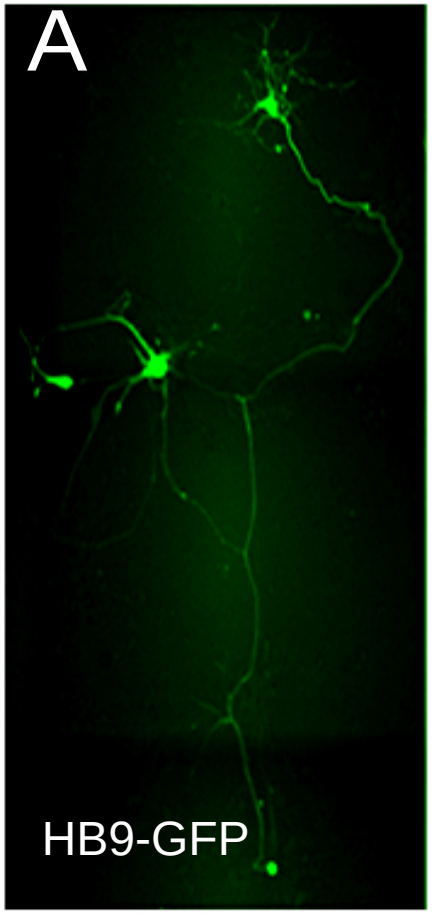
We can reliably generate motor neurons from MND patient cultured stem cells

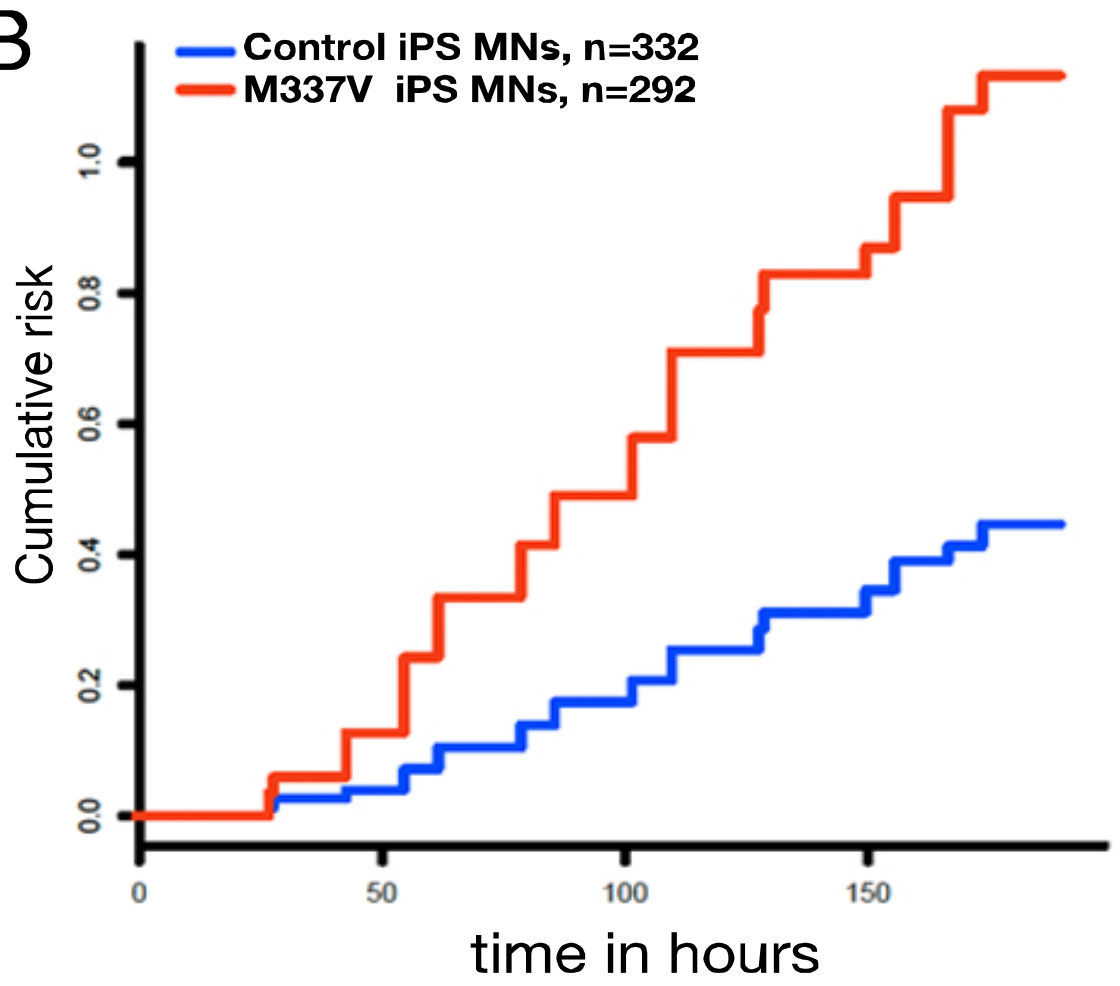


They behave like normal neurons: They are electrically responsive, spontaneously active, make networks and neuromuscular junctions.

Bilican et al., PNAS. 2012

Mutant motor neurons die in prolonged culture



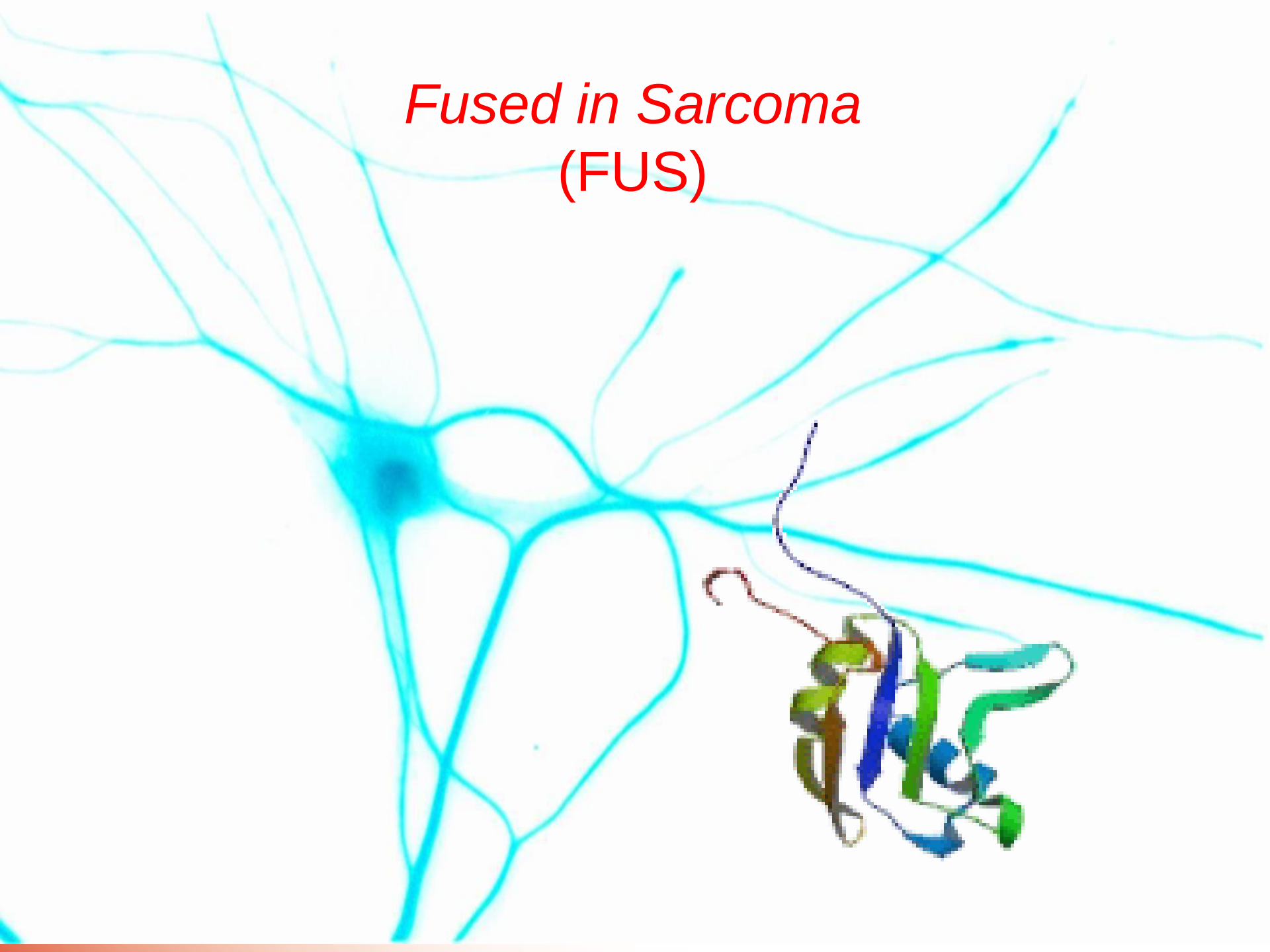


Motor neurons expressing a specific reporter were observed over 200 hours

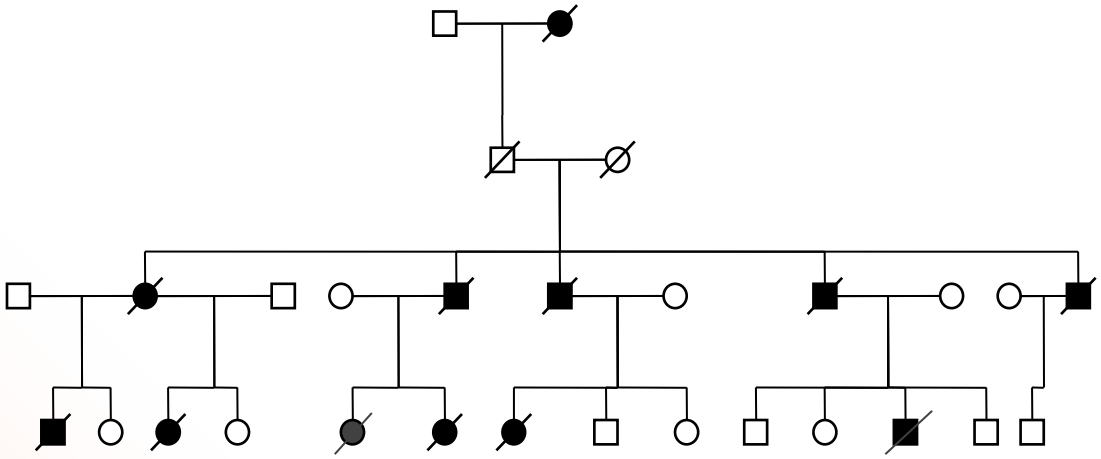
Those expressing M337V mutant TDP-43 die spontaneously at a faster rate

Bilican et al., PNAS. 2012

Fused in Sarcoma
(FUS)



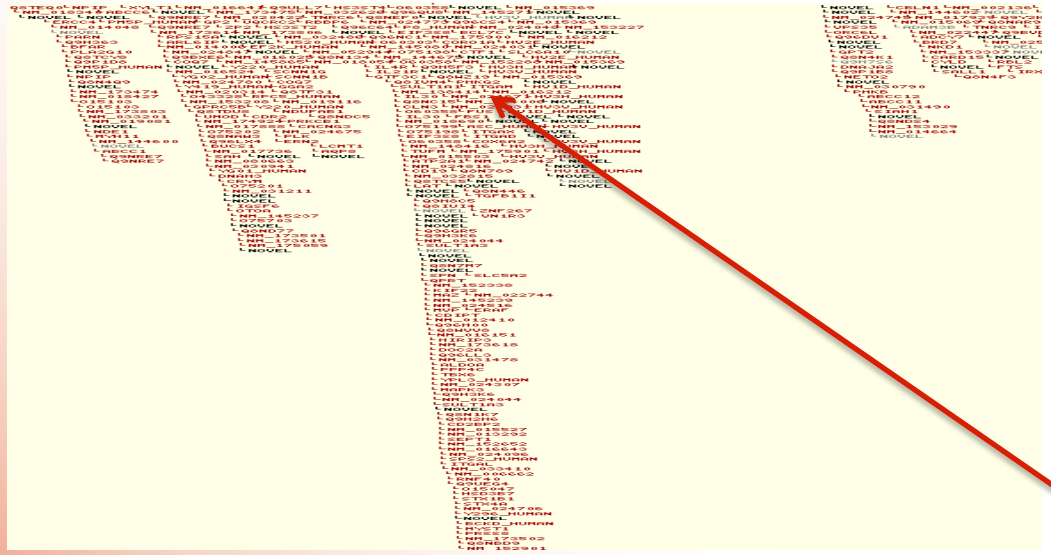
MND family from Essex UK linked to Chr 16



Age at onset ~38 years

Mean survival 13 months

Starts with arm weakness

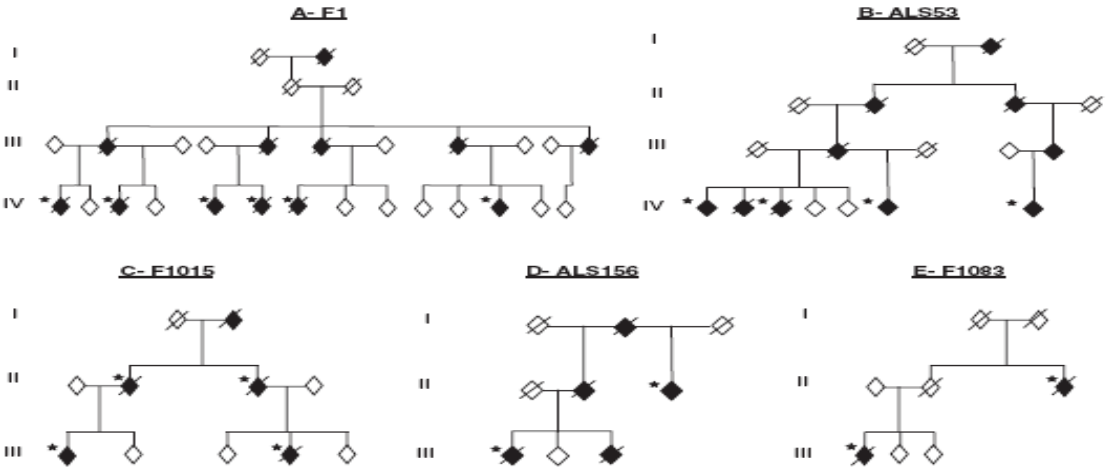


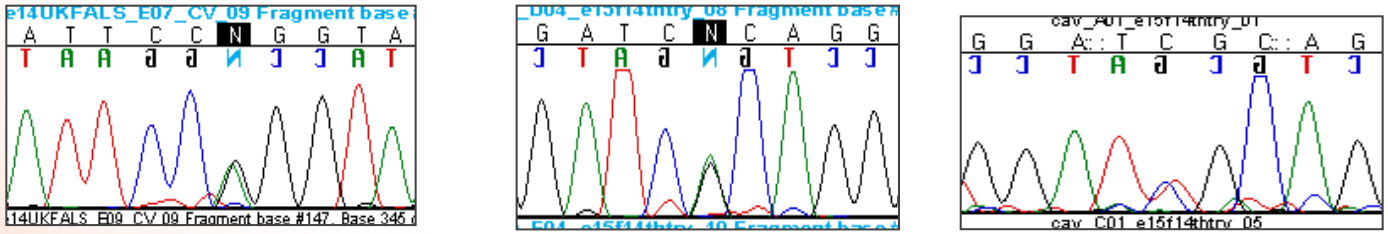
Used 400 markers to show that the gene responsible resided on Chromosome 16 but which one of >400 genes

In a long-term collaboration with US colleagues we eventually identified the causative gene Fused in Sarcoma (FUS)

Ruddy et al AJHG 2003

FUS mutations detected in familial MND





TTTGGCCCTGGCAAGATGGATTCCAGGGGTGAGCACAGACAGGATCGCAGGGAGAGGCCGTATTAA

-F--G--P--G--K--M--D--S--**R**--G--E--H--R--Q--D--**R**--R--E--R--P--Y--*-

Arg 515 Gly

Arg 521 Cys

Arg 521 His

Vance et al., *Science* 2009

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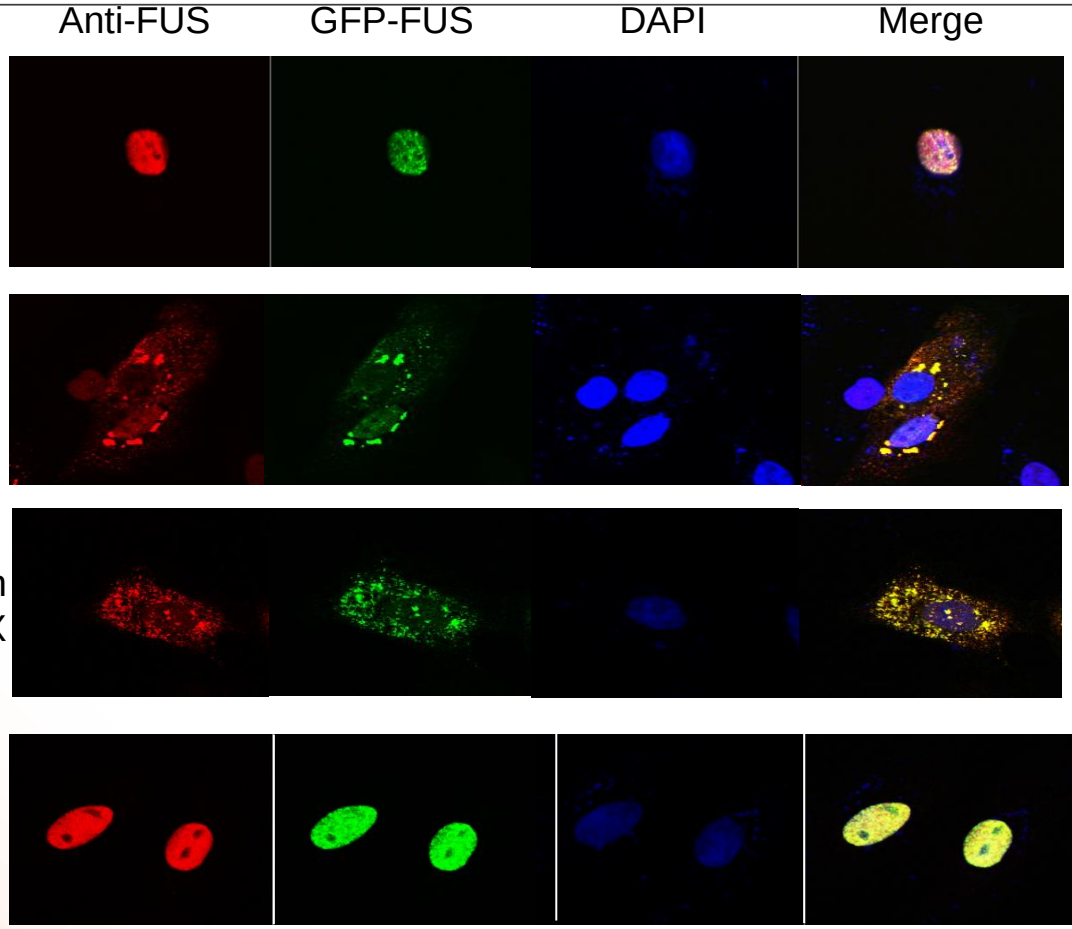
FUS C-term contains a nuclear localising signal

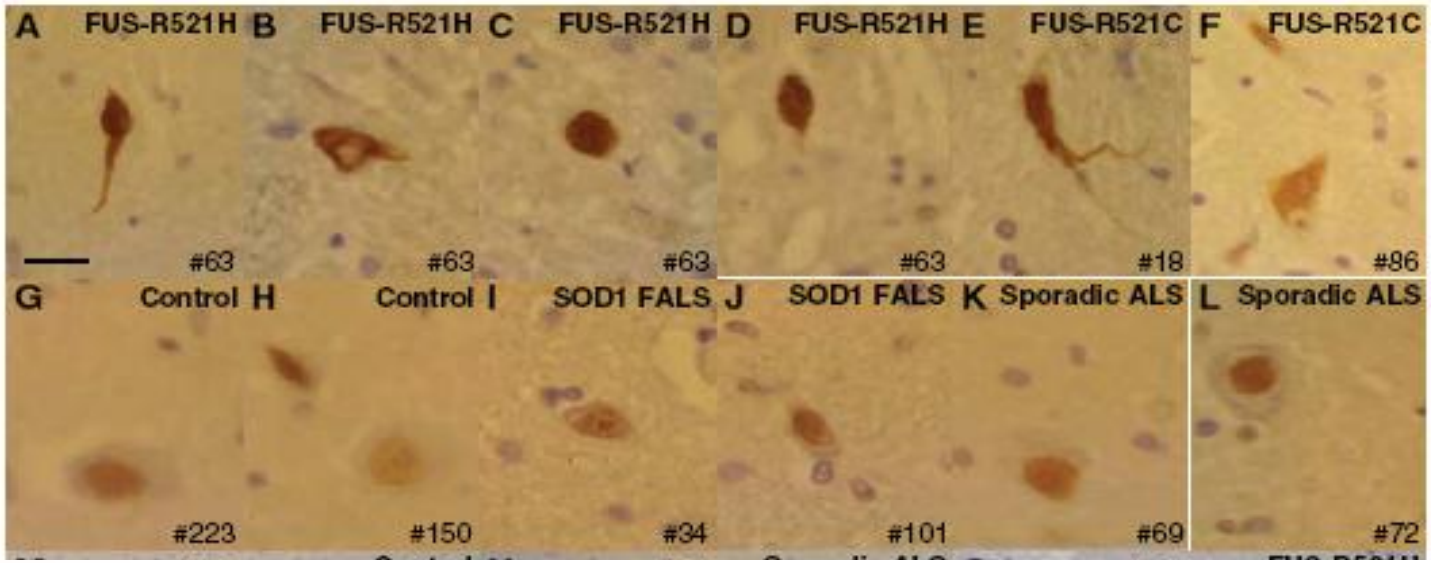
CV1 cell line

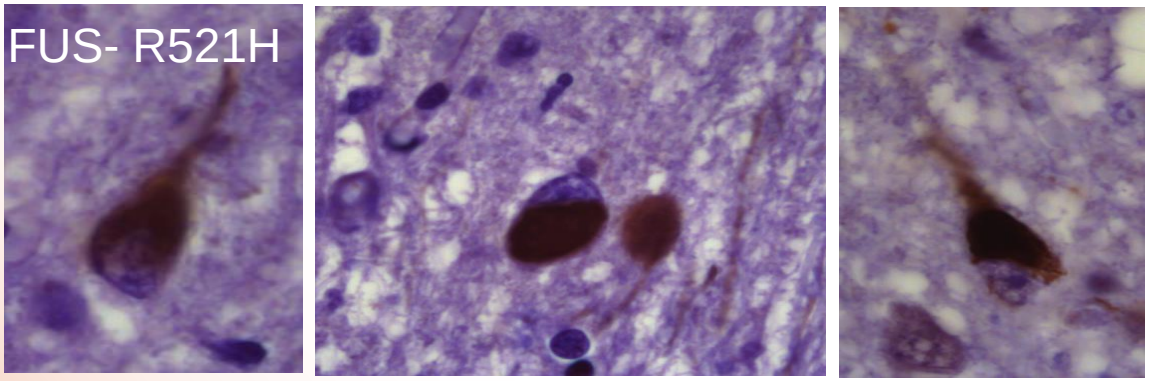


Excision or mutation at the very end of FUS protein inhibits nuclear importation

Addition of a functional end to mutated FUS rescues the nuclear import of FUS

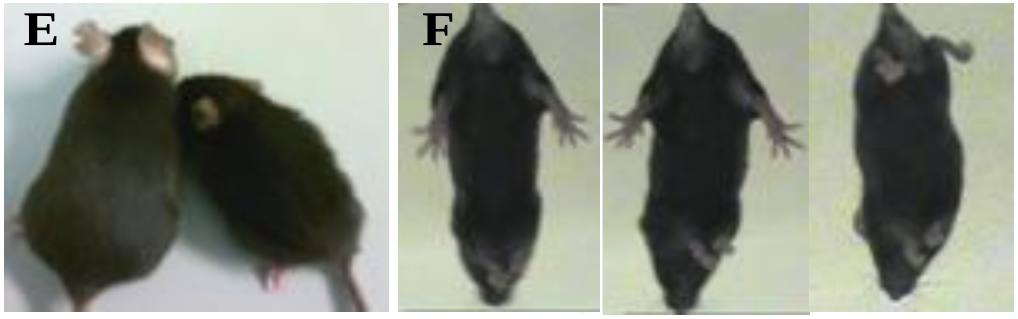
FUS aggregates in MND due to FUS mutations





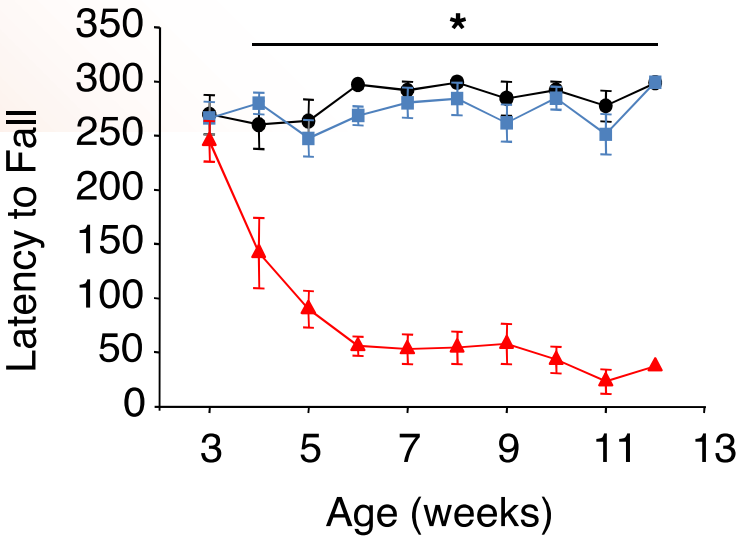
Motor neurons with large FUS inclusions appear to have nuclear clearing of FUS

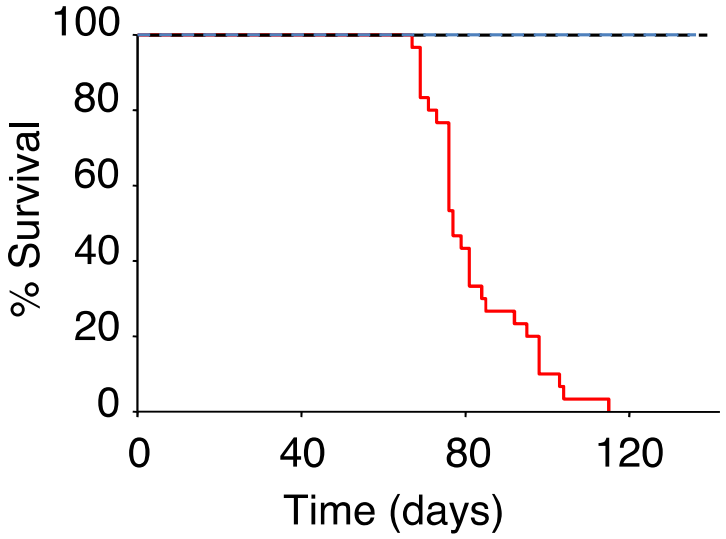
Transgenic mouse model of WT FUS



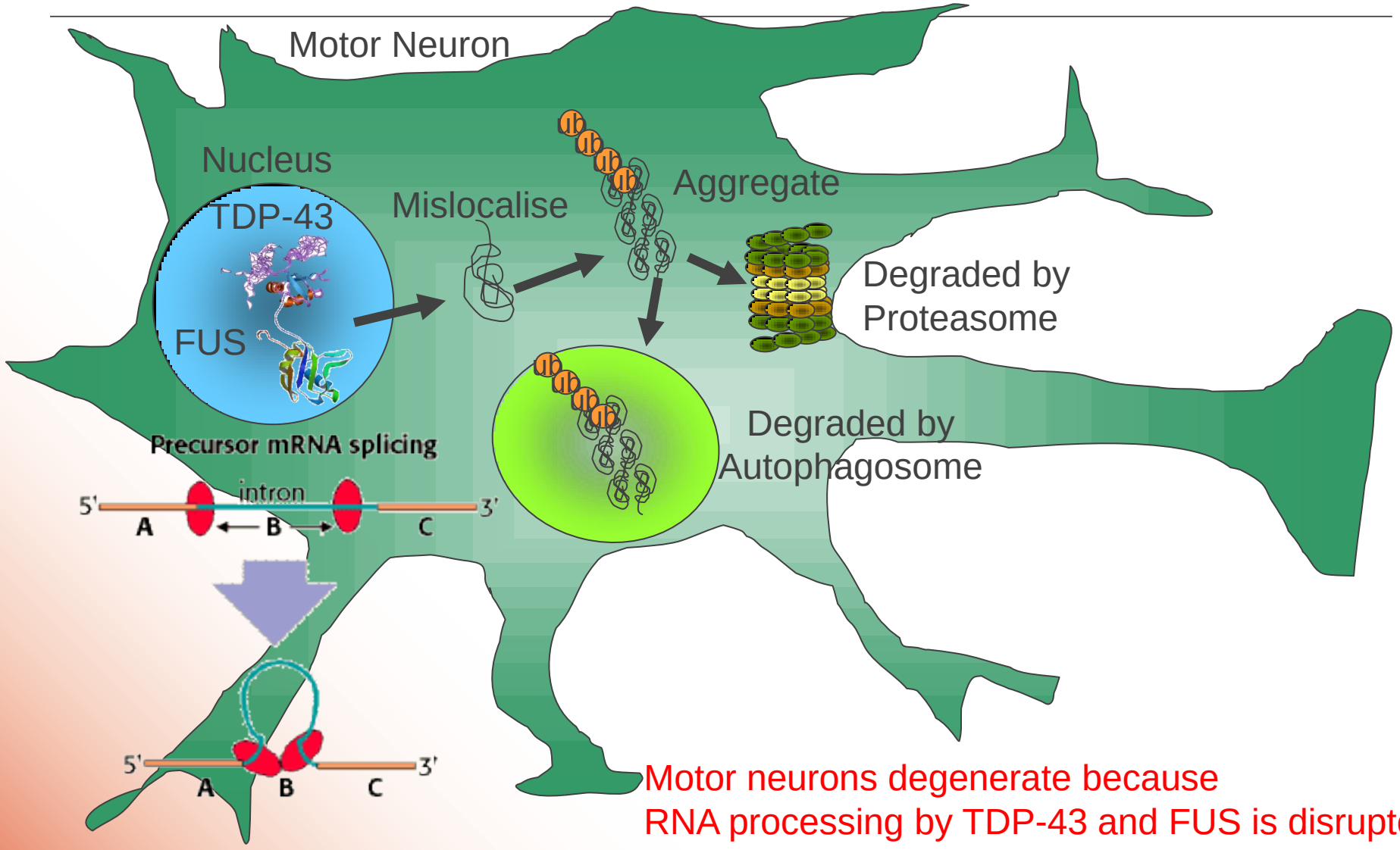
Homozygous hFUS mice
fail to gain weight,
develop paralysis
die prematurely

Histologically they show increased cytoplasmic FUS in motor neurons





TDP-43 and FUS disease mechanisms



Motor neurons degenerate because
RNA processing by TDP-43 and FUS is disrupted

Shaw, *Neuron* 2010

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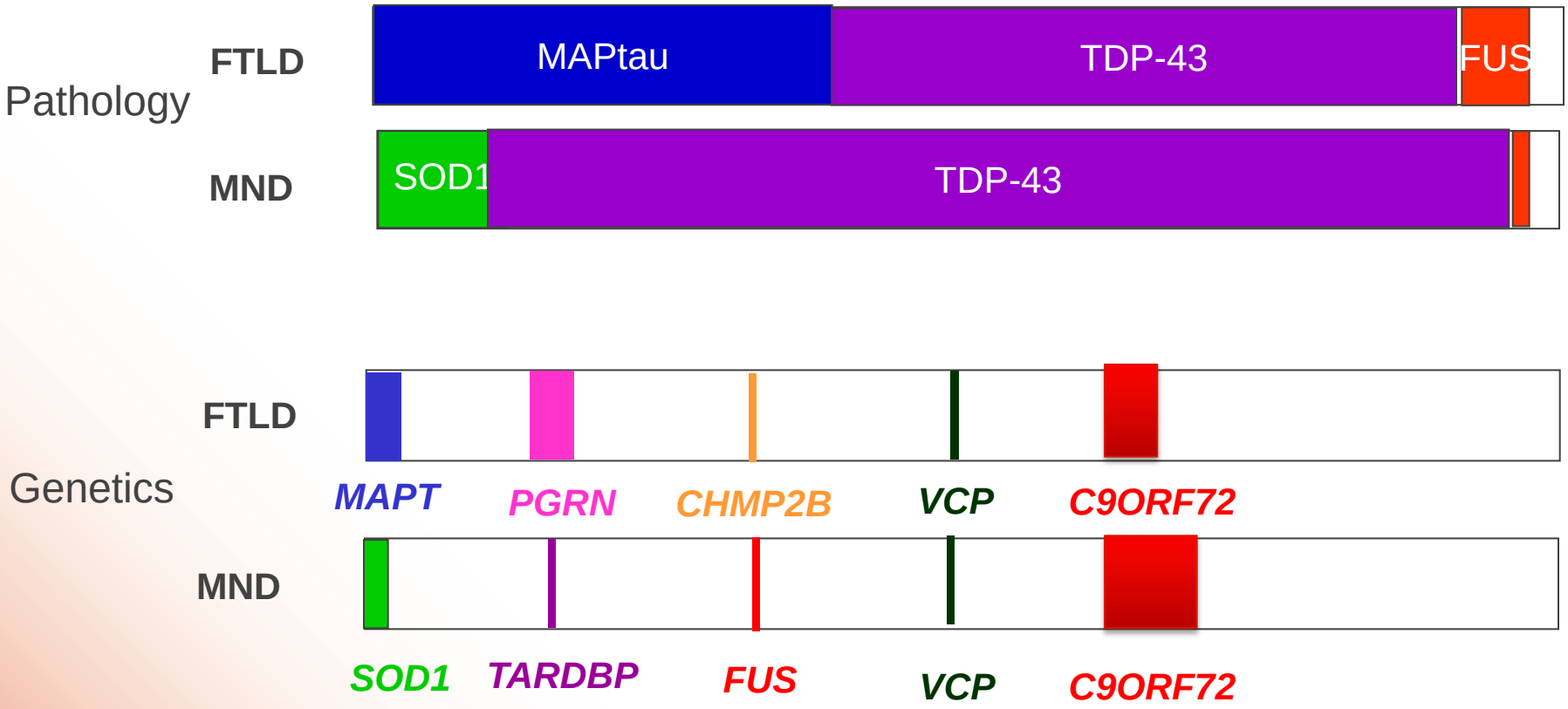
Pathology and Genetics of MND and FTD



Gene Discovery> Disease Models > Drug Discovery

Zebra fish

Fruit Fly

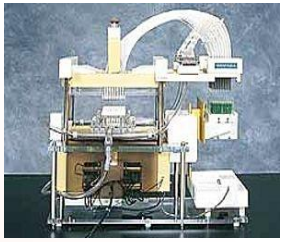
Mouse

New Gene Discovery

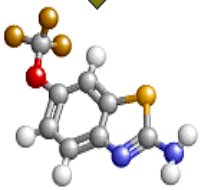
Cell Lines

Human Brain Tissue

New Drug Discovery



Automated cell cult



Compound libraries

Drugs that slow or reverse disease

Who's in Charge

Sophie Morris

Vicky Strzelczyk

The Gene Hunters

Jack Miller

Bradley Smith

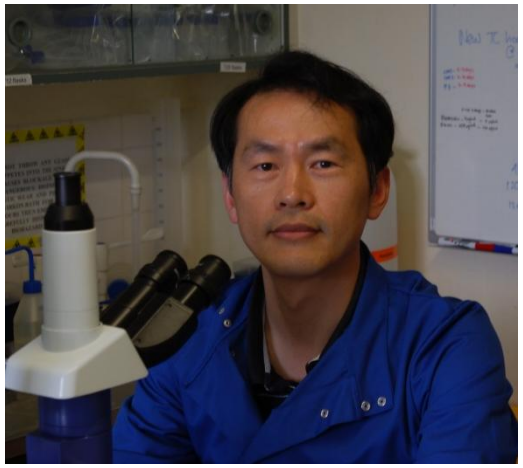
Lauren Johnson

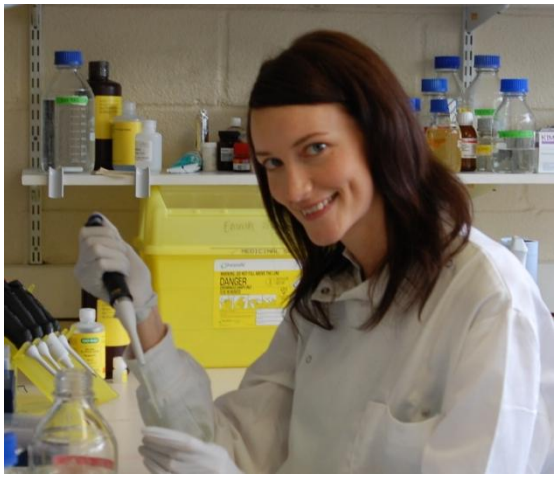
Caroline Vance

Stephen Newhouse

The Cell Line Modellers

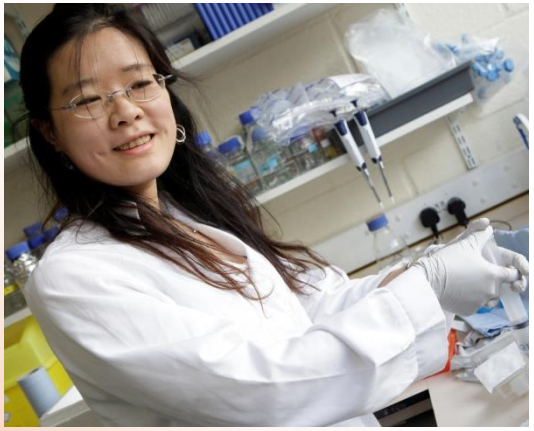
Younbok Lee

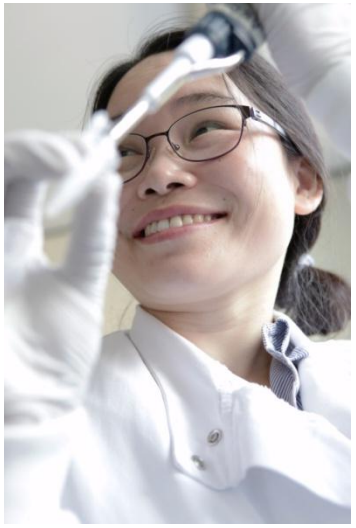




Emma Daniel

Han-Jou Chein





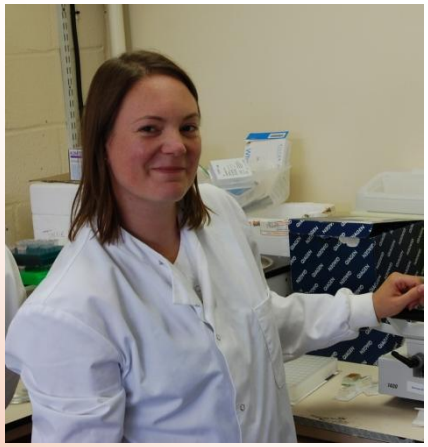
Doyoung Lee

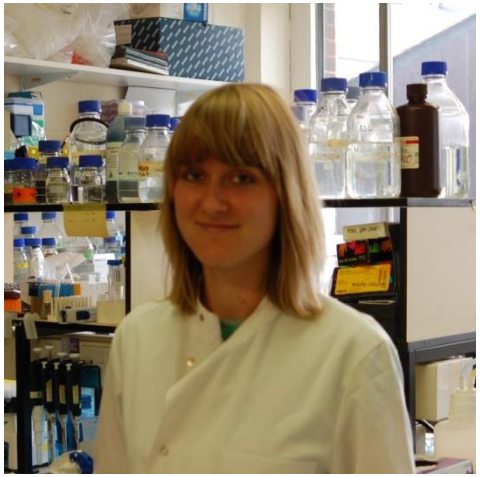
The Molecular Pathologists

Boris Rogelj

Tibor Hortobagyi

Claire Troakes

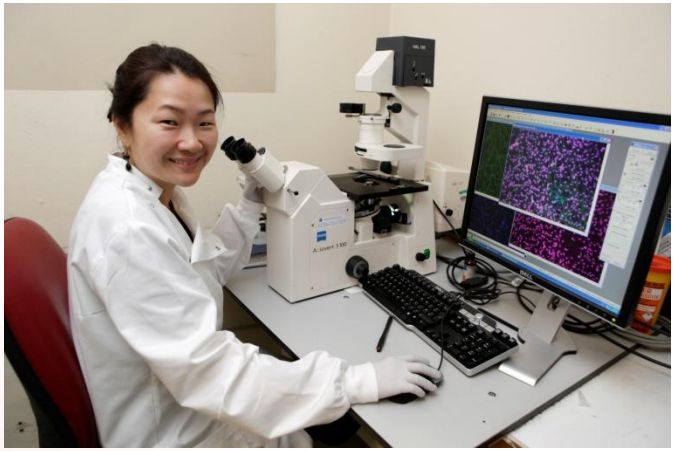


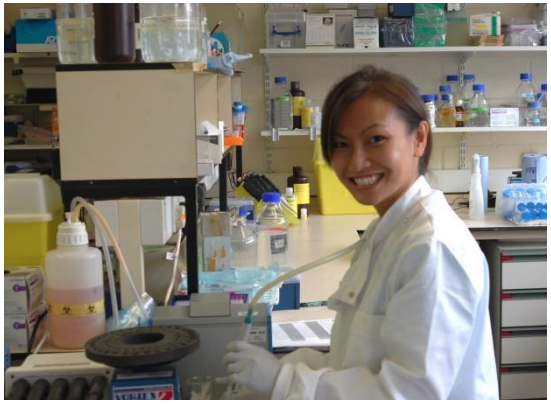


Maya Stalekar

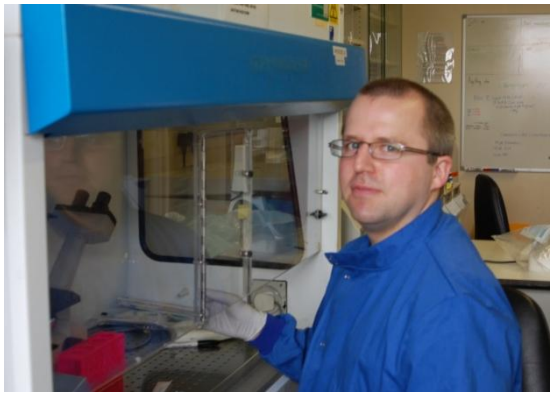
The Stem Cell Modellers

Agnes Nishimura





Carole Shum

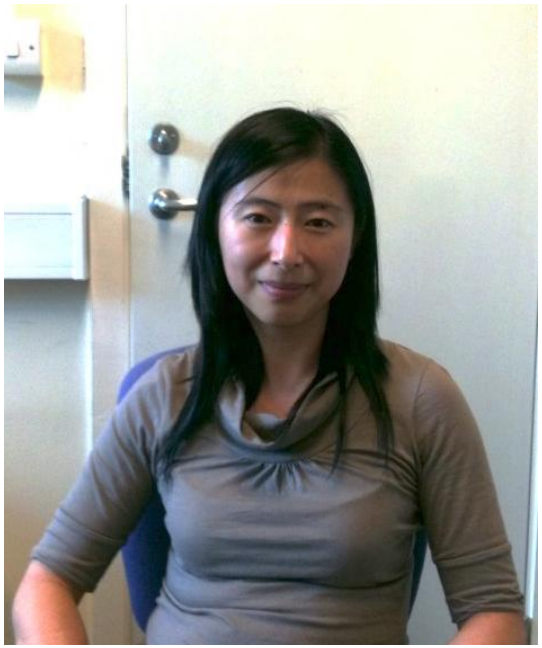


Jamie Wright

The Mouse Modellers

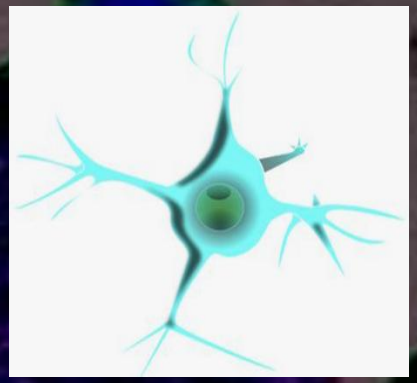
Jackie Mitchell

Lin Liu

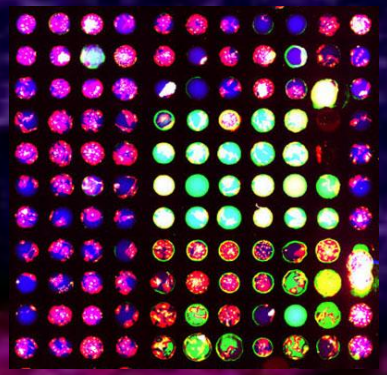


MND is not incurable

With greater effort, smarter experiments and increased funding we will discover what causes MND.



Only then can we develop treatments that can really cure this disease.



Colleagues, Collaborators and Cash

Collaborators

Nigel Leigh, Chris Miller, Ammar Al-Chalabi	
Jean-Marc Gallo, Noel Buckley, Corrine Houart	KCL
John Hardy	UCL
Bob Brown, John Landers	UMass
Jackie De Belleroche	Imperial
Peter Andersen	Umea
Garth Nicholson	Sydney
Don Cleveland	San Diego
Ian Wilmut, Siddarthan Chandran	Edinburgh
Tom Maniatis	Columbia
Stuart Pickering-Brown, David Mann	Manchester
Jernej Ule	Cambridge

Work Supported by

MND Association
Medical Research Council
American ALS Association
Welton Trust
Wellcome Trust
Heaton Ellis Trust
Psychiatry Research Trust
Alzheimer Research UK

The last word(s) on motor neuron disease

Finding out more about MND (also known as ALS) and global research efforts

http://www.mndassociation.org/life_with_mnd/what_is_mnd/index.html

http://www.alsa.org/

http://www.heatonellistrust.com/

Living with MND, the patients perspective

http://www.healthtalkonline.org/Nerves_and_brain/motorneuronedisease/

http://www.youtube.com/watch?v=KeHs7lalfjY