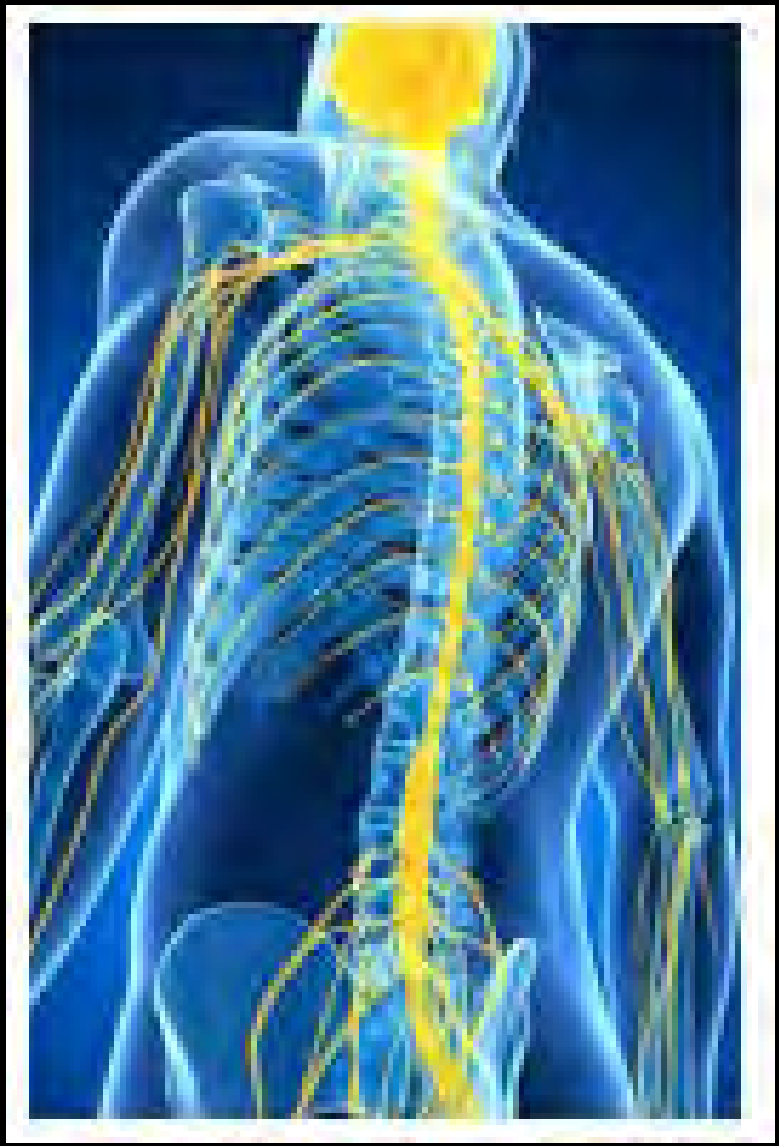
Humanising mouse models to understand neurodegeneration

Elizabeth Fisher,

UCL, London, UK

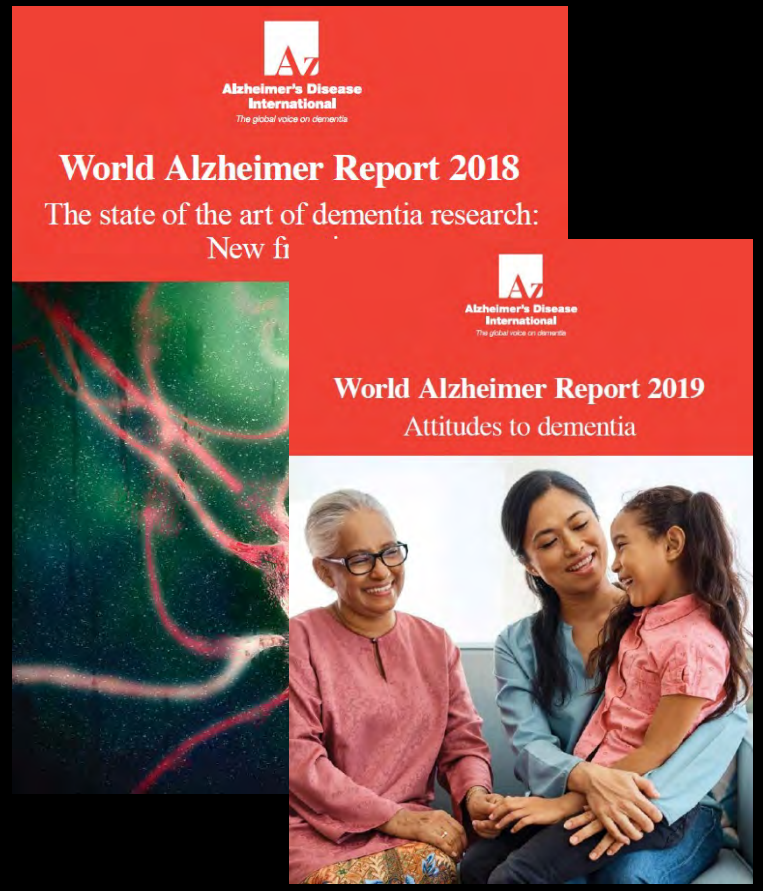
Neurodegeneration

- **Brain** (Alzheimer disease) and/or **spinal cord** (motor neuron disease)
- **With/without dementia** (Parkinson disease)
- **Genetic** (Huntington disease) or '**sporadic**' (most Alzheimer disease)



Neurodegeneration

- 50 million people with dementia, globally
- $1 trillion per year
- AD, No.1 killer in England and Wales
- AD, new case every 3 seconds globally
- 10 million people have Parkinson's disease



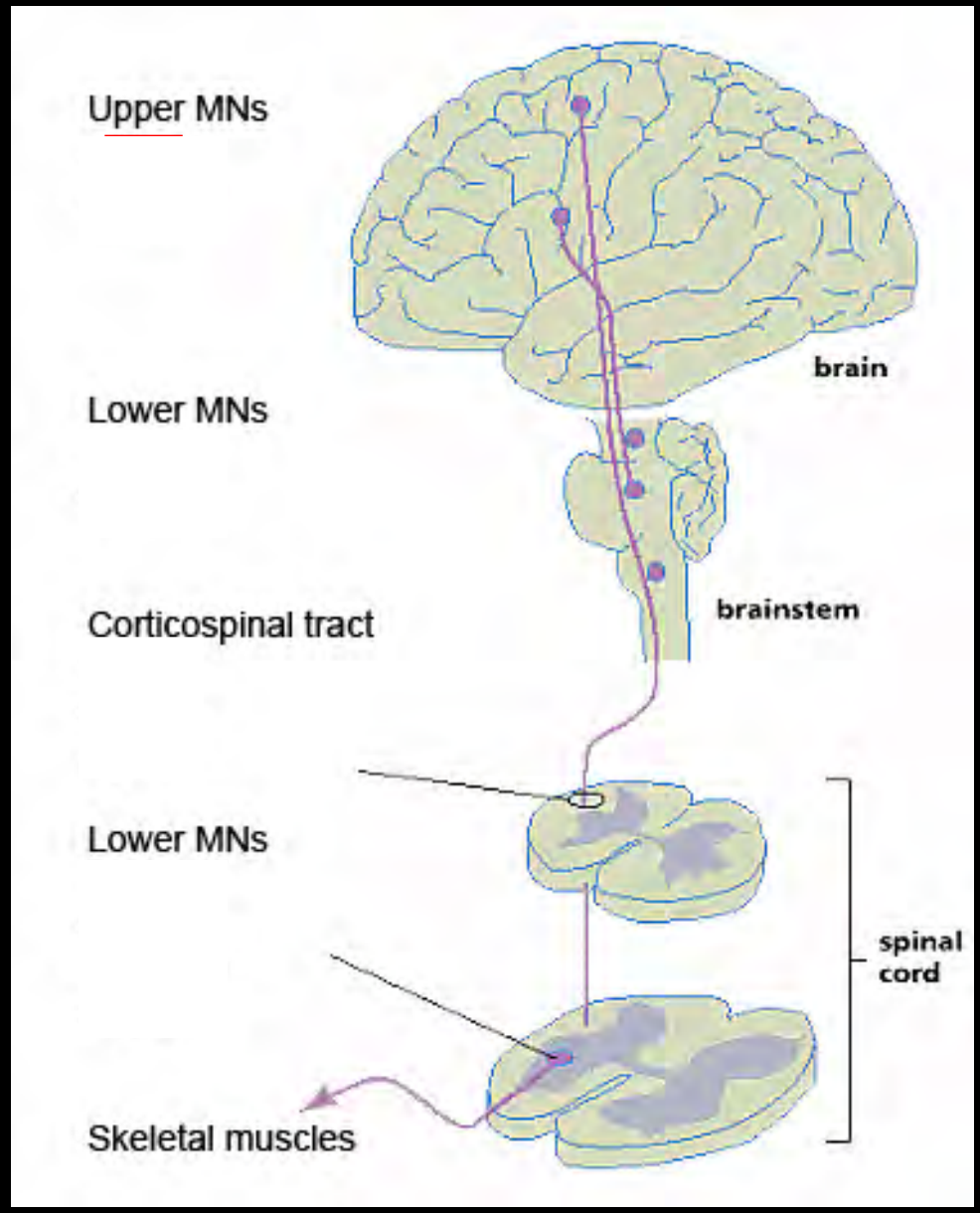
- 66% people with dementia are in low-, middle income countries
- 1 in 10,000 newborns born with Type 1 spinal muscular atrophy, will die usually before 5 years old

Amyotrophic lateral sclerosis
ALS, motor neuron disease

- 1:300 lifetime risk

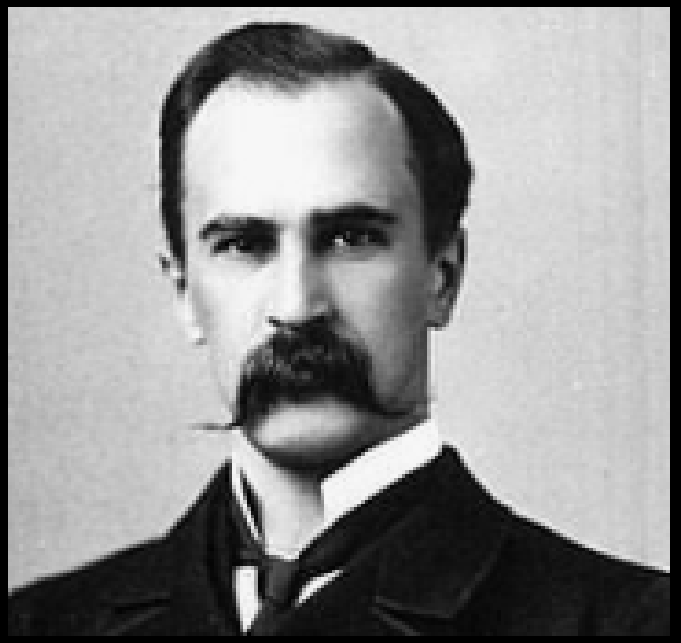
- Loss upper & lower motor neurons
- Focal progressive weakness
- Cognitive changes…50% of patients…..
  ....15% frontotemporal dementia (**FTD**)

- Mean age of onset 55 years
- Death ~3-5 years from diagnosis
- No treatment/cure



ALS

1874 French neurologist **Jean-Martin Charcot**

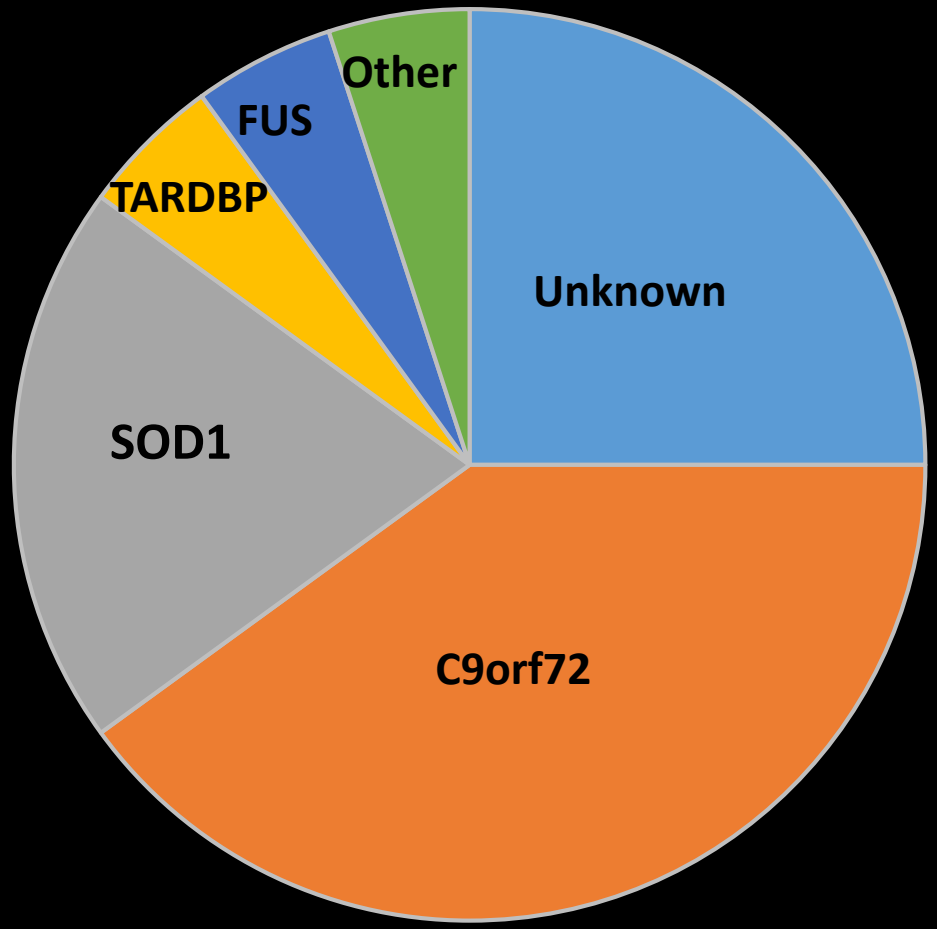


1880 Canadian physician Sir **William Osler**
recognized Farr family of Vermont
dominant ALS, Erastus Farr, farmer, died 1835

ALS ~90% sporadic, ~10% **familial**
…***genetics – a route in…***

- *SOD1, superoxide dismutase*,
- 1993, Bob Brown, Harvard, USA
- Dominant, toxic gain of function

More than 25 different genes for ALS …***genetics – a route in…***



Find the gene,
create the mouse model,
understand the mechanisms,
target the treatment…

Amyotrophic lateral sclerosis

Research resources

Mouse models

Primary neuronal models

Human iPS cells

Human imaging

Rat models

Zebra fish models

Human brain and spinal cord samples

GWAS studies

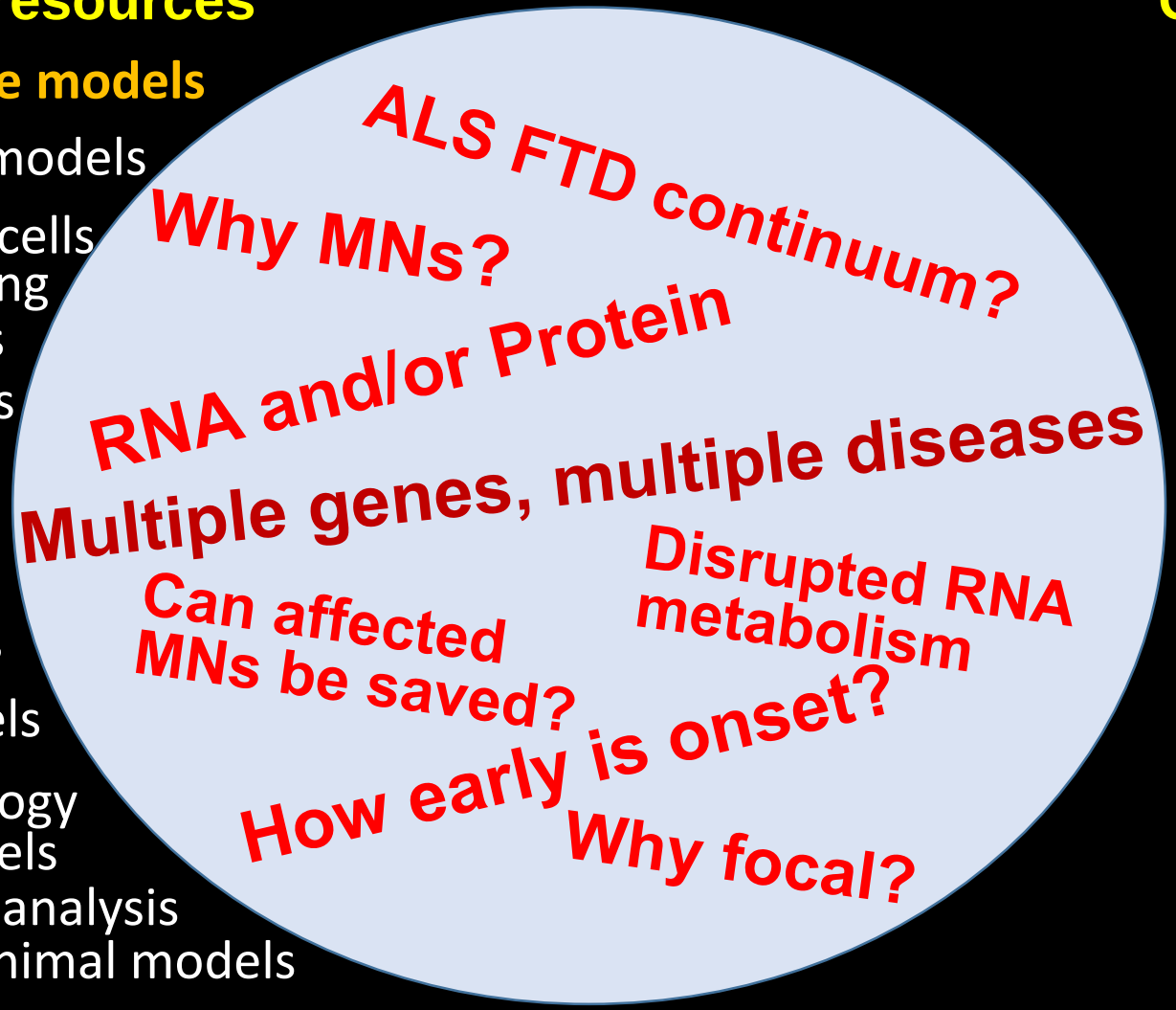
Biochemical models

Epidemiology

Fly models

Metadata analysis

Other animal models



Outputs

Mechanisms

Therapies

Cures

Why work with disease *models*?

- Difficult: human tissue at all disease stages
- Difficult: human tissue from embryo through to old age
- Difficult: human tissue QUICKLY for RNA/protein studies

- Difficult: ALS involves interactions between cell types

- Difficult: do primary drug screens

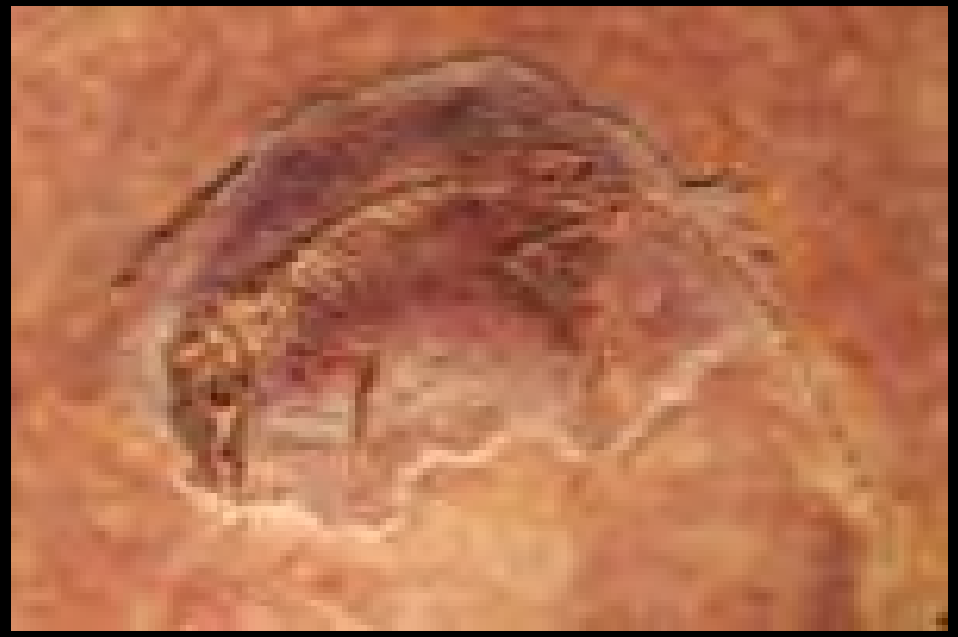
Mouse and humans:
this is <u>grandma</u>

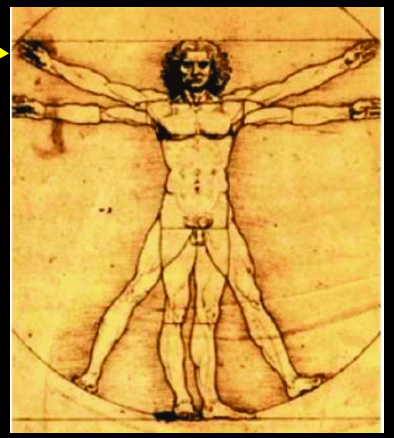
Eomaia scansoria (*climbing dawn mother*)
early Cretaceous

125 million years old

fossil from China

10 cm in length, weight ~25g.





The mouse:
a cousin,
only 75 million years from humans

From Wikipedia…

Mice are not mini-humans

- 1% mouse, human genes not in the other species
- Gene copy number e.g. SMN1, SMN2 humans, Smn mice
- Splice variants ~3.4 per human gene, ~2.4 per mouse gene
- Biochemistry e.g. human SOD1 more ‘aggregatable’ than mouse SOD1
- Anatomy e.g. upper to lower motor neurons monosynaptic in humans, not in mice
- Lifespan, heart rate, etc.

BUT,

Evolutionarily conserved MECHANISMS

Fisher and Bannerman
Sci. Transl. Med. **11**, eaaq1818
(2019) 22 May 2019

Mouse models

- Transgenic mice
- Knock out mice
- Knock in mice
- ENU mutants
- Chromosome engineered mice
- Gene edited mice
- Conditional mutants
- Inducible mutants
- Chimeras mouse:mouse, mouse:human

Transgenic mouse models of ALS…

- Fast phenotype, SOD1 G93A,136 days on C57BL/6J

- Overexpression artefacts
- Insertion site mutations

Genome Research
2019

Large-scale discovery of mouse transgenic integration sites reveals frequent structural variation and insertional mutagenesis

Leslie O. Goodwin,[1] Erik Splinter,[2] Tiffany L. Davis,[1] Rachel Urban,[1] Hao He,[3] Robert E. Braun,[1] Elissa J. Chesler,[1] Vivek Kumar,[1] Max van Min,[2] Juliet Ndukum,[1] Vivek M. Philip,[1] Laura G. Reinholdt,[1] Karen Svenson,[1] Jacqueline K. White,[1] Michael Sasner,[1] Cathleen Lutz,[1] and Stephen A. Murray[1]

[1]The Jackson Laboratory, Bar Harbor, Maine 04609, USA; [2]Cergentis B.V., 3584 CM Utrecht, The Netherlands; [3]The Jackson Laboratory for Genomic Medicine, Farmington, Connecticut 06032, USA

SOD1 transgenics…translation

- 2% ALS patients…
- SOD1 appears to be an outlier

- Antisense oligo therapies
  n.b. need ‘humanized’ mice…

Endogenous gene models… *physiological* expression*

- **Chemical mutagenesis**
- **Gene targeting**

- Slow phenotypes…

*ALS:

RNA binding proteins exquisitely dosage sensitive (despite autoregulation)

- **Chemical mutagenesis**
- Gene targeting

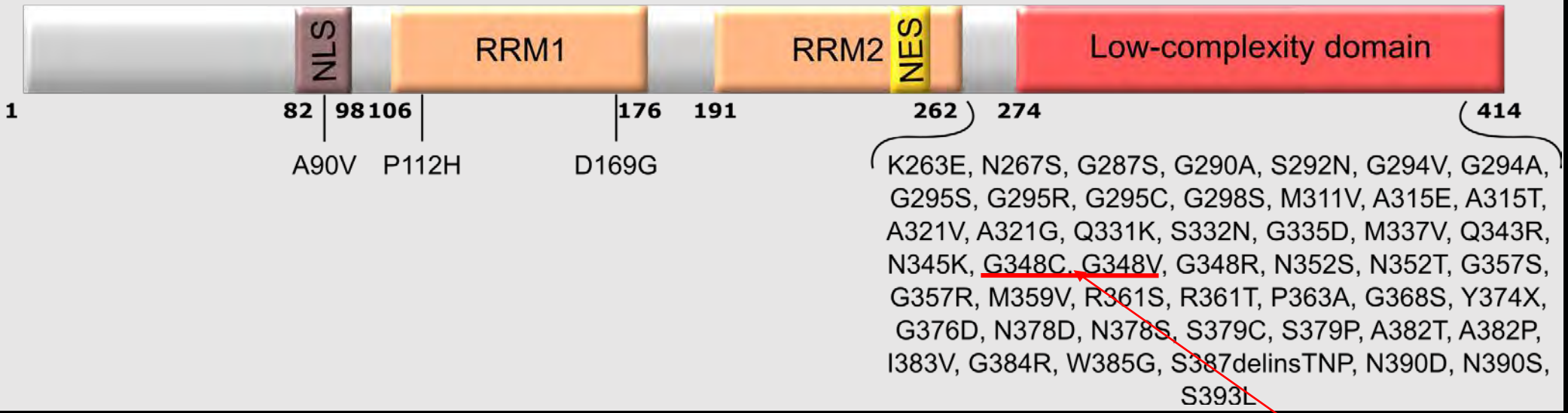
Chemical mutagenesis

- *N*-ethyl-*N*-nitrosourea, **ENU**
-
- F1 males carrying
  ~50 random point mutations

- 10,000 DNAs for screening, sperm frozen

Chemical mutagenesis

MRC Harwell



Human TDP43 ALS mutations

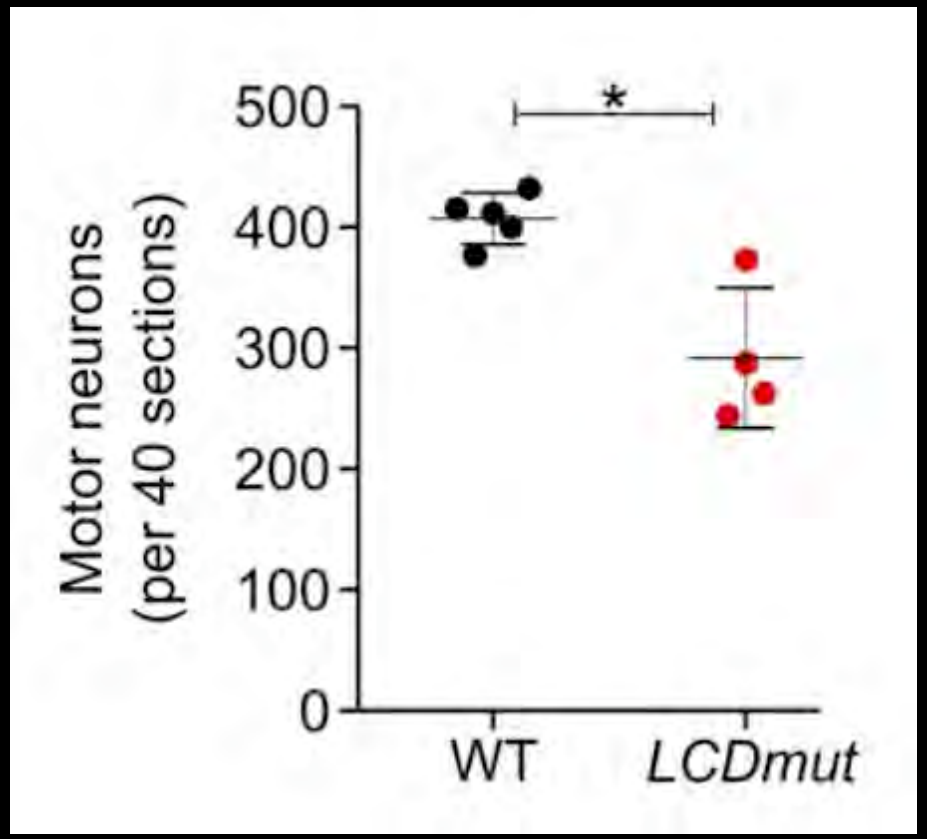
Allelic series *Tardbp* point mutations

M323K

Prasanth Sivakumar

TDP43 LCDmut GOF
spinal cord motor neurons die

Behavioural, physiological, histopathological deficits by 24 months, progressive



Motor neuron loss
Gold Standard for our ALS models

Bernadett Kalmar, Pietro Fratta (UCL)
Abraham Acevedo Arozena (Tenerife)

LCD mutant TDP-43

skiptic exons, GOF

Prasanth Sivakumar, Pietro Fratta (UCL)
Abraham Acevedo Arozena (Tenerife)

TDP43-ALS patient fibroblasts G298S, A382T

skiptic exons, GOF

Prasanth Sivakumar, Pietro Fratta (UCL)
Abraham Acevedo Arozena (Tenerife)

Published online: May 15, 2018

Article

SOURCE DATA | TRANSPARENT PROCESS | OPEN ACCESS

THE EMBO JOURNAL

Mice with endogenous TDP-43 mutations exhibit gain of splicing function and characteristics of amyotrophic lateral sclerosis

Pietro Fratta[1,†,‡,*], Prasanth Sivakumar[1,‡], Jack Humphrey[1,2,‡], Kitty Lo[2,‡], Thomas Ricketts[3,‡], Hugo Oliveira[3,‡], Jose M Brito-Armas[4], Bernadett Kalmar[1], Agnieszka Ule[1], Yichao Yu[5], Nicol Birsa[1], Cristian Bodo[1], Toby Collins[1], Alexander E Conicella[6], Alan Mejia Maza[1], Alessandro Marrero-Gagliardi[4], Michelle Stewart[7], Joffrey Mianne[7], Silvia Corrochano[3], Warren Emmett[2], Gemma Codner[7], Michael Groves[1], Ryutaro Fukumura[8], Yoichi Gondo[8], Mark Lythgoe[5], Erwin Pauws[9], Emma Peskett[9], Philip Stanier[9], Lydia Teboul[7], Martina Hallegger[10], Andrea Calvo[11], Adriano Chiò[11], Adrian M Isaacs[1,12], Nicolas L Fawzi[13], Eric Wang[14], David E Housman[14], Francisco Baralle[15], Linda Greensmith[1], Emanuele Buratti[15], Vincent Plagnol[2], Elizabeth MC Fisher[1,†] & Abraham Acevedo-Arozena[3,4,†,**]

TDP43

The EMBO Journal e98684 | 2018

Pietro Fratta (UCL)
Abraham Acevedo Arozena (Tenerife)

- Chemical mutagenesis
- Gene targeting

Knock in the *human* gene

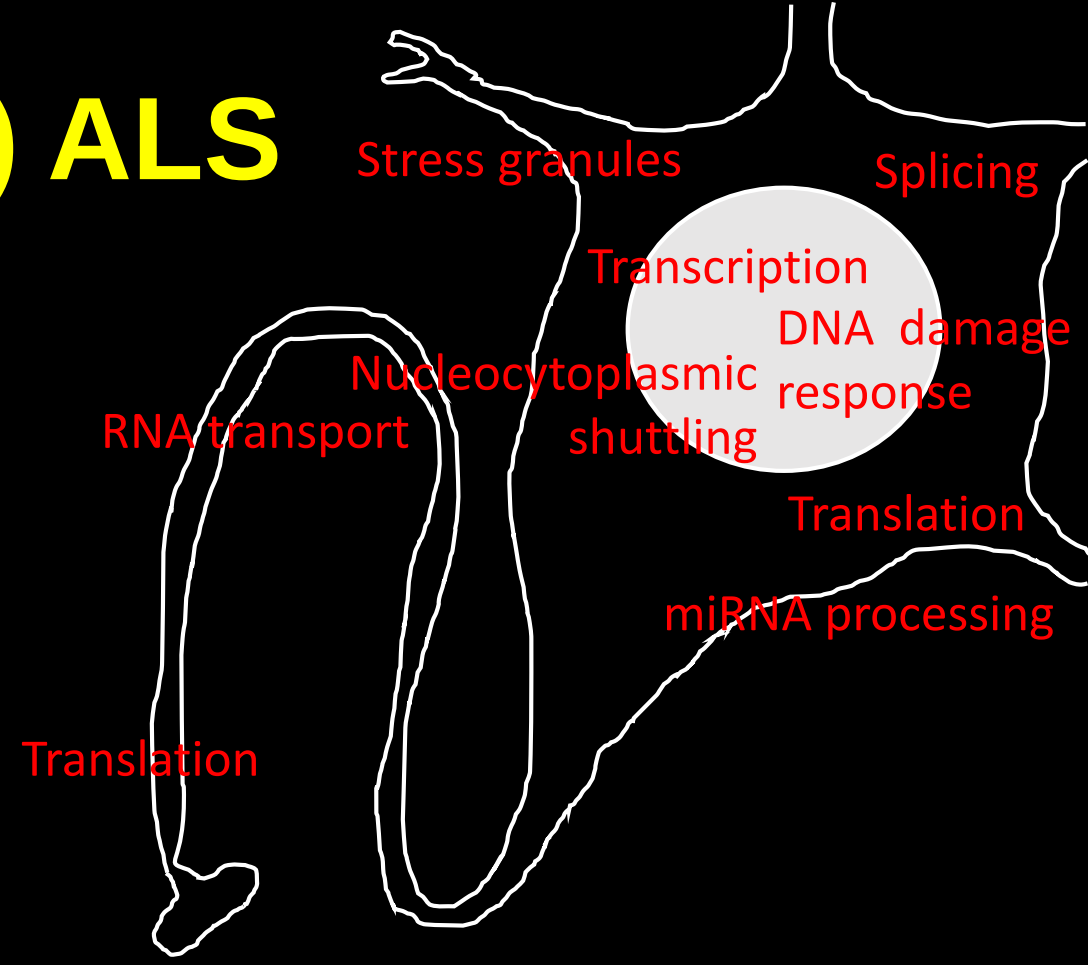
‘Genomic humanisation’

- Human protein chemistry
  APP, SOD1 more ‘aggregatable’
- Human mRNA splice complexity/regulatory sequences

Zhu, F., Nair, R.R., Fisher, E.M.C.*, Cunningham, T.J. (2019) Humanising the mouse genome piece by piece. Nature Comms, **doi.org/10.1038/s4167-019-09716-7**

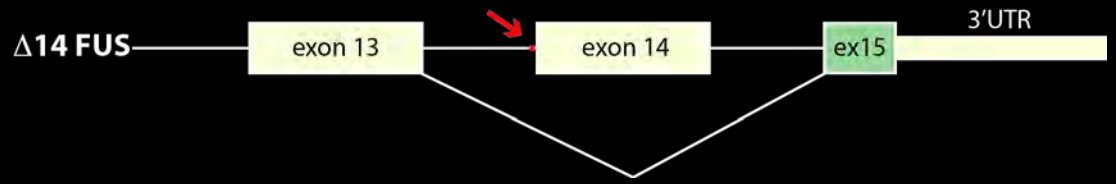
FUS (Fused in sarcoma) ALS

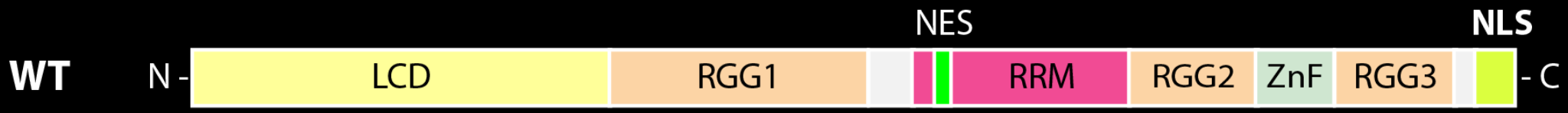
- ~5% fALS, rarely FTD
- Dominant
- FET protein/RBP
- ALS mutations, C-terminal

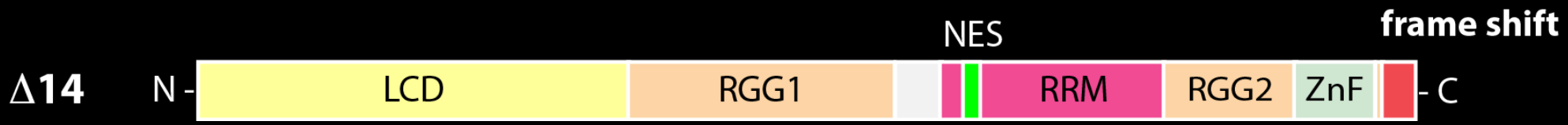


FUS knockin partially humanised

- FUS ‘Delta14’ human ALS; onset 20 y.o., duration 22 months
- Exon 14 missed, frame shift exon 15





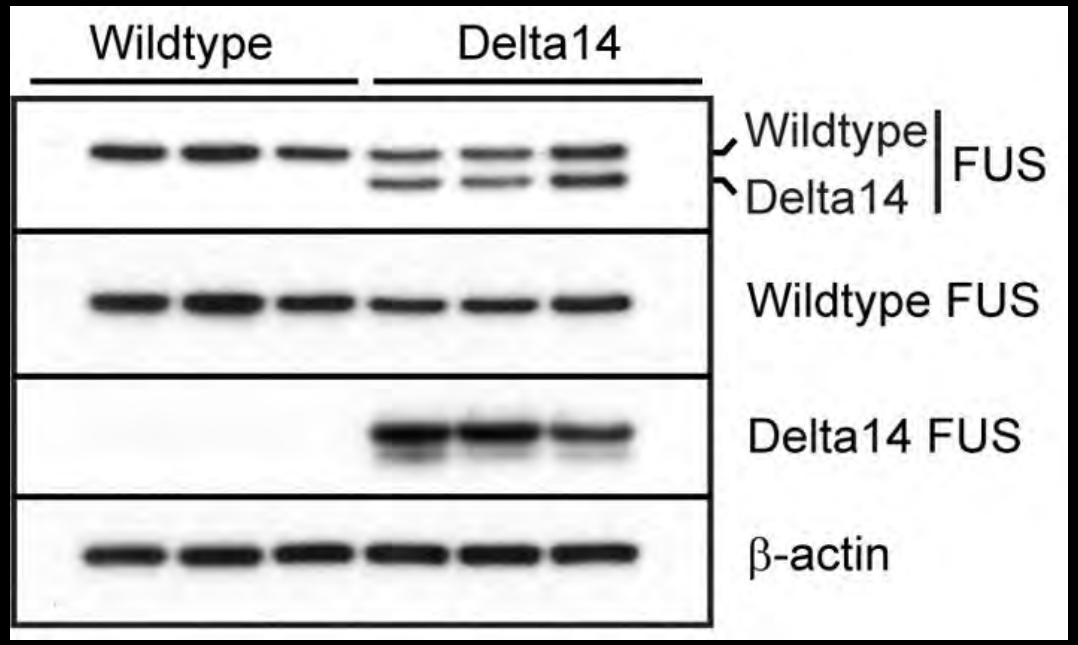


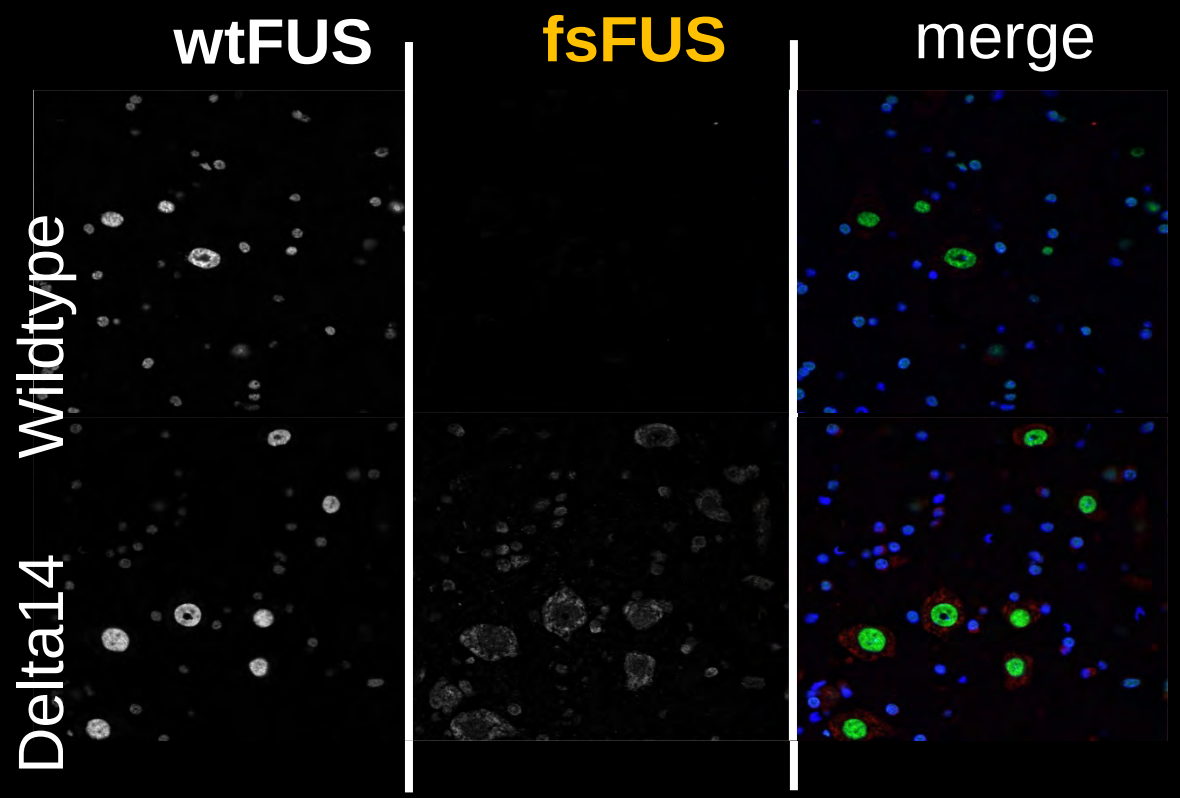
FUS Delta14 antibody!!

Anny Devoy, Pietro Fratta

FUS Delta14 *heterozygotes*

- Physiological expression mRNA/protein
- Mislocalisation





9 month lumbar spinal cord

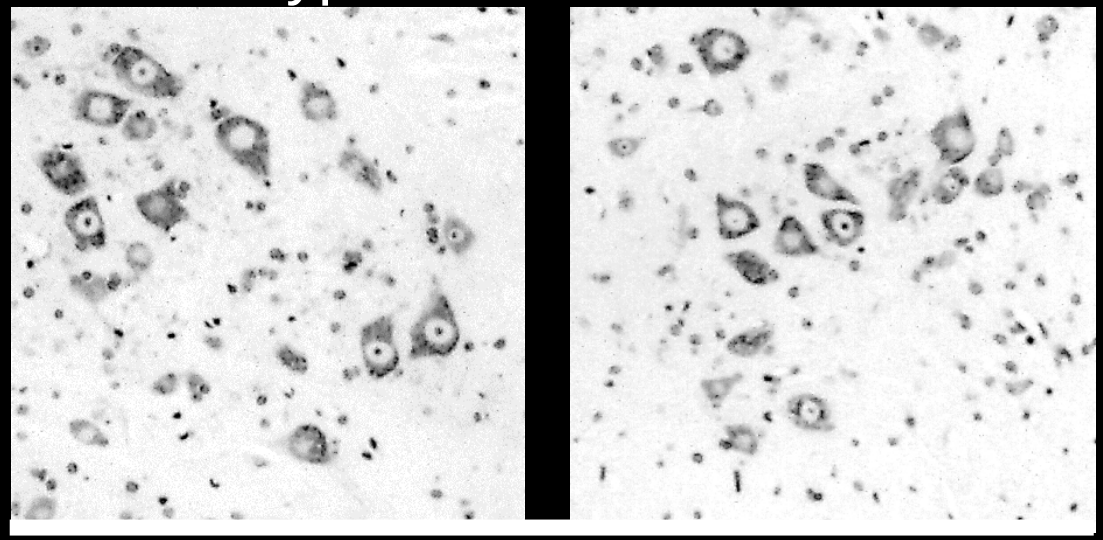
Anny Devoy, Bernadett Kalmar, Pietro Fratta

FUSDelta14 heterozygotes progressive loss motor neurons

Wildtype

FUSDelta14

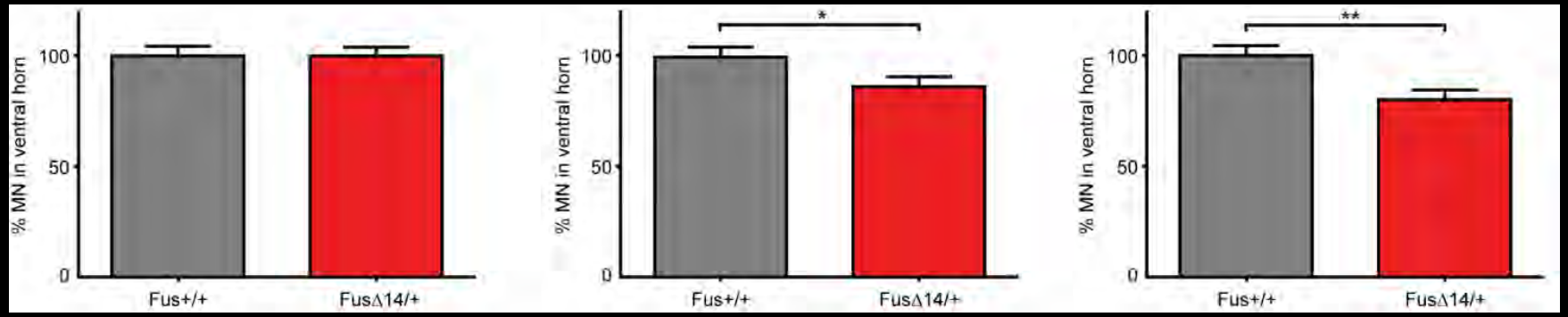
Lumbar L3-4 spinal cord
18 months



3 months

12 months

18 months

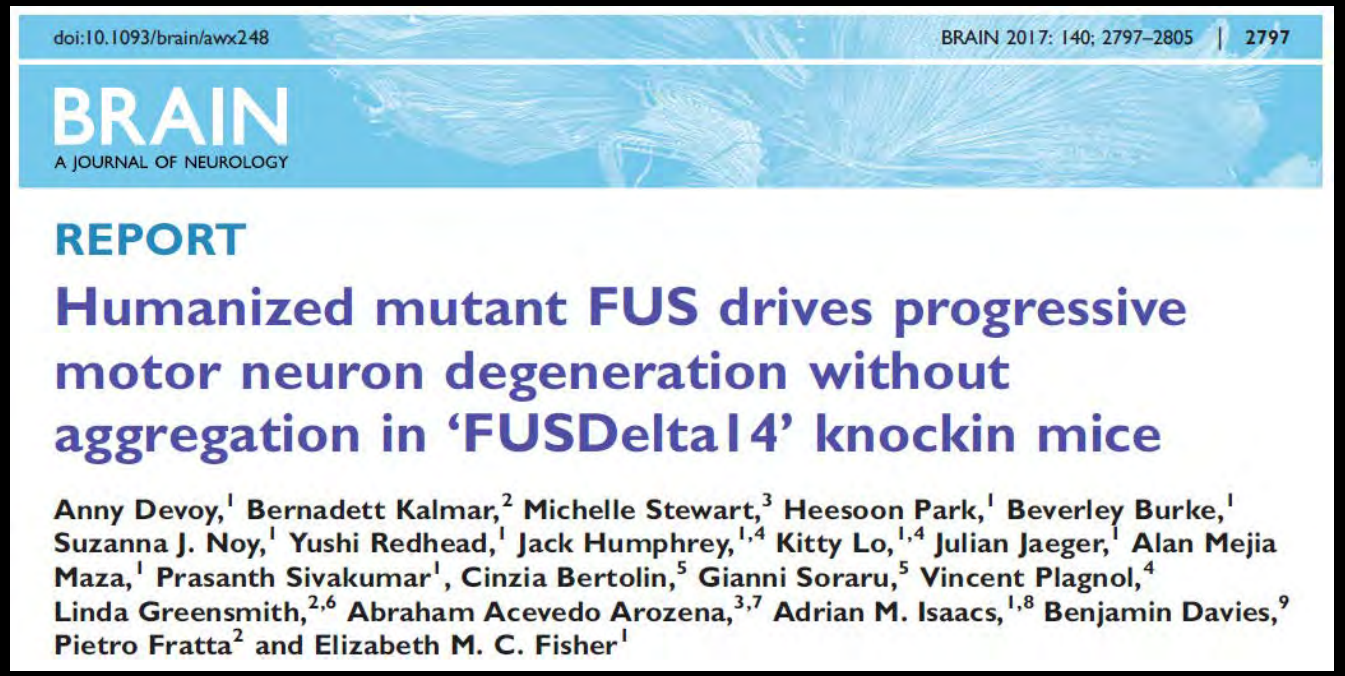


0%

14%

20%

FUSDelta14 mouse



doi:10.1093/brain/awx248 BRAIN 2017: 140; 2797–2805 | 2797

BRAIN
A JOURNAL OF NEUROLOGY

REPORT

Humanized mutant FUS drives progressive motor neuron degeneration without aggregation in 'FUSDelta14' knockin mice

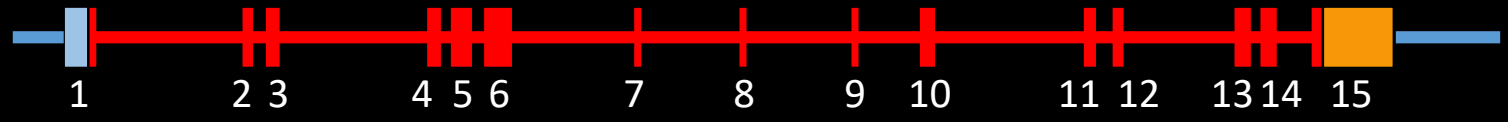
Anny Devoy,[1] Bernadett Kalmar,[2] Michelle Stewart,[3] Heesoon Park,[1] Beverley Burke,[1] Suzanna J. Noy,[1] Yushi Redhead,[1] Jack Humphrey,[1,4] Kitty Lo,[1,4] Julian Jaeger,[1] Alan Mejia Maza,[1] Prasanth Sivakumar[1], Cinzia Bertolin,[5] Gianni Soraru,[5] Vincent Plagnol,[4] Linda Greensmith,[2,6] Abraham Acevedo Arozena,[3,7] Adrian M. Isaacs,[1,8] Benjamin Davies,[9] Pietro Fratta[2] and Elizabeth M. C. Fisher[1]

- Partial humanisation
- Dominant
- NMJ, muscle changes
- Mid-life onset MN death underway by 12 months
- Looks an extremely good model
- EARLY DISEASE EVENTS

- Freely available from EMMA

Devoy et al, (2017) Brain 140:2797–2805

FUS Fully humanised



- ATG to end of 3’ UTR

- Hom wildtype (~7 months, n=23)
- P525L breeding onto C57BL/6J, to N5
- Q519Ifs poor health….few mice

Tom Cunningham, Anny Devoy, Lydia Teboul

Knock in the *human* gene ‘Genomic humanisation’

- FUS ✓
- SOD1 ✓
- TDP43
- C9orf72

Zhu, F., et al. (2019). Nature Comms, **doi.org/10.1038/s4167-019-09716-7**

REVIEW ARTICLE

https://doi.org/10.1038/s41467-019-09716-7 OPEN

Humanising the mouse genome piece by piece

Fei Zhu[1,3], Remya R. Nair[2,3], Elizabeth M.C. Fisher[1] & Thomas J. Cunningham[2]

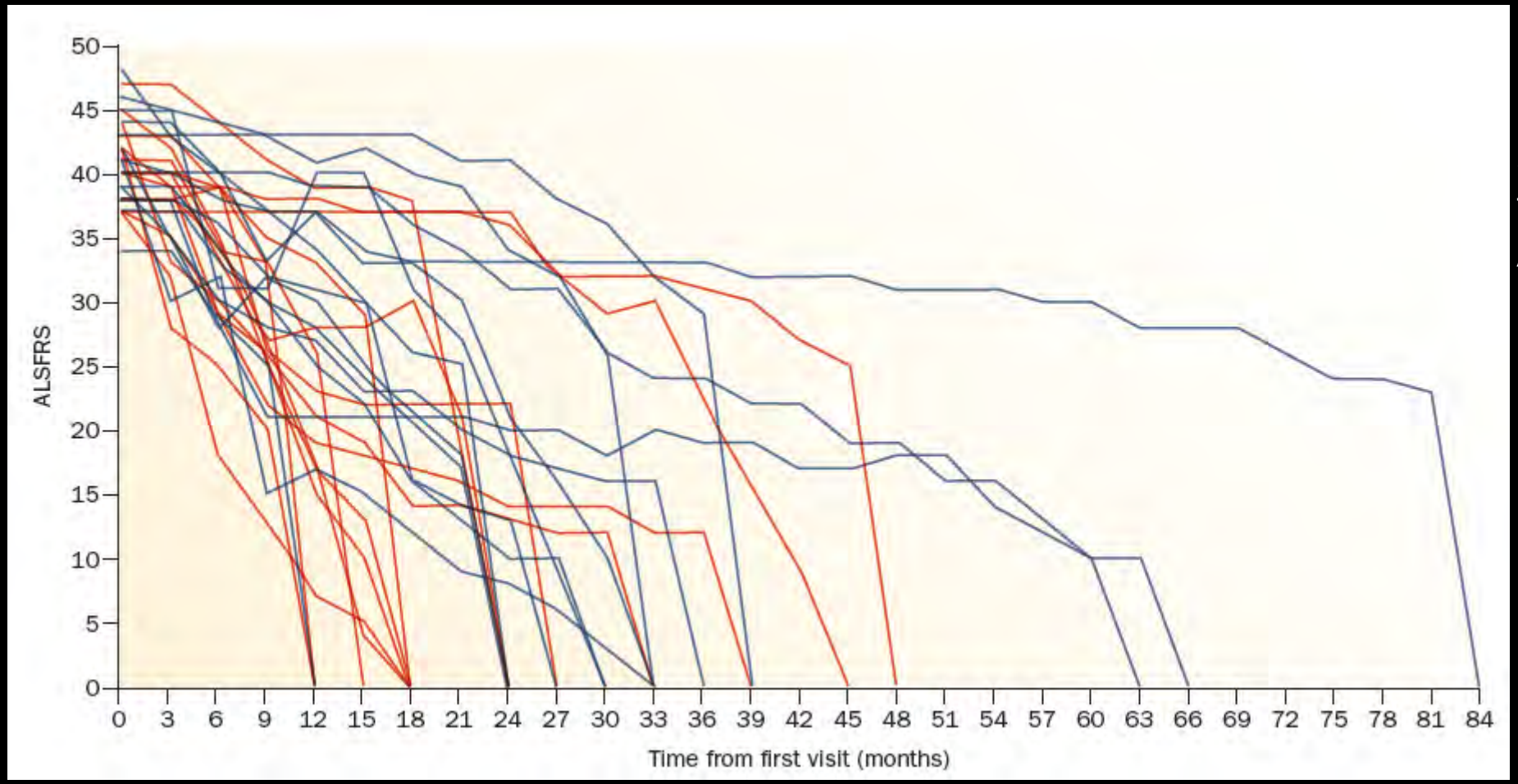
What are we?

- Genetics
- Environment
- Ageing
- Luck
- Metaphysics...

- **Variability!**

by Emily Young

ALS is highly *variable*



30 random patients,
first visit to death,
ALS Functional Rating Scale

Swinnen and Robberecht, The phenotypic variability of ALS. *Nat. Rev. Neurol* (2014)

Translation…*genetic variation*

- Mice: we minimise genetic variation
- Humans: we’re rather genetically variable
- Sexual dimorphism
- Change our mouse genetic backgrounds, known variability

Genetic variation

Mouse genetic backgrounds

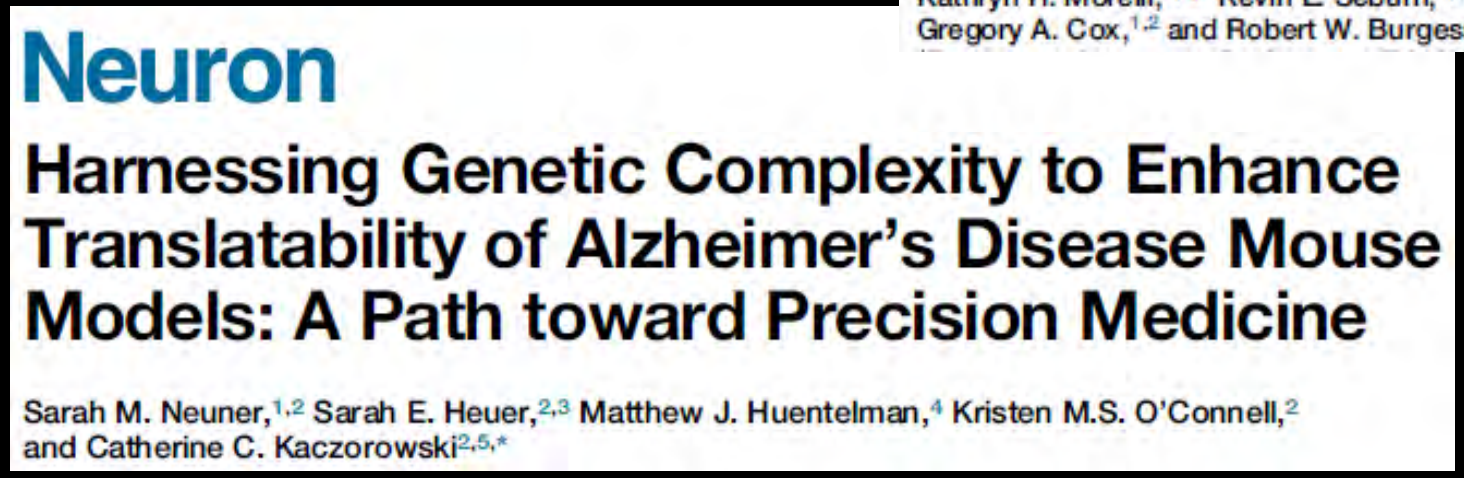
known variability,

IDENTIFY MODIFYING LOCI

Cell Reports

Severity of Demyelinating and Axonal Neuropathy Mouse Models Is Modified by Genes Affecting Structure and Function of Peripheral Nodes

Kathryn H. Morelli,[1,2,3] Kevin L. Seburn,[1,3] David G. Schroeder,[1] Emily L. Spaulding,[1,2] Loiuse A. Dionne,[1] Gregory A. Cox,[1,2] and Robert W. Burgess[1,2,4,*]



Neuron

Harnessing Genetic Complexity to Enhance Translatability of Alzheimer's Disease Mouse Models: A Path toward Precision Medicine

Sarah M. Neuner,[1,2] Sarah E. Heuer,[2,3] Matthew J. Huentelman,[4] Kristen M.S. O'Connell,[2] and Catherine C. Kaczorowski[2,5,*]

Environmental variation

Stress in grandparents, stress in utero, early life stress, birth order, temperature, food, microbiome, environmental enrichment, loneliness, who's in your cage, etc., affect phenotype [*Mind body dualism is dead, sorry Decartes, read John Bowlby*]

Fisher and Bannerman *Sci. Transl. Med.* (2019)

SCIENCE TRANSLATIONAL MEDICINE | REVIEW

NEURODEGENERATIVE DISEASE

Mouse models of neurodegeneration: Know your question, know your mouse

Elizabeth M. C. Fisher[1]* and David M. Bannerman[2]*

Many mutant mouse strains have been developed as models to investigate neurodegenerative disease in humans. However, variability in results among studies using these mouse strains has led to questions about the value of these models. Here, we appraise various mouse models for dissecting neurodegenerative disease mechanisms and emphasize the importance of asking appropriate research questions. In therapeutic studies, we suggest that understanding variability among and within mouse models is crucial for preventing translational failures in human patients.

INTRODUCTION

Neurodegenerative diseases are common, largely untreatable, and certainly incurable and create a huge health and social burden worldwide. For example, in 2018, more than 50 million people worldwide had dementia, of which ~70% was caused by Alzheimer's disease (AD) with an overall cost of $1 trillion (*1*). Currently, ~66% of those with dementia live in low- or middle-income countries (*1*). For neurodegenerative movement disorders, ~10 million people worldwide currently suffer from Parkinson's disease (PD) (*2*). These diseases are not necessarily illnesses of older age, for example, type 1 spinal muscular atrophy (SMA) is the biggest single genetic

Clearly, a multiplicity of models, including genetically modified mice and three-dimensional cellular systems, is required to understand neurodegenerative diseases. For example, human iPSCs provide cellular models and robust in vitro readouts that could be used for high-throughput analysis such as drug screens, which are not feasible in mice. However, mouse models remain essential because they enable us to take a holistic approach over the life span of an animal, giving us access to in vivo systemic interactions, between cell types (for example, glia and neurons), tissues (for example, muscle and neurons), and whole animal systems (for example, the immune system and the nervous system). They also provide access to develop-

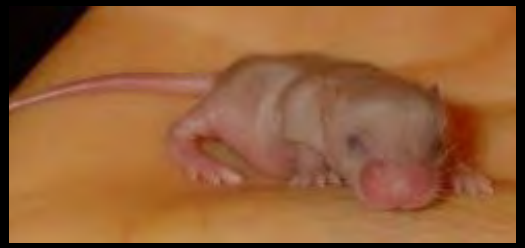
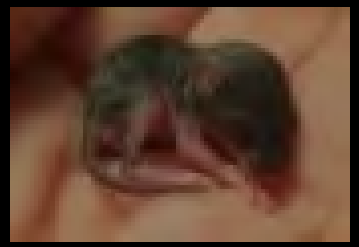
*SOD1*G93A transgenic…

Nature August 2019

Potential roles of gut microbiome and metabolites in modulating ALS in mice

Eran Blacher[1,11], Stavros Bashiardes[1,11], Hagit Shapiro[1,11], Daphna Rothschild[2,3,11], Uria Mor[1], Mally Dori-Bachash[1], Christian Kleimeyer[1], Claudia Moresi[1], Yotam Harnik[1], Maya Zur[1], Michal Zabari[4], Rotem Ben-Zeev Brik[1], Denise Kviatcovsky[1], Niv Zmora[1], Yotam Cohen[1], Noam Bar[2,3], Izhak Levi[2,3], Nira Amar[1], Tevie Mehlman[5], Alexander Brandis[5], Inbal Biton[6], Yael Kuperman[6], Michael Tsoory[6], Leenor Alfahel[7], Alon Harmelin[6], Michal Schwartz[8], Adrian Israelson[7], Liisa Arike[9], Malin E. V. Johansson[9], Gunnar C. Hansson[9], Marc Gotkine[4,12*], Eran Segal[2,3,12*] & Eran Elinav[1,10,12*]

Amyotrophic lateral sclerosis (ALS) is a complex neurodegenerative disorder, in which the clinical manifestations may be influenced by genetic and unknown environmental factors. Here we show that ALS-prone *Sod1* transgenic (*Sod1*-Tg) mice have a pre-symptomatic, vivarium-dependent dysbiosis and altered metabolite configuration, coupled with an exacerbated disease under germ-free conditions or after treatment with broad-spectrum antibiotics. We correlate eleven distinct commensal bacteria at our vivarium with the severity of ALS in mice, and by their individual supplementation into antibiotic-treated *Sod1*-Tg mice we demonstrate that *Akkermansia muciniphila* (AM) ameliorates whereas *Ruminococcus torques* and *Parabacteroides distasonis* exacerbate the symptoms of ALS. Furthermore, *Sod1*-Tg mice that are administered AM are found to accumulate AM-associated nicotinamide in the central nervous system, and systemic supplementation of nicotinamide improves motor symptoms and gene expression patterns in the spinal cord of *Sod1*-Tg mice. In humans, we identify distinct microbiome and metabolite configurations—including reduced levels of nicotinamide systemically and in the cerebrospinal fluid—in a small preliminary study that compares patients with ALS with household controls. We suggest that environmentally driven microbiome-brain interactions may modulate ALS in mice, and we call for similar investigations in the human form of the disease.


Ageing and luck

- Capture snapshots over time, for disease trajectory
- Lessons from modelling cancer…

Mice...ideally…

- Match the model to the question
- What is a good mouse model? MN degeneration
- Physiological models for early disease processes but need funding… and cellular phenotypes…

- Human:mouse chimeras
- New analyses (e.g. Home cage videos with machine learning)

- Human trial stratification

UCL Institute of Neurology
Alan Meija Maza
Anny Devoy

Adrian Isaacs
Carmelo Milioto

Linda Greensmith
Bernadett Kalmar

Giampietro Schiavo

Pietro Fratta
Prasanth Sivakumar
Jack Humphrey
Kitty Lo
Vincent Plagnol
Nicol Birsa
Matthew Bentham

MRC Harwell
Tom Cunningham
Lydia Teboul
Michelle Stewart
Peter Joyce
Tom Ricketts
Hugo Oliveira
Asif Nukhuda
Charlotte Tibit
Samanta Gasco
Remya Nair

ICGEB, Trieste
Francisco Baralle
Emanuele Buratti

Wellcome Trust Centre for Human Genetics
Ben Davies

Hospital Universitario de Canarias
Abraham Acevedo