Motor neuron disease genetics

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Honorary Consultant Neurologist

Bridgend, Wales 14-11-2023

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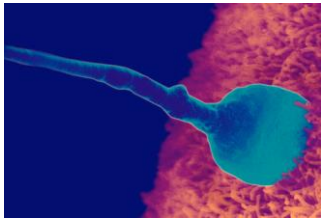
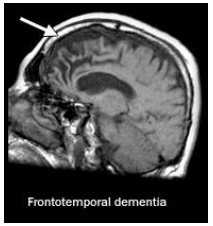
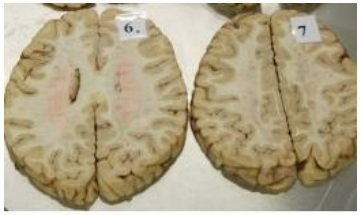
Dept of Basic and Clinical Neuroscience

Routes to therapies for ALS

Biomarkers







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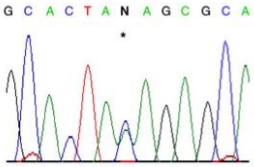
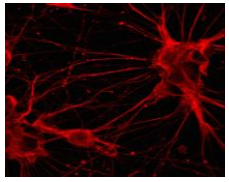
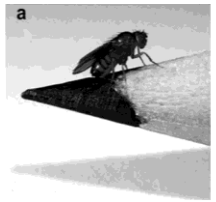
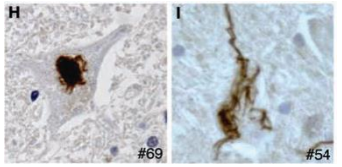


Genetics

‘Modelling’







Pathology

Artificial but useful distinction between fALS and sALS

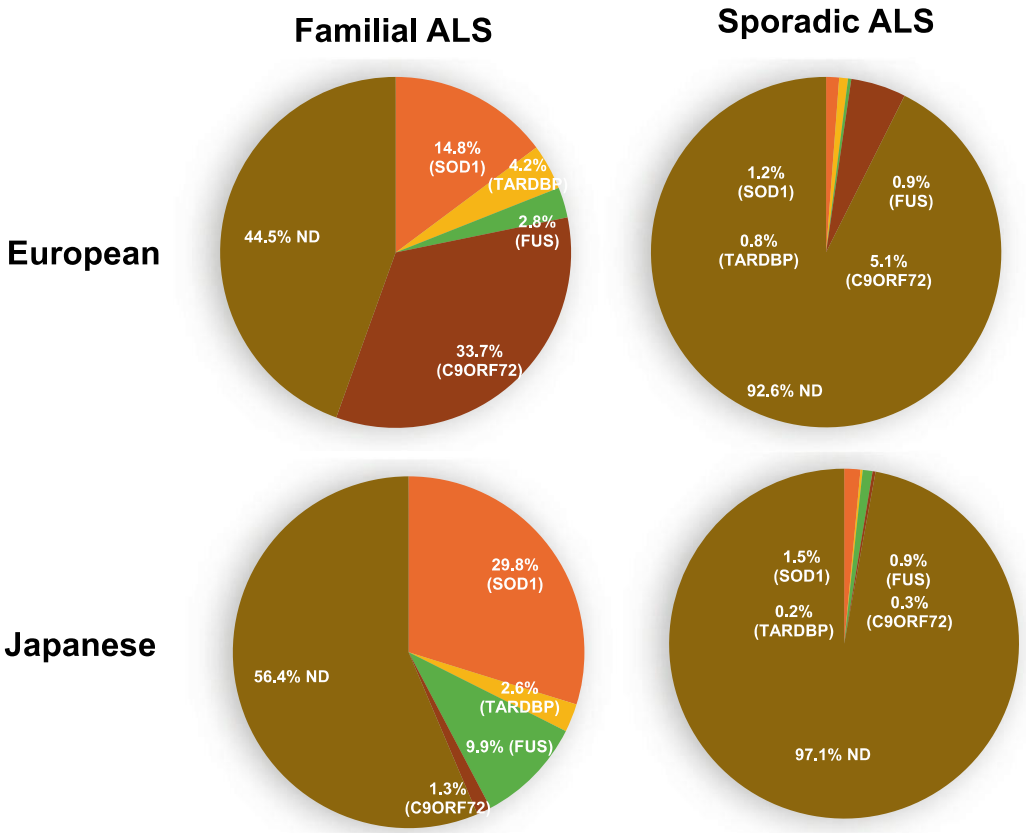
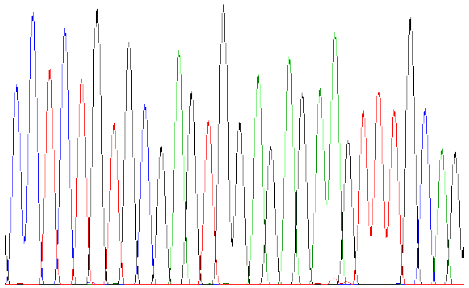


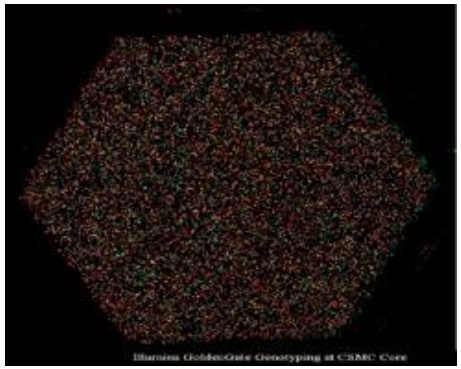
Fig. 1 Racial/ethnic difference of amyotrophic lateral sclerosis (ALS) causative genes [28, 29, 93]. The pie charts of ALS causative genes in Europeans and Japan are shown, color-coded with *SOD1, TARDBP, FUS, C9ORF72*, and not determined (ND) in these four genes. Mutations were identified in 55.5% of Europeans with familial ALS and 43.6% of Japanese individuals with familial ALS. In sporadic ALS, only 7.4% of mutations were identified in Europe and 2.9% in Japan. The difference between European and Japanese is largely due to the difference in the frequency of *C9ORF72* mutation, *SOD1*, and *FUS* being more common in Japanese and *TARDBP* being more common in European

Suzuki et al J Hum Genetics 2023

Gene sequencing advances



2000
Sanger sequencing
ABI 3100
1 persons genome
15 years
$2 Billion



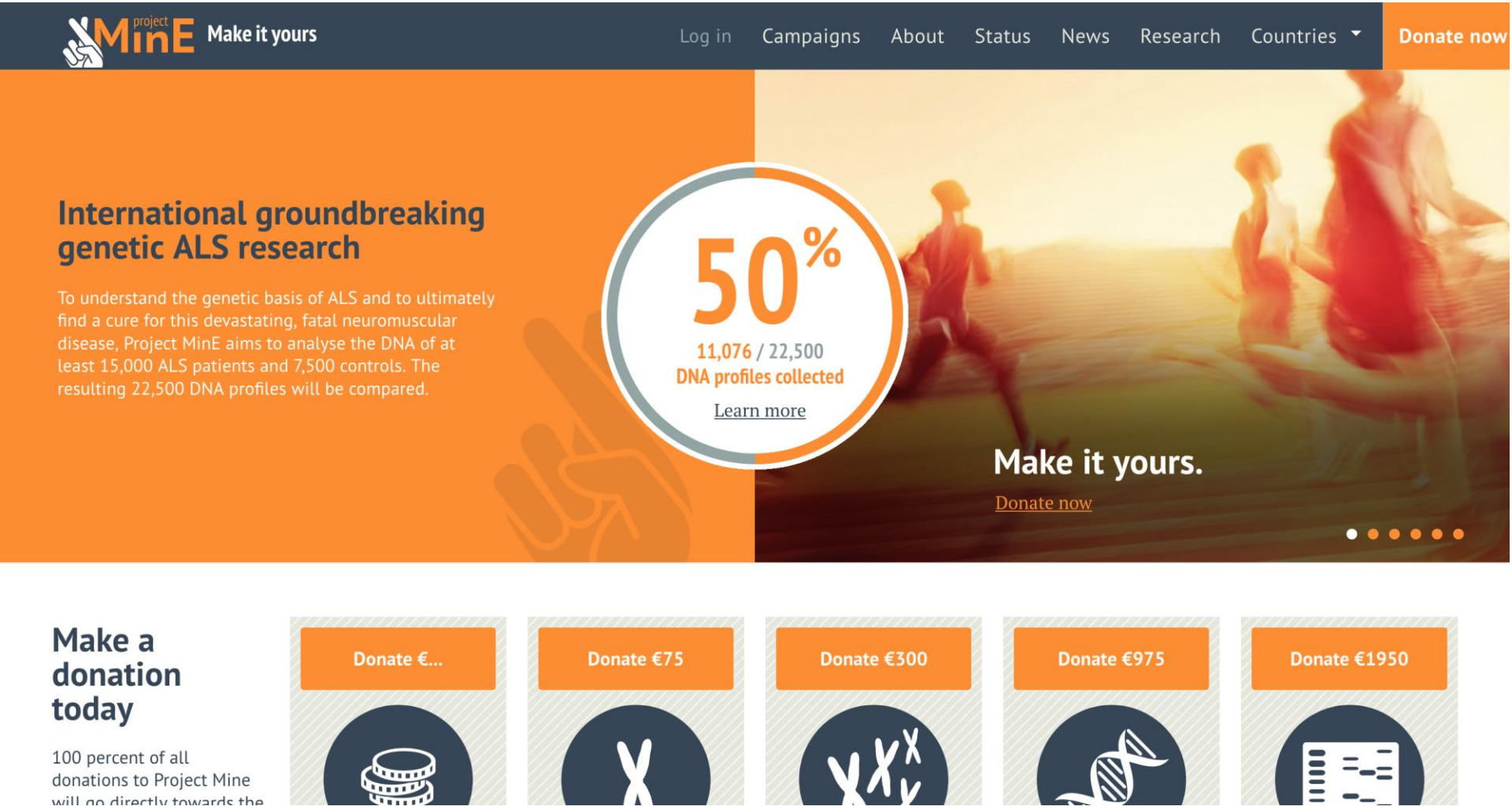
2022
Sequencing by synthesis
Illumina HiSeq 2000
1 persons genome
5 hours and 2 minutes (Jan 2022)
$399

Sequencing DNA is now 25,000 times faster and 50,00,000 times cheaper

New sequencing technologies have the power to identify all ALS genes

Mutant ALS genes can be identified in >10% of apparently sporadic ALS

WGS – accelerating gene discovery



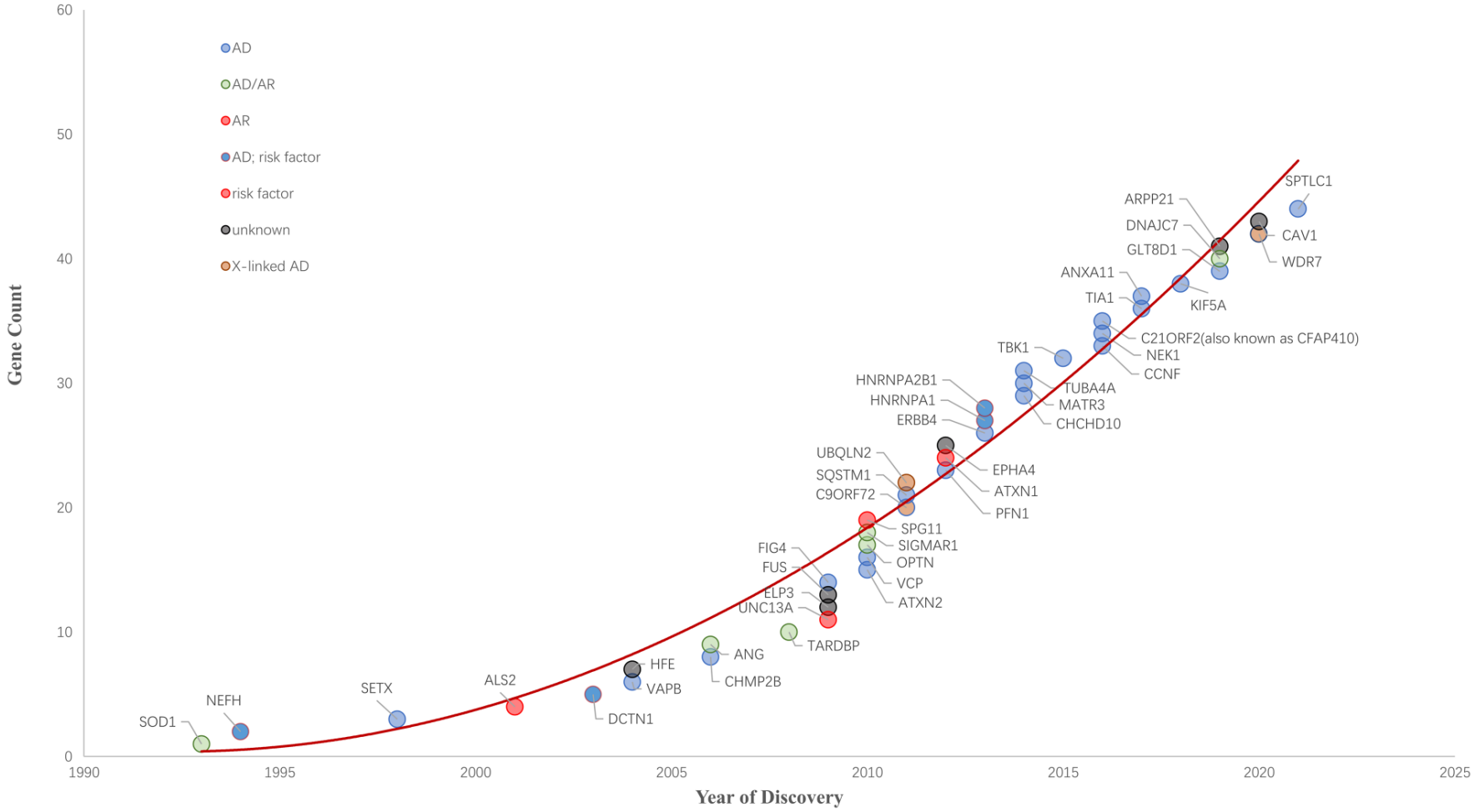
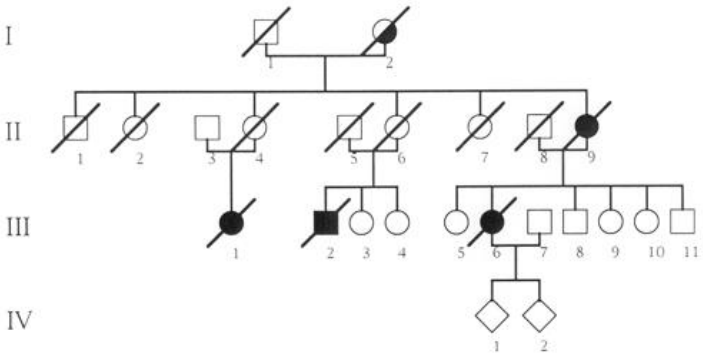


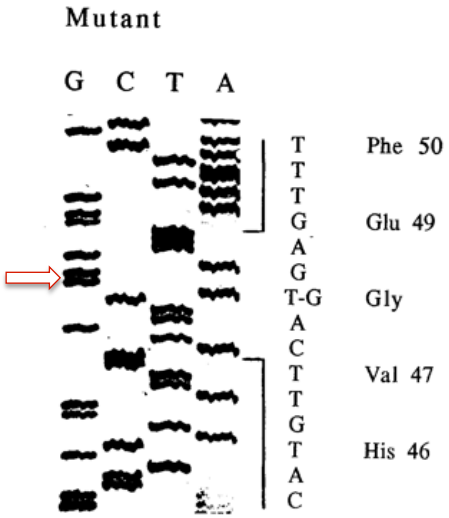
FIGURE 1
ALS Gene Discovery from 1990 to 2022. The cumulative number of ALS-related genes discovered is growing rapidly. ALS-related genes are plotted and their respective inheritance patterns are represented by different colored circles.

Wang et al Front. Neurosci 2023

ALS1- SOD1 (1993). The first ALS gene



I 2	died 1936	aged 39 years	Rapidly progressive "crippling disease"
II 9	died 1975	aged 56 years	Motor neurone disease (limb)
III 1	died 1980	aged 38 years	Motor neurone disease (limb)
III 2	died 1987	aged 58 years	Motor neurone disease (limb)
III 6	died 1994	aged 54 years	Motor neurone disease (limb)

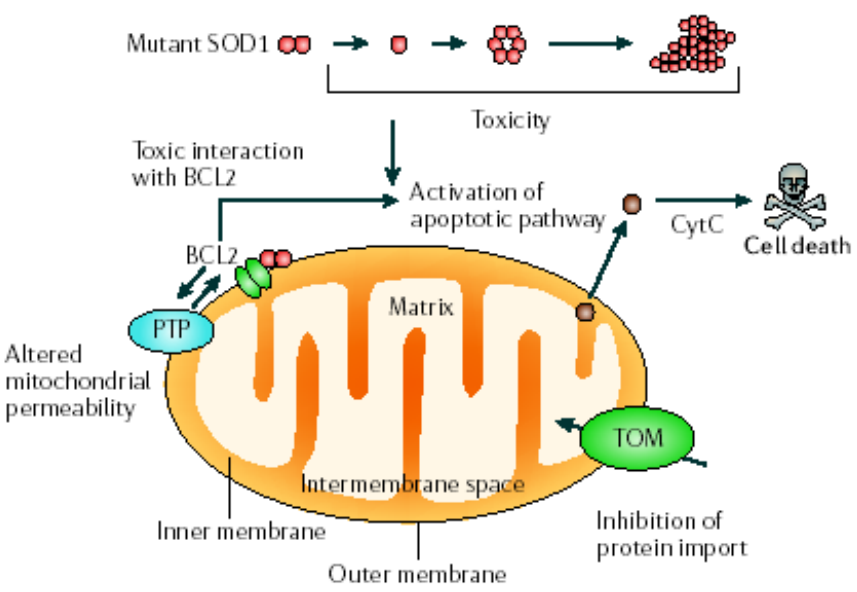


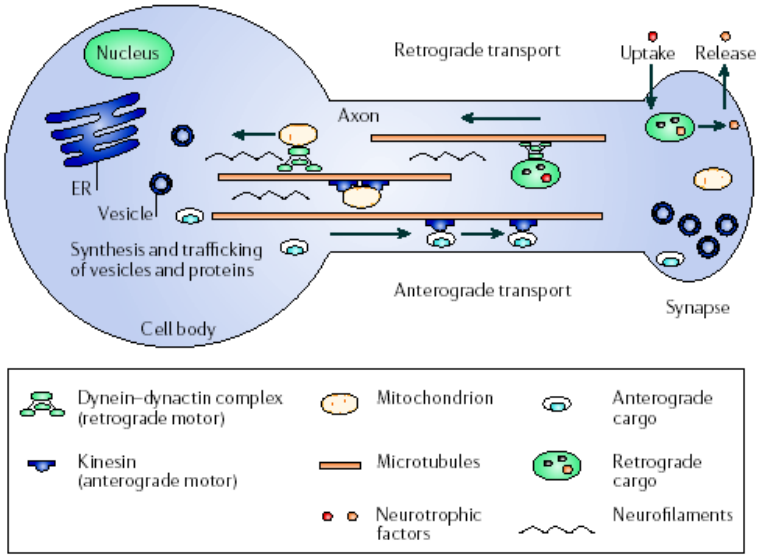
Raymond Van den Bergh, Wu-Yen Hung†, Thomas Bird††, Gang Deng†, Donald W. Mulder‡‡, Celestine Smyth§§, Nigel G. Laing§§, Edwin Soriano†, Margaret A. Pericak-Vance|||, Jonathan Haines¶¶, Guy A. Rouleau§, James S. Gusella¶¶, H. Robert Horvitz|| & Robert H. Brown Jr* #**

* Day Neuromuscular Research Laboratory, Massachusetts General Hospital, Room 6627, MGH-East, Building 149, 13th Street, Charlestown, Massachusetts 02129, USA; † Department of Neurology, Northwestern University Medical School, Chicago, Illinois 60611, USA; ‡ Eleanor Roosevelt Institute for Cancer Research and the University of Colorado Health Science Center, Denver, Colorado 80206, USA; § Center for Research in Neuroscience, McGill University, and the Montreal General Hospital Research Institute, Montreal PQ H3G 1A4, Canada; || Howard Hughes Medical Institute, Department of Biology, Massachusetts Institute of Technology, Boston, Massachusetts 02139, USA; ¶ Laboratory of Genetics and Aging, Neuroscience Center, Massachusetts General Hospital, Boston, Massachusetts 02129, USA; # Department of Neurology, Northshore University Hospital, Manhasset, New York 11030, USA; ** Department of Neurology, Universitaire Ziekenhuizen, Leuven 3000, Belgium; †† Department of Neurology, University of Washington School of Medicine, Seattle, Washington 98195, USA; ‡‡ Department of Neurology, Mayo Clinic, Rochester, Minnesota 55905, USA; §§ Australian Neuromuscular Research Institute, Nedlands, Western Australia, Australia; ||| Department of Medicine (Neurology), Duke University Medical Center, Durham, North Carolina 27710, USA; ¶¶ Molecular Neurogenetics Laboratory, Neuroscience Center, Massachusetts General Hospital, Boston, Massachusetts 02129, USA

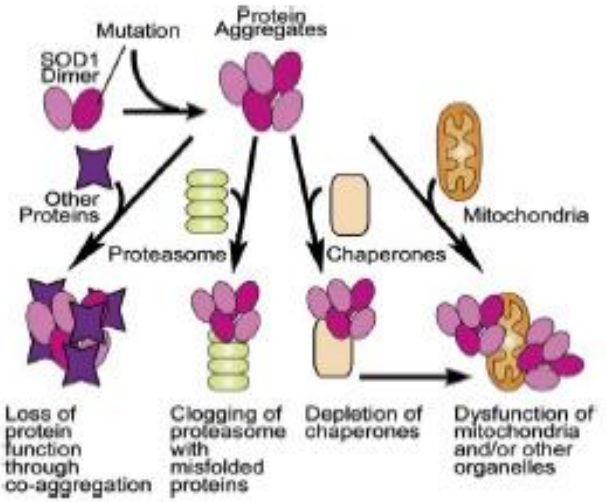
AMYOTROPHIC lateral sclerosis (ALS) is a degenerative disorder

SOD1: Toxic gain of function

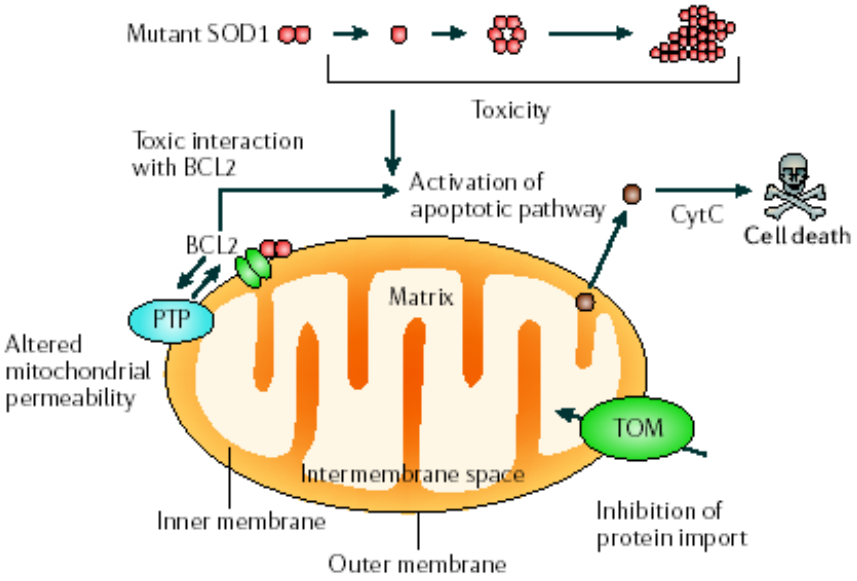


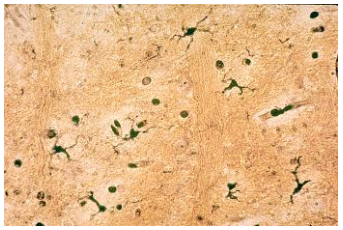


- Mitochondrial dysfunction
- Excitotoxicity
- (Oxidative stress)
- Inflammation
- Impaired axonal transport
- SOD1 misfolding, protein aggregation
- Proteasomal inhibition
- Apoptosis

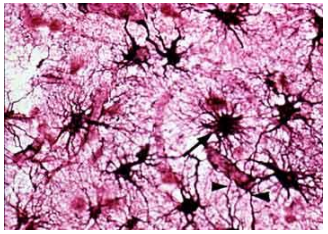


SOD1: Toxic gain of function





SOD1 mutant Microglia: **promote late disease progression**



SOD1 mutant Astrocytes: **promote motor neuron death, activate microglia, decrease survival**

- Mitochondrial dysfunction
- Excitotoxicity
- (Oxidative stress)
- Inflammation
- Impaired axonal transport
- SOD1 misfolding, protein aggregation
- Proteasomal inhibition
- Apoptosis

Ilieva et al *J Cell Biology* 2009

BUT, 95% of ALS is not characterised by SOD1 pathology, but by **ubiquitinated** inclusions

Ubiquitin deposits in anterior horn cells in motor neurone disease

P.N. Leigh[1], B.H. Anderton[2], A. Dodson[3], J.-M. Gallo[2], M. Swash[4] and D.M. Power[2]

Neuroscience Letters, 93 (1988) 197–203
Elsevier Scientific Publishers Ireland Ltd.

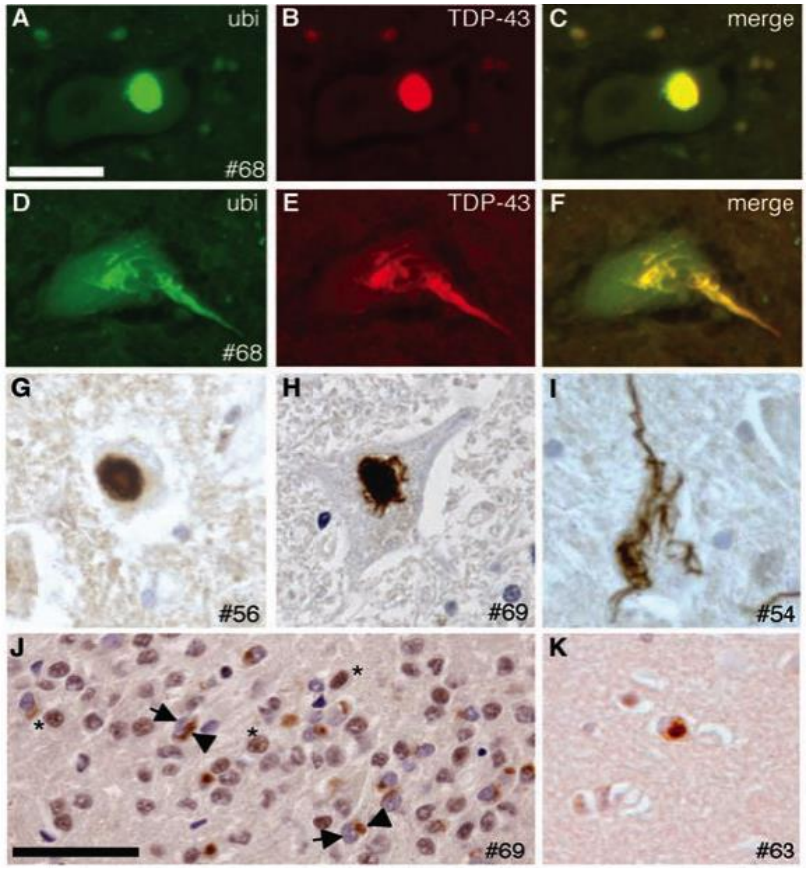
Familial frontotemporal dementia with ubiquitin-positive, tau-negative inclusions

A. Kertesz, MD, FRCPC; T. Kawarai, MD; E. Rogaeva, PhD; P. St. George-Hyslop, MD, FRCPC; P. Poorkaj, PhD; T.D. Bird, MD; and D.G. Munoz, MD, FRCPC

NEUROLOGY 2000;54:818–827

The major ubiquitinated protein in 95% of ALS and 50% of FTD is **TDP-43**

- *TARDBP* gene
- Highly conserved
- ~nuclear protein
- DNA & RNA binding
- RNA processing
- Autoregulates



Neumann et al. 2006

ALS-FTD

- Mislocalisation (nuclear depletion +/- cytoplasmic aggregation)
- Fragmentation
- Phosphorylation
- Ubiquitination
- Autoregulation may be disturbed
- Cryptic exons appear

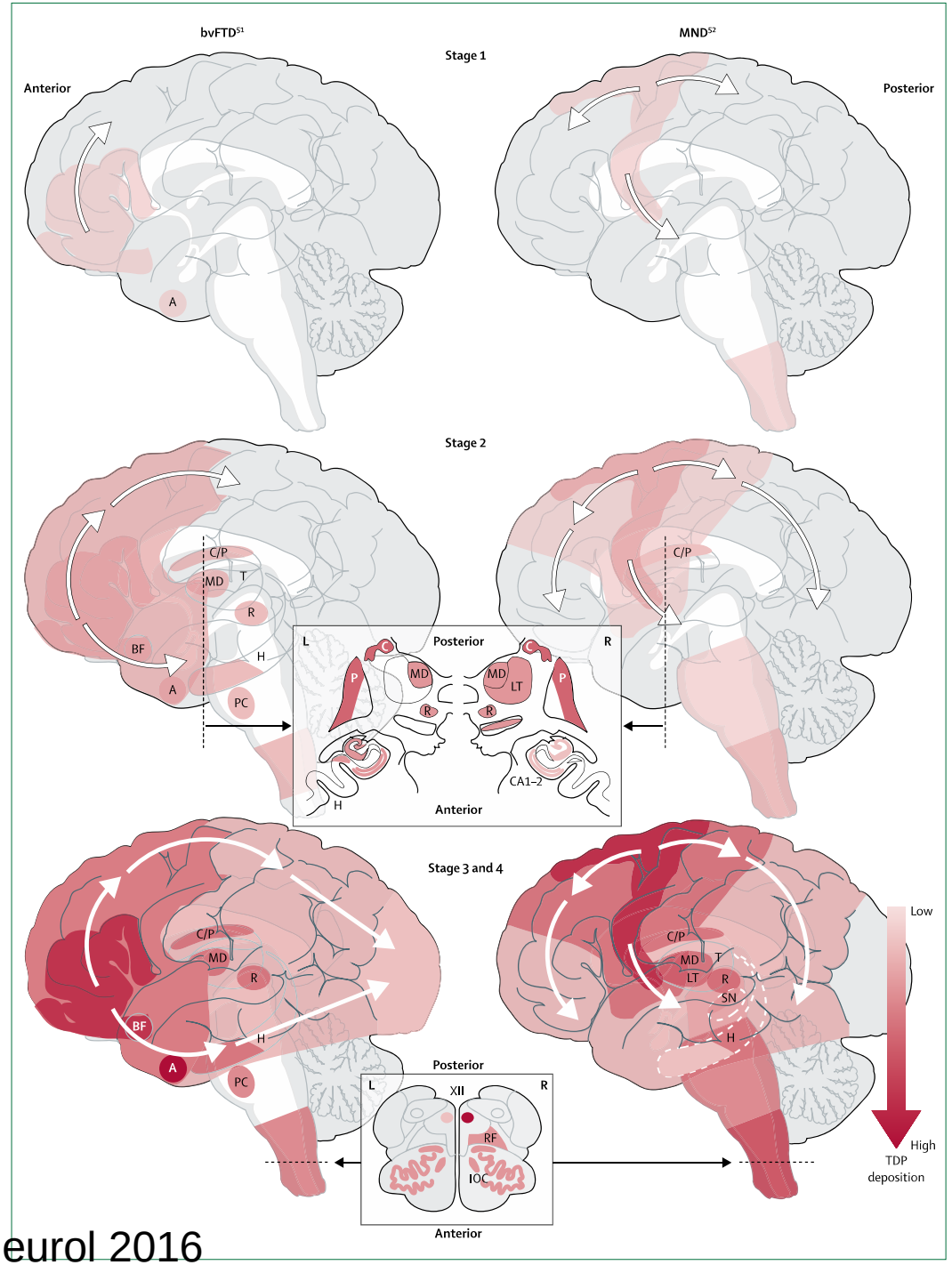
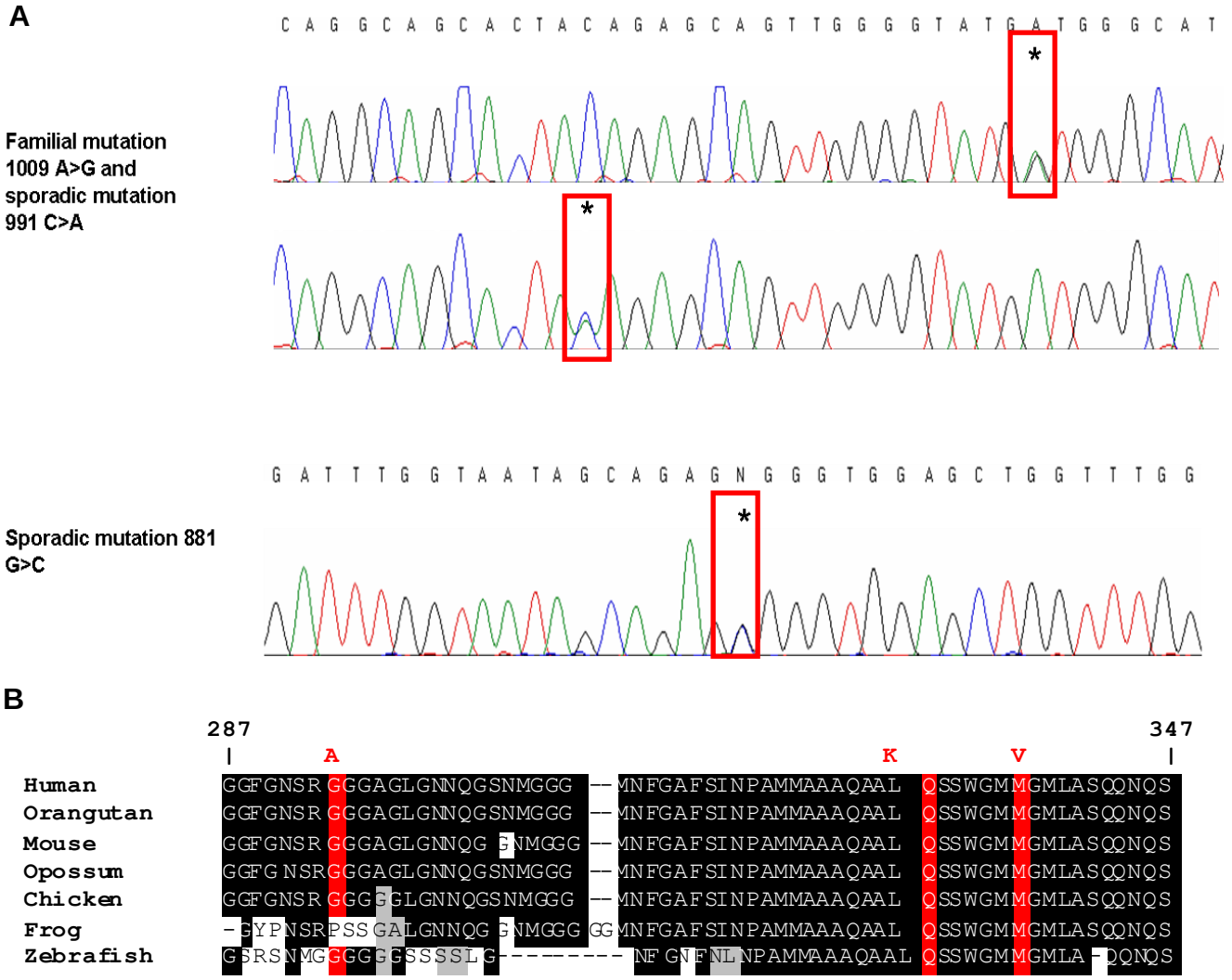


Figure 3: **Stages of TDP-43 deposition in behavioural variant-frontotemporal dementia compared with motor neuron disease**

Behavioural variant-frontotemporal dementia (bvFTD) with types A and B TAR DNA binding protein (TDP-43) deposition begins (stage 1) with deposits in the anterior and basal aspects of the frontal lobe and amygdala (A). By stage 2, the pathology has increased in the anterior frontal and basal forebrain (BF) and spread further into other frontal lobe and anterior temporal lobe regions, including the hippocampus (H), the caudate nucleus and putamen (C/P), and the mediodorsal nucleus (MD) of the thalamus (T), red nucleus (R), precerebellar nuclei (PC), and dorsomedial medullary regions. By stage 3, these regions have increased deposition and cortical, brainstem, and spinal motor regions become involved. By stage 4 the visual cortex also has TDP-43 deposition. Adapted from Brettschneider and colleagues,[51] by permission of Springer. Motor neuron disease (MND) with TDP-43 deposition begins (stage 1) in upper motor neurons in the cortex or lower motor neurons in the spinal cord and lower brainstem. By stage 2, TDP-43 pathology has progressed to the posterior frontal and anterior parietal regions, brainstem reticular formation (RF), and PC and R. By stage 3, the anterior frontal and basal forebrain, the C/P, MD and lateral thalamus (LT), and substantia nigra (SN) are involved, with stage 4 cases also having TDP-43 deposition in the anterior temporal lobe, including the H. CA1–2=cornu armmonis subregions 1 and 2 of the hippocampus. XII=hypoglossal nucleus. IOC=inferior olivary complex.

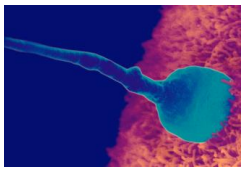
Burrell et al Lancet Neurol 2016

TARDBP mutations occur in ALS, indicating a mechanistic role for TDP-43 in neurodegeneration



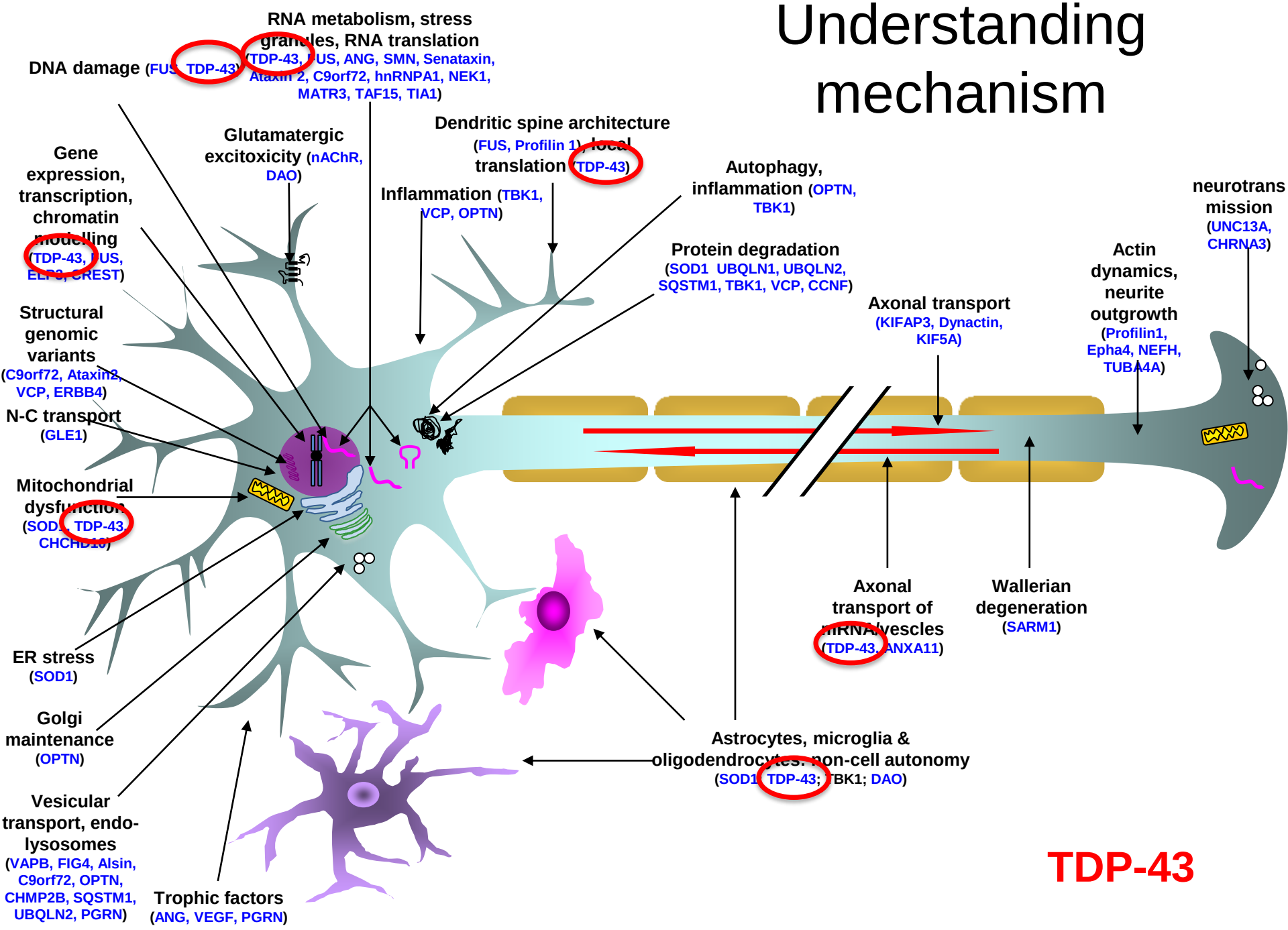
Sreedharan et al 2008

Modelling disease-linked TDP-43 mutations may allow us to understand mechanisms of disease



Li et al J of Neurology 2022

Understanding mechanism



TDP-43

C9orf72 – the commonest genetic cause of ALS and FTD

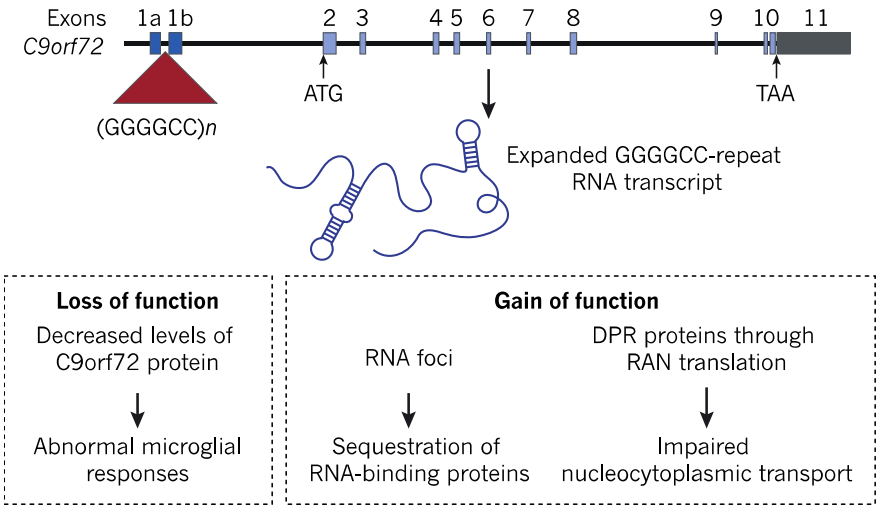


Figure 4 | Proposed mechanisms for the development of C9 ALS–FTD. Expansion of an intronic hexanucleotide repeat (GGGGCC) in *C9orf72* from fewer than 23 copies to hundreds or thousands of copies causes C9 ALS–FTD. This mutation results in a modest reduction in the levels of C9orf72 protein (left) that seems insufficient to cause disease but might contribute to its progression through abnormal microglial responses. Meanwhile, the expression of sense and antisense RNA transcripts that contain the expanded repeat probably drive a toxic gain of function (right). The two main gain-of-function modes that are implicated are: toxicity through the sequestration of RNA-binding proteins in RNA foci by the expanded GGGGCC repeat RNA transcript; and the production of DPR proteins through RAN translation, leading to toxicity through several cellular targets such as membraneless organelles and nuclear pores.

Taylor, Brown, Cleveland 2016

Possible Mechanisms of C9 toxicity

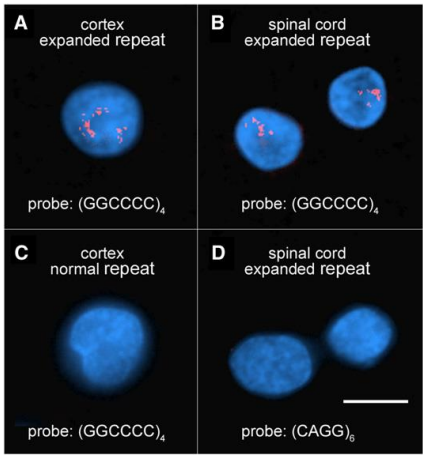
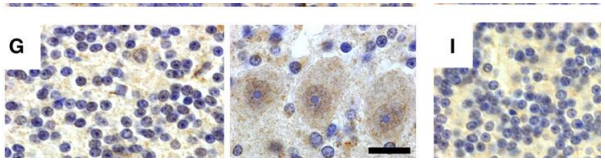
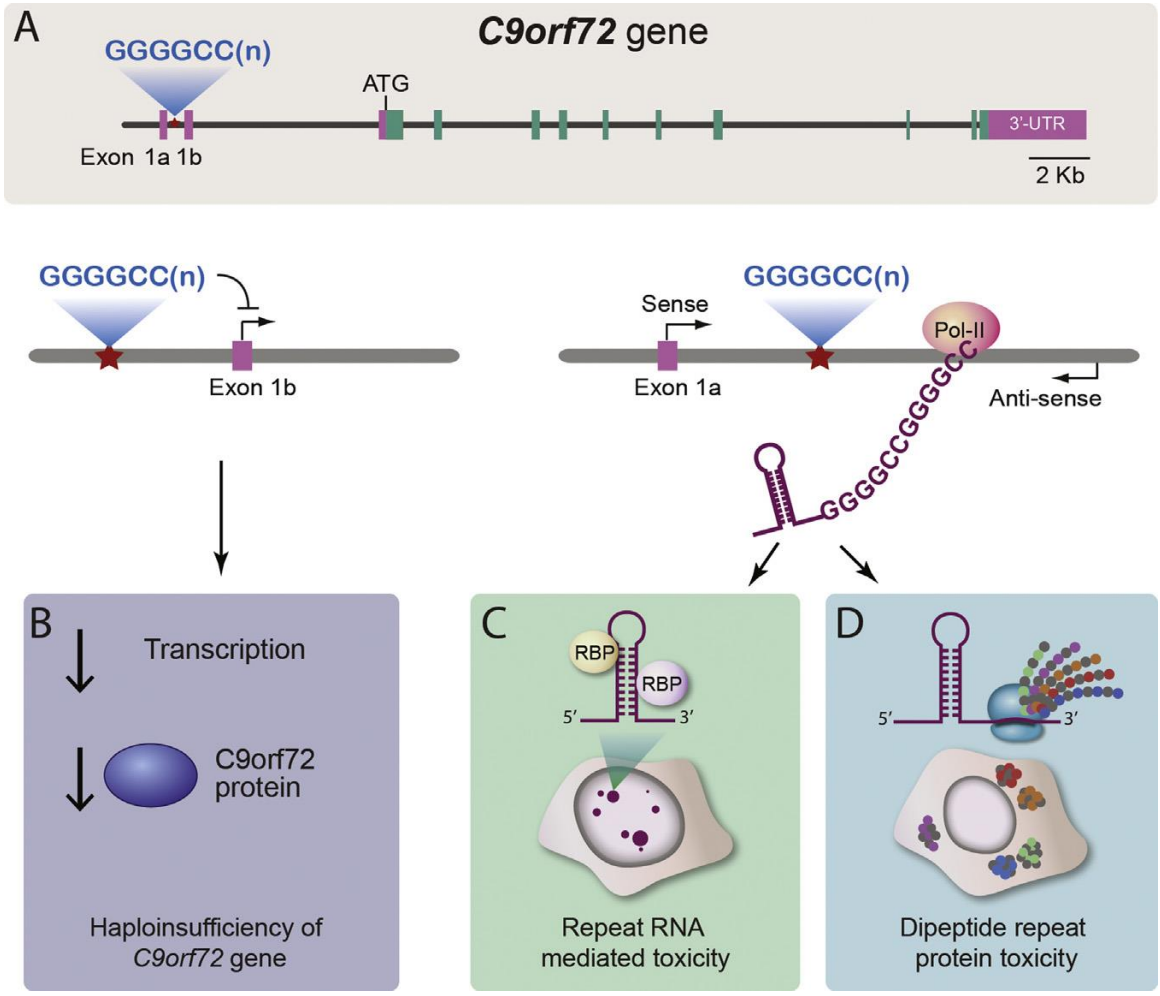


Figure 5. Expanded GGGGCC Hexanucleotide Repeat Forms Nuclear RNA Foci in Human Brain and Spinal Cord





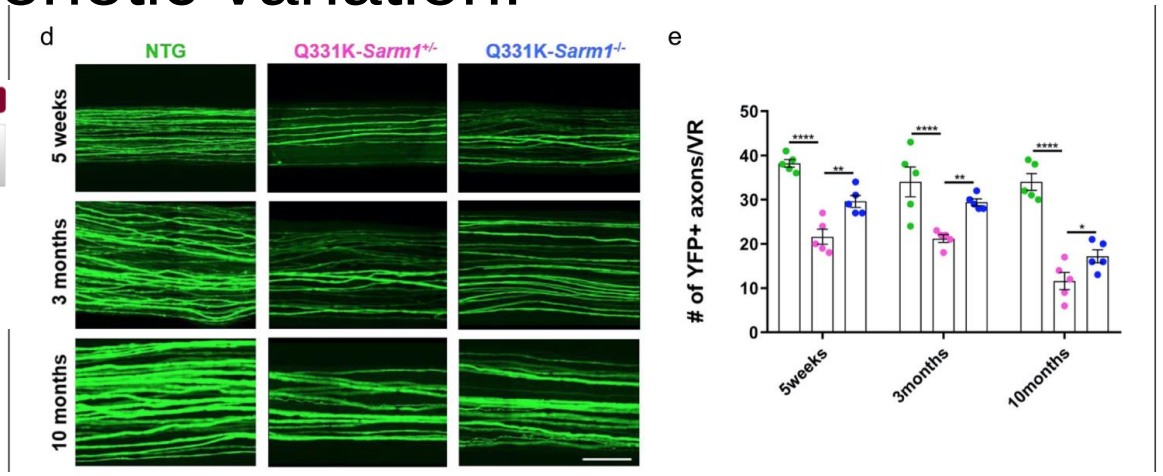
Gitler and Tsuiji Brain Res 2016

Axon degeneration – target for therapy and source of genetic variation!

RESEARCH Open Access

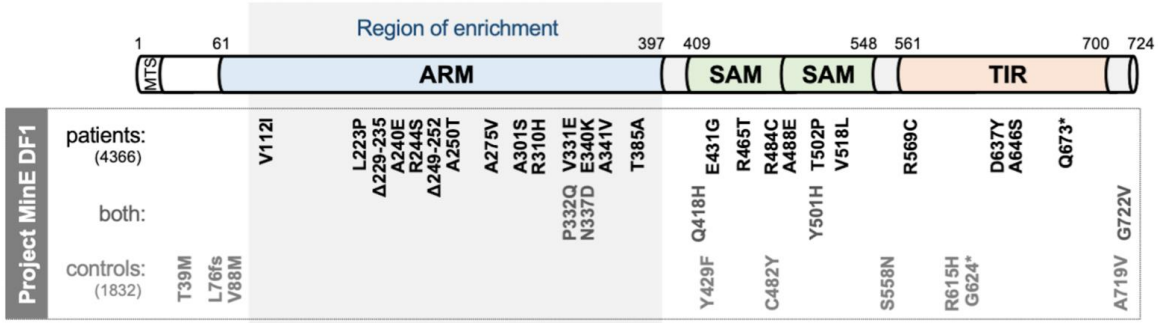
Sarm1 deletion suppresses TDP-43-linked motor neuron degeneration and cortical spine loss

Matthew A. White[1†], Ziqiang Lin[1,2†], Eugene Kim[3], Christopher M. Henstridge[4], Emiliano Pena Altamira[1], Camille K. Hunt[1], Ella Burchill[1], Isobel Callaghan[1], Andrea Loreto[5], Heledd Brown-Wright[6], Richard Mead[6], Camilla Simmons[3], Diana Cash[3], Michael P. Coleman[5,7] and Jemeen Sreedharan[1*]



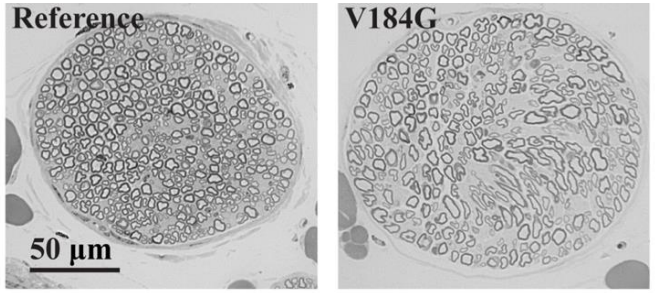
Enrichment of *SARM1* alleles encoding variants with constitutively hyperactive NADase in patients with ALS and other motor nerve disorders

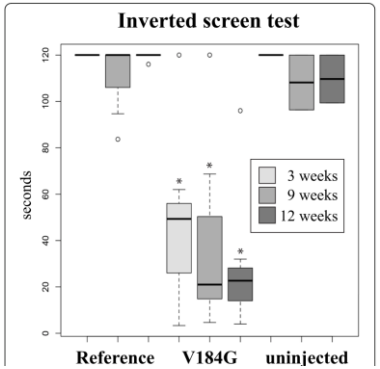
Jonathan Gilley[1*], Oscar Jackson[1], Menelaos Pipis[2], Mehrdad A Estiar[3,4], Ammar Al-Chalabi[5,6], Matt C Danzi[7], Kristel R van Eijk[8], Stephen A Goutman[9], Matthew B Harms[10], Henry Houlden[2], Alfredo Iacoangeli[5,11,12], Julia Kaye[13], Leandro Lima[13,14], Queen Square Genomics[2], John Ravits[15], Guy A Rouleau[3,4,16], Rebecca Schüle[17], Jishu Xu[17], Stephan Züchner[7], Johnathan Cooper-Knock[18], Ziv Gan-Or[3,4,16], Mary M Reilly[2], Michael P Coleman[1*]



Constitutively active SARM1 variants that induce neuropathy are enriched in ALS patients

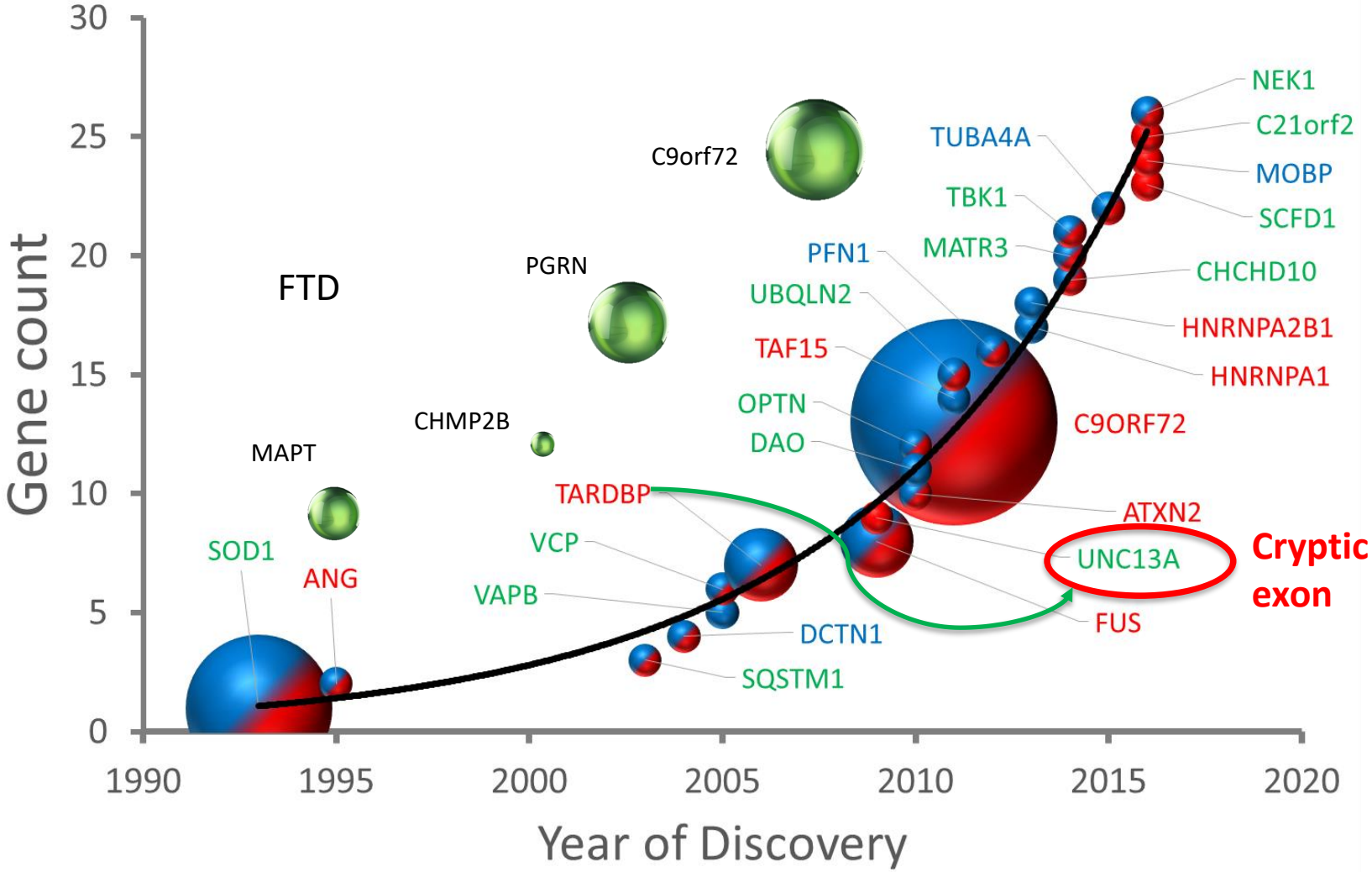
A. Joseph Bloom[1*], Xianrong Mao[1], Amy Strickland[1], Yo Sasaki[1], Jeffrey Milbrandt[1*] and Aaron DiAntonio[2*]





Genetic clues are linking together

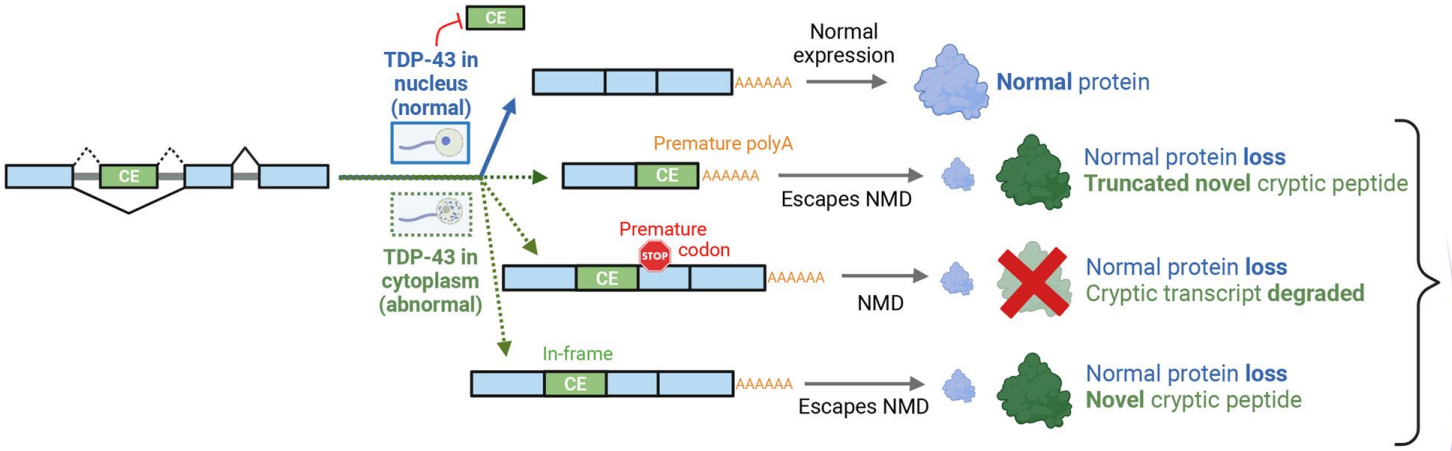
SARM1



Cytoskeleton **RNA processing** **Protein degradation**

Al-Chalabi and Brown 2017 (modified)

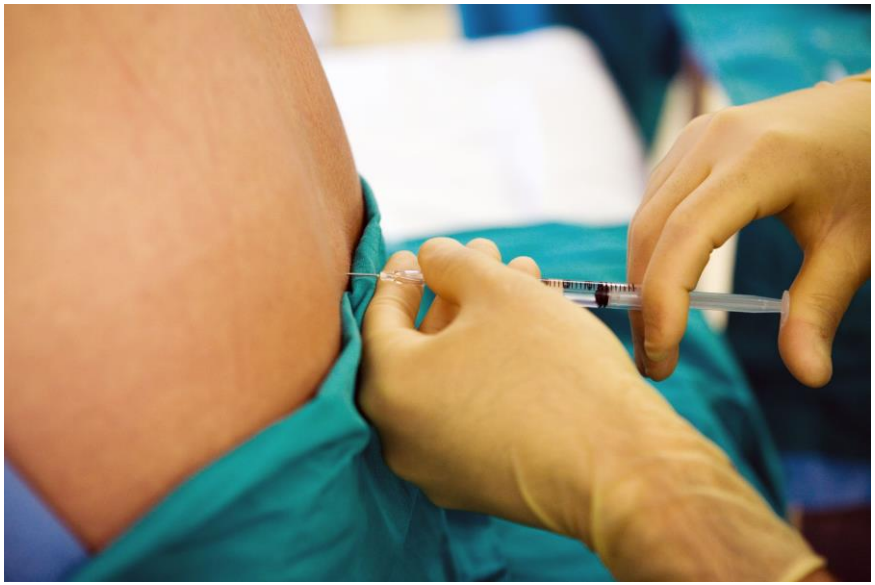
Genetic clues are linking together



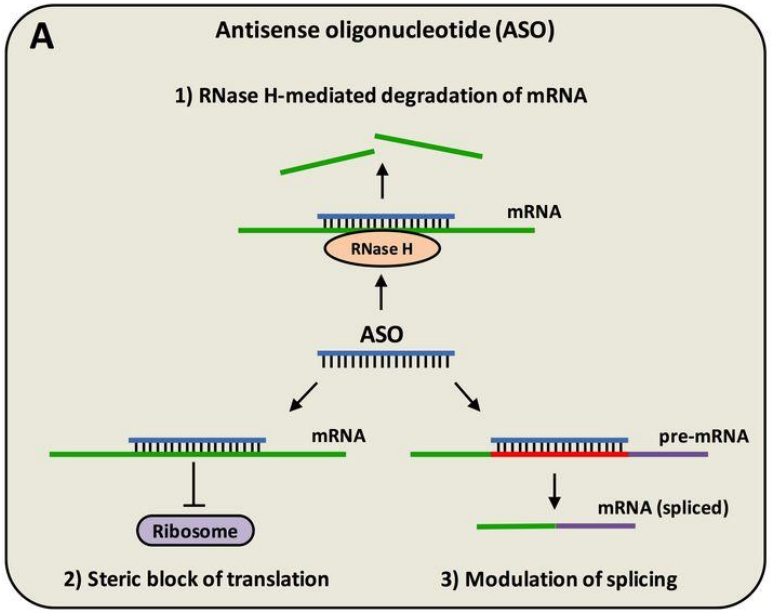
PATHOGENIC PLAYERS	BIOMARKERS	THERAPEUTICS
***STMN2* CE:** Impact on neuronal health	**RNA biomarkers**	**Restoration of functional protein levels via viral vector delivery**
***UNC13A* CE & risk SNPs:** Impact on disease progression	**Protein biomarkers**	**Splicing modification:** E.g., ASOs CasRx U7 snRNAs Small molecules
? CEs in other genes: Yet to be explored		

Mehta et al Mol Neurodegen 2023

Gene therapies with ASO : “Shoot the messenger”



Antisense drug delivery through a lumbar puncture



Rossor et al 2018

Mutation target: SOD1

Trial type: double-blind placebo-controlled trial

Intervention: Anti-sense oligonucleotide (Tofersen, BIIB067) – reduces SOD1 synthesis

Sponsor: Biogen

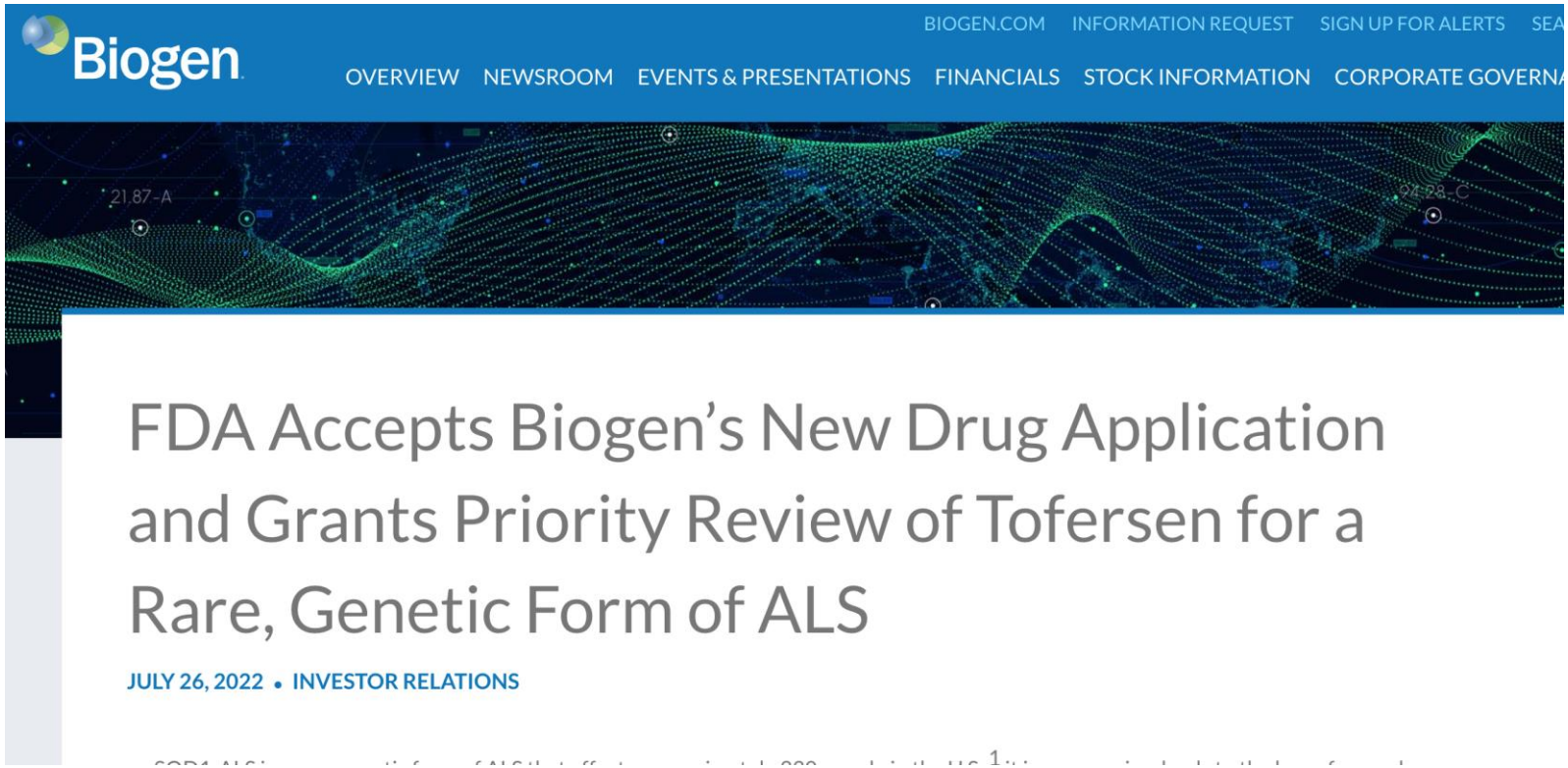
Phase III VALOR completed. Open-Label Extension (OLE) ongoing.

108 patients participated in VALOR

95 patients in the OLE

Tofersen also available gratis as part of an Early Access Programme (EAP)

TOFERSEN (**Qalsody**) THE FIRST ‘CURE’ FOR (SOD1) ALS!



Tofersen presymptomatic clinical trial - NCT04856982

ClinicalTrials.gov Identifier: NCT04856982

RECRUITING

A Study of BIIB067 When Initiated in Clinically Presymptomatic Adults With a Confirmed Superoxide Dismutase 1 Mutation (ATLAS)

Information provided by Biogen (Responsible Party)

Last Updated: September 5, 2022

This has changed our genetic practice – R58 panel increasingly being done
Can't do SOD1 only*
Should also do C9orf72??

FUSION

IONIS

Trial type: double-blind placebo-controlled trial

Intervention: Anti-sense oligonucleotide (Jacifusen) – reduces FUS expression

Sponsor: Ionis

Trial Phase I-III in progress. Period 1 single ascending dose, Period 2 multiple ascending dose

Part 1: Enrollment target 49 patients worldwide Cohort A, 18 patients Cohort B. 61w double-blind treatment period

Part 2: Enrollment target of 77 patients worldwide. 80w Open Label Extension

~10 patients given the product as part of an Early Access Programme (EAP) prior to trial commencement (to be included in Part 2 enrollment)

King's College Hospital NHS
NHS Foundation Trust

KCL MND Biobank

Trial type: research tissue bank

Study overview: Central resource of samples and information from patients with MND and healthy controls

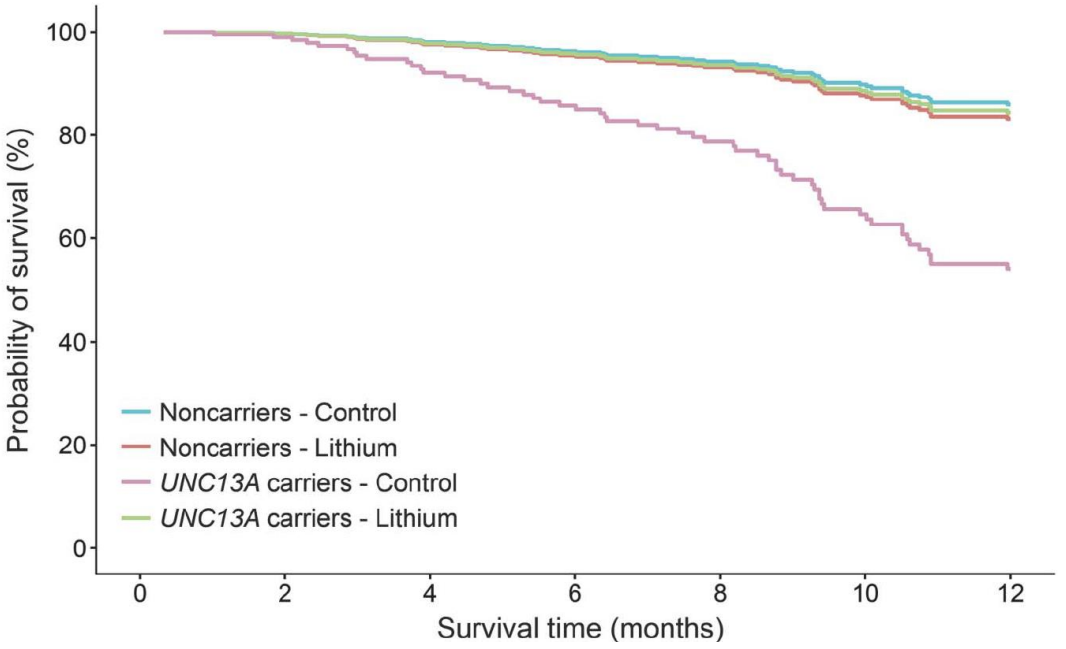
Sponsor: King's College London and King's College Hospital

Data collected: blood for genomic, viral and biochemical testing along with demographic and clinical data. Saliva kits to expand. CSF and skin added to ethics

Aim: to build a resource of at least 2,000 participants

PRELUDE trial of lithium in MND: MAGNET TRIAL

- Original lithium trials were all negative
- Genetic subgroup with rapid disease
  - Shows benefit of lithium
  - Only in people carrying a variation in the *UNC13A* gene
  - 'MAGNET' (Multi-arm, Adaptive, Group-sequential trial NETwork) is the first international platform trial focused on finding effective treatments for MND. It will test multiple treatments simultaneously.
  - Kings currently only recruiting site in UK



Conclusions

Genetic forms of ALS (5-10%) can tell us much about apparently sporadic ALS (90-95%)

SOD1, TDP-43 (and FUS) and C9orf72: three waves of research have dominated ALS and led to major breakthroughs

Technological advances: Linkage > GWAS > WGS

Genetics > Mechanism > Clinical trials > Genetics!

- SOD1 gene therapy
- UNC13a and lithium
- HAART and HLA status

Questions?

Dilemmas and ethical implications

- Access to gene therapy
- Mutations identified in sporadic ALS
- Variants of uncertain significance
  - ATXN2 point mutation
  - SOD1 splice site mutation
- Private genetic testing – UK and international

UK MND Research Institute

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About us

The UK Motor Neuron Disease Research Institute is a national network of MND centres working together to understand how and why MND happens, what might work as a treatment, and testing possible treatments in clinical trials. Researchers across the country are carrying out world-leading MND research in a coordinated way to accelerate the search for a cure.