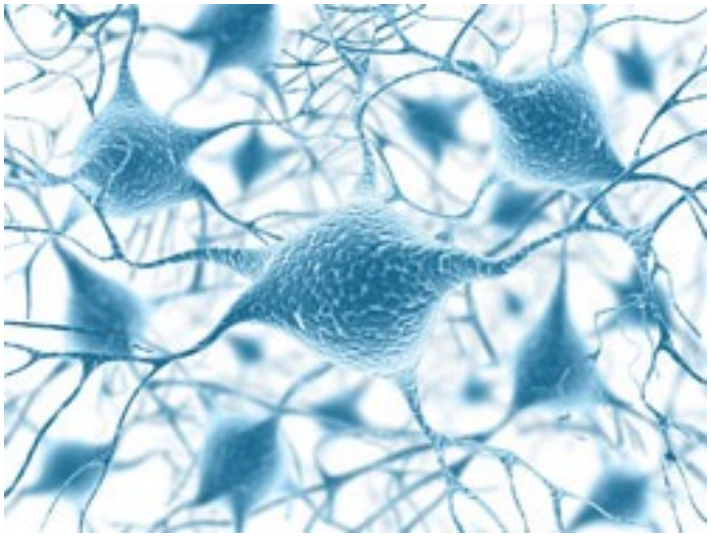
MOTOR NEURON DISEASE II

Pathophysiology, genetics, proteins, therapies

Bernard Schneider

October 2025



Lecture plan

1. **Motor system**
   - Overview
2. **Motor Neuron Diseases**
   - Clinical presentation
   - Molecular pathology
3. **Amyotrophic Lateral Sclerosis**
   - Clinical presentation, epidemiology, etiology
   - Non-cell autonomous mechanisms
   - Molecular pathology: RNA metabolism

Motor neuron diseases: Amyotrophic Lateral Sclerosis

- First described by Jean-Martin Charcot (1869)
- = ‘Lou Gehrig’s disease’ in the US
- Motoneuron degeneration in motor cortex, brain stem and spinal cord
- No cure

"a" for without

"myo" for muscle

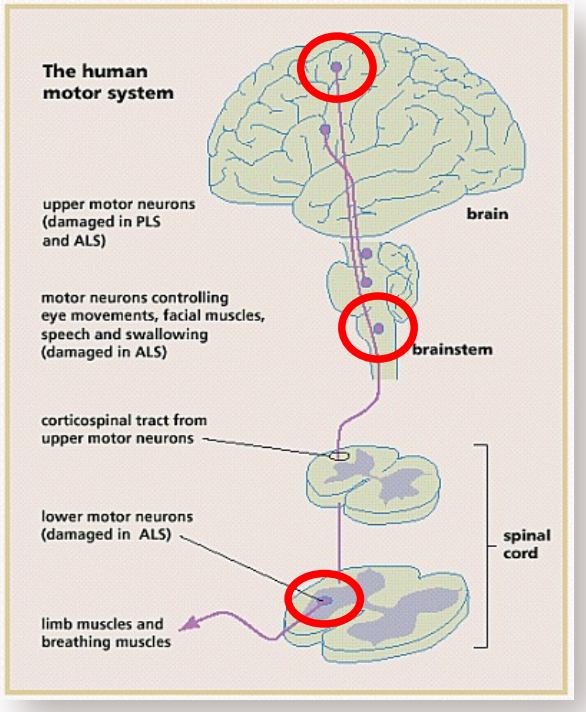
"trophic" for nourishment

"lateral" for side (of the spinal cord)

"sclerosis" for hardening or scarring in area following deterioration

J.M. Charcot

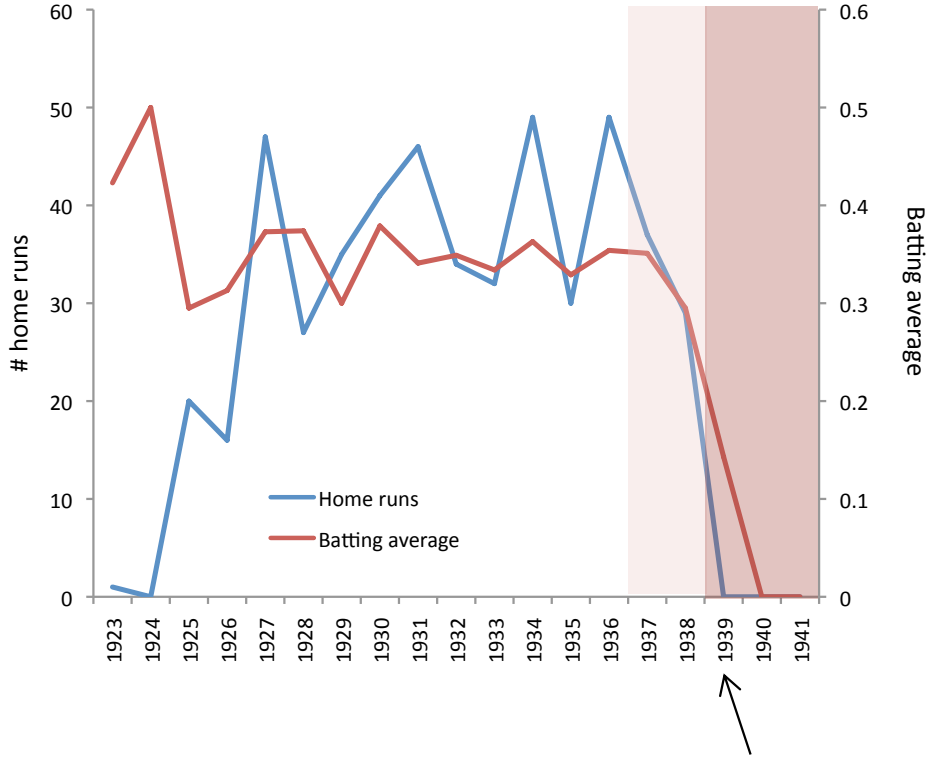
Lou Gehrig



Motor neuron diseases: ALS

Lou Gehrig – New York Yankee baseball player

1903 - 1941

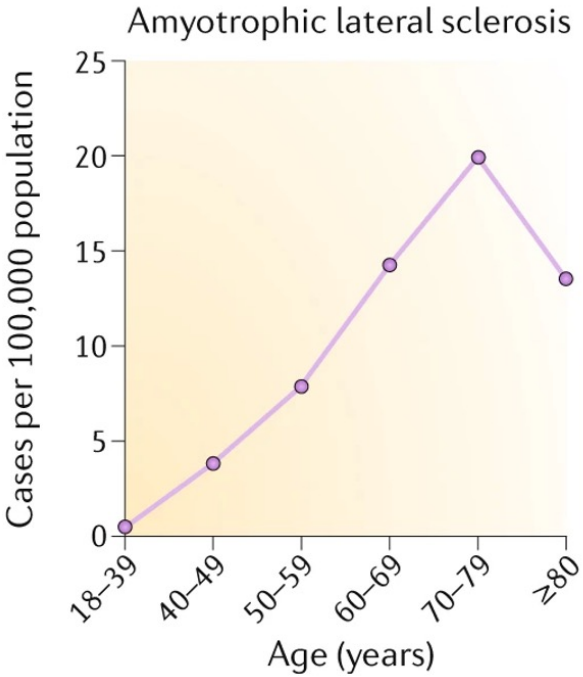


Diagnosed with ALS in 1939

Motor neuron diseases: ALS

Epidemiology of ALS

- Most common motor neuron disorder
  - Incidence: 1.5 - 2.7 in 100,000
  - Prevalence: 2.7 - 7.4 in 100,000
  - **Lifetime risk: 1:250 – 1:400**
  - >350,000 patients worldwide
- **Mean age at onset: 56 yrs** (rare cases < 20)
- Affects 40 – 70 yrs old people
- 120,000 people diagnosed each year worldwide
- 90% Caucasian
- Male:Female ratio 1.3 – 1.5:1 (mainly in young onset cases)



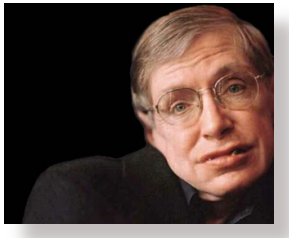
- *Nature Reviews Neurology volume 15, pages 565–581 (2019)*

Motor neuron diseases: ALS

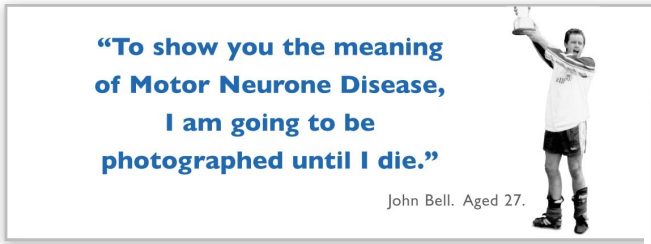
Disease progression

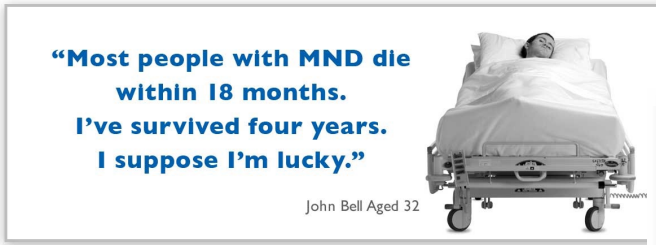
- Rapid progression
- No remission
- Death from respiratory failure
- Median survival: 3-5 yrs
- 10% live ≥10 yrs

- Rare survival beyond 20 years



Stephen Hawking
(diagnosed in 1963)
1942-2018



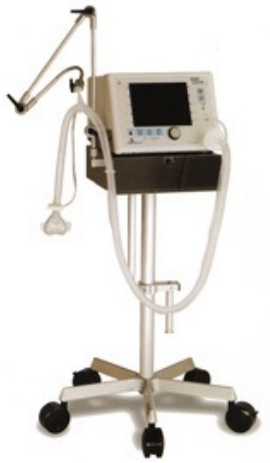


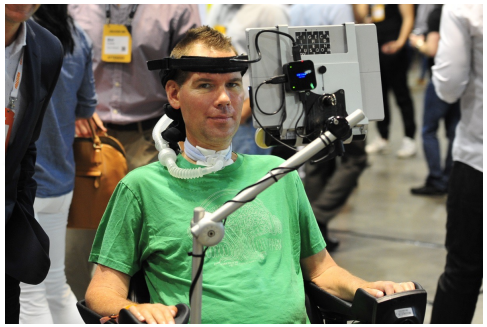
Motor neuron diseases: ALS

Supportive care in ALS

- By the time patients are given a definitive diagnosis of ALS, it is estimated that they have already lost 60% of motoneurons.
- Finding biomarkers is critical to allow for early intervention.
- No cure available
- Available treatments: riluzole, radicava
- Locked-in
- Voice amplification, speech generating devices, eye tracking
- Respiratory failure is the most common cause of death.

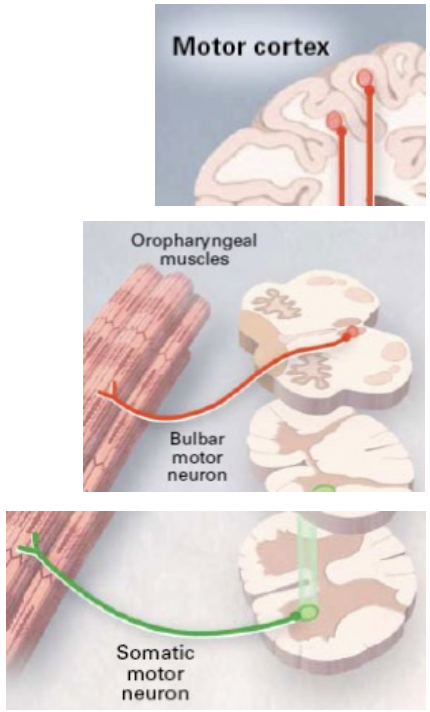
  ⇒ Respiratory assistance





Motor neuron diseases: ALS Symptoms

Progression rate



Initial symptoms

Upper motor neuron →
Stiffness (spasticity)
Brisk tendon reflexes
Abnormal reflexes
Lack of coordination, dexterity

Bulbar motor neuron →
Speech difficulty
Swallowing difficulty
Tongue fasciculation

Lower motor neuron →
Respiratory muscle weakness
Generalized weakness

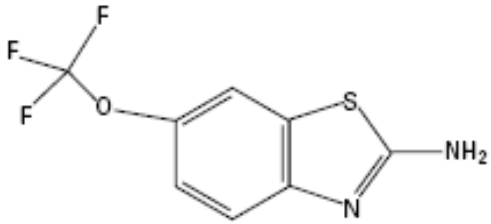
Twitching muscles (fasciculation)
Asymmetric limb weakness
Muscle cramps
Foot drop

- the progression pattern suggests disease spread among contiguous pools of motor neurons.

Motor neuron diseases: ALS

ALS: treatments

- Rilutek® (riluzole): FDA approved drug
- increases lifespan by **2 months**
- Although riluzole reduces glutamate excitotoxicity in some models, its mode of action remains unclear.

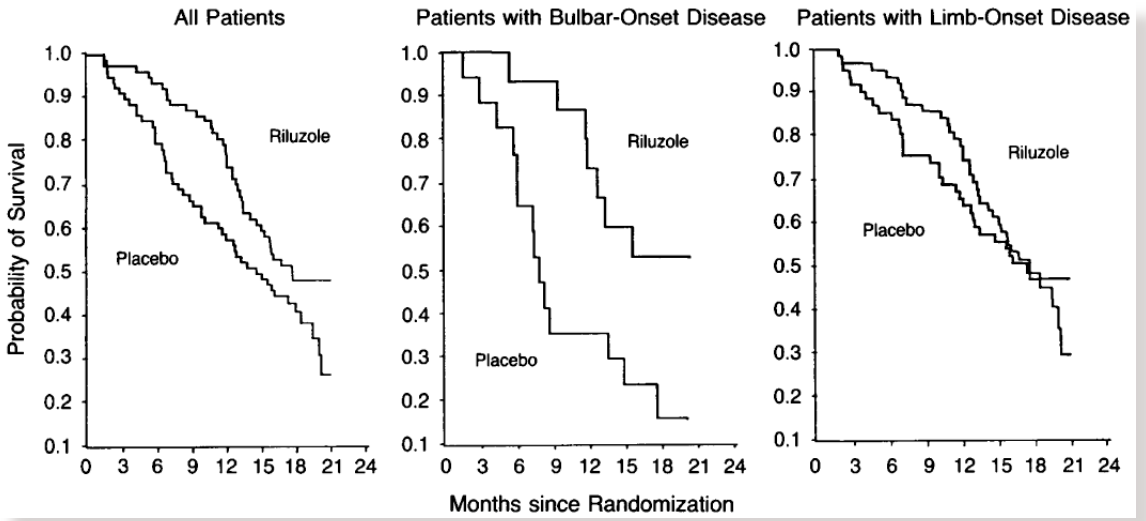


The New England
Journal of Medicine

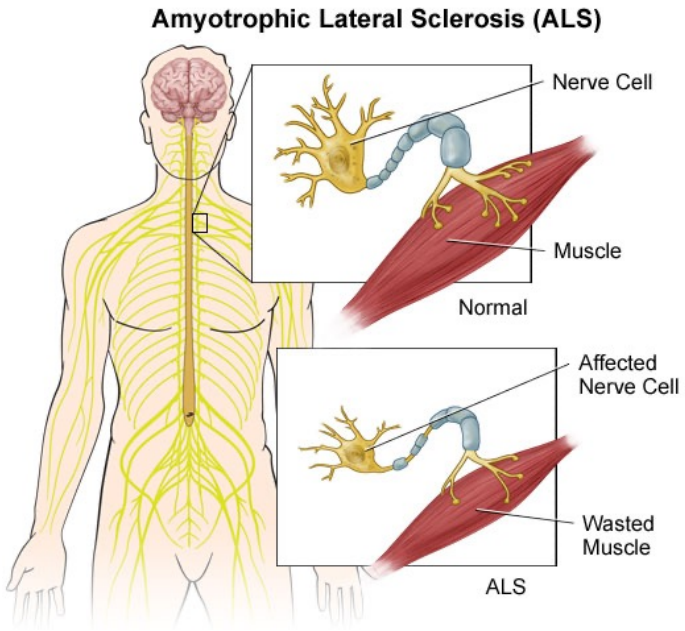
Volume 330 MARCH 3, 1994 Number 9

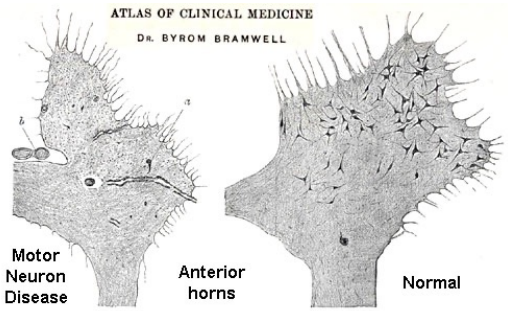
A CONTROLLED TRIAL OF RILUZOLE IN AMYOTROPHIC LATERAL SCLEROSIS

G. Bensimon, L. Lacomblez, V. Meininger, and the ALS/Riluzole Study Group*

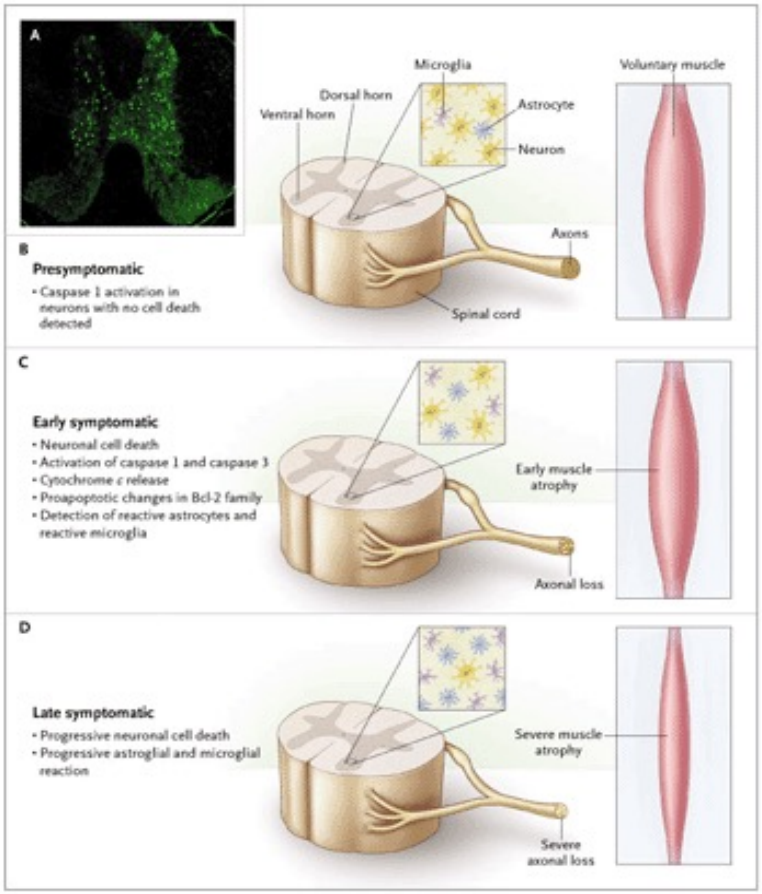


Motor neuron diseases: ALS





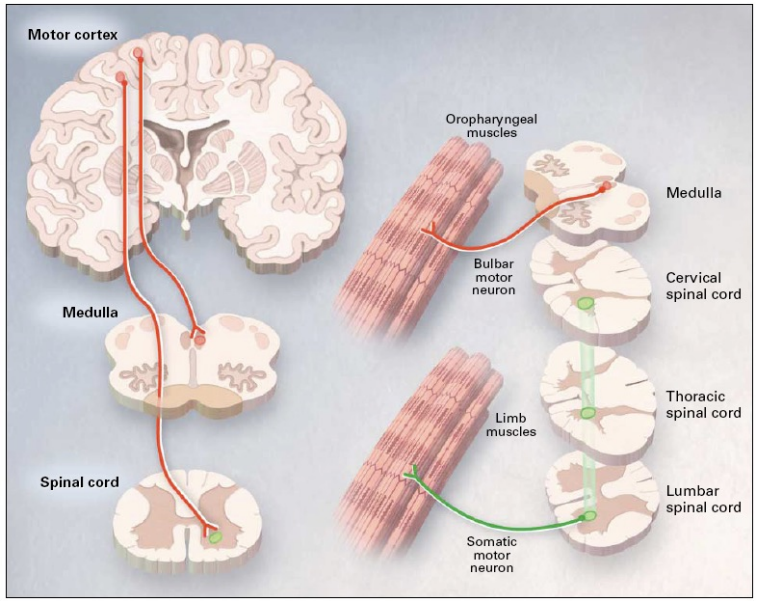
Pathology



Motor neuron diseases: ALS

Selective vulnerability of motoneurons

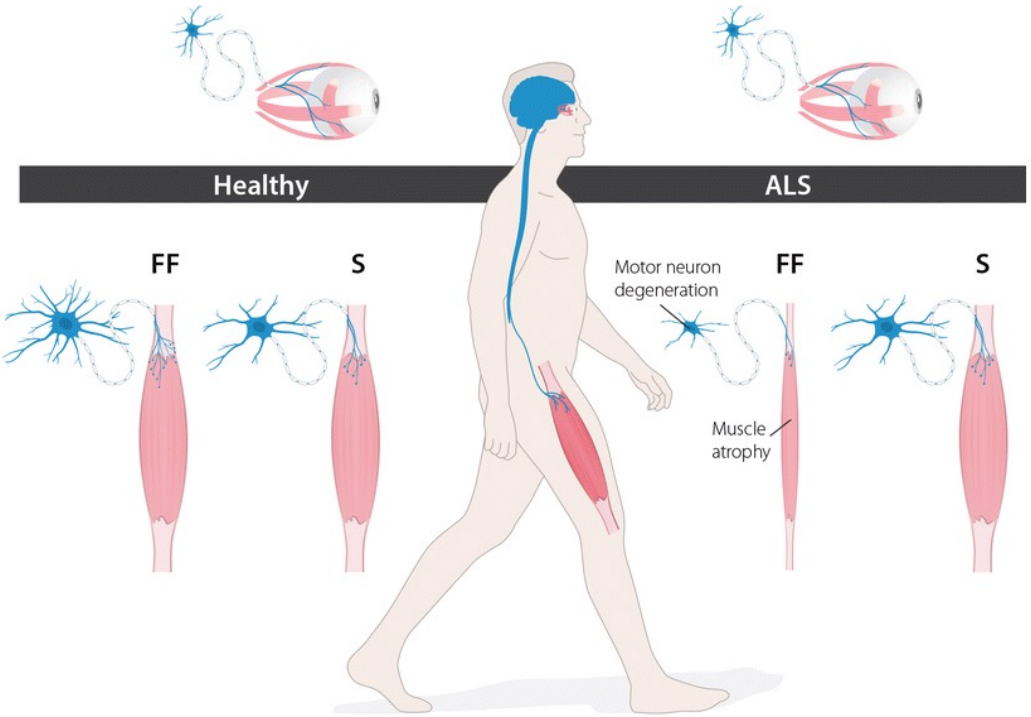
- **Disease selectively affecting motor neurons**
- Cognitive dysfunction or decline is observed but remains a minor feature of the disease:
  - dementia reported in less than 15% of the ALS patients
  - cognitive impairment in >40% of patients



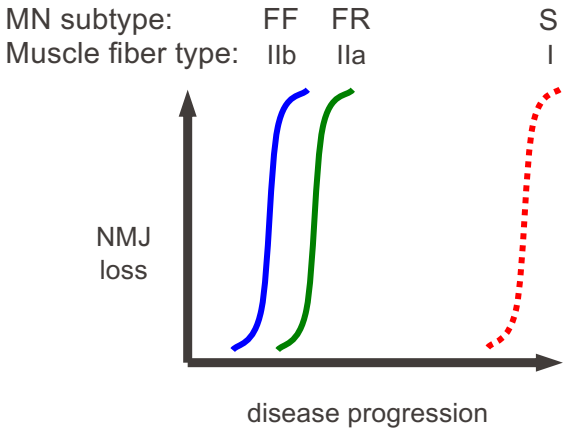
Motor neuron diseases: ALS

Vulnerable and resistant motor neurons

- Sequential loss of motoneurons in ALS: FF ⇒ FR ⇒ S
- Some subpopulations of motoneurons are highly resistant to the disease: oculomotor nucleus, Onuf's nucleus (sphincter).



NMJ denervation by MN subtype
Pun S et al., Nat Neuroscience 2006



FF: fast-fatigable (strength)
FR: fatigue-resistant
S: slow (endurance)

- Nijssen, J., Comley, L.H. & Hedlund, E. Acta Neuropathol (2017) 133: 863.

Motor neuron diseases: question 7

Researchers have compared the transcriptional profile of motoneurons that are resistant or vulnerable to disease. What is your opinion on this approach?

A. This is irrelevant as these pools of neurons differ in their integration in the local circuit

B. This is an effective approach to identify protective genes.

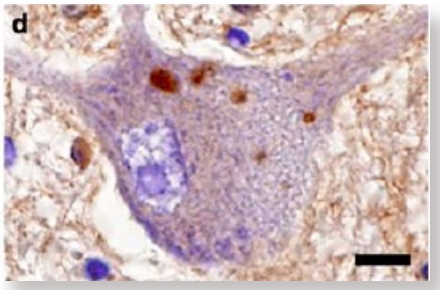
C. This approach may identify mechanisms that are inherent to motoneurons but will fail to identify possible cause of disease in muscle or glial cells.

D. The vulnerability of neurons is unlikely to be the same in humans and in animal models.

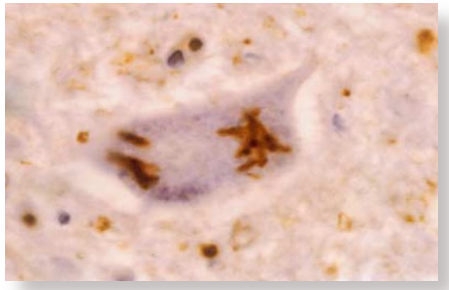
Motor neuron diseases: ALS

Protein pathology

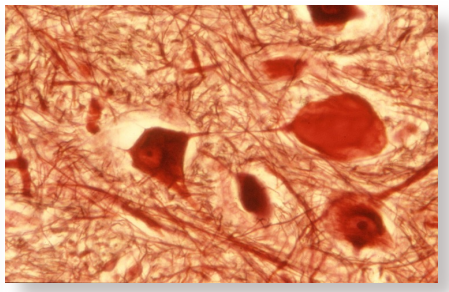
- Accumulation of phosphorylated neurofilaments
- Inclusion bodies
- Ubiquitin-positive inclusions



Bunina bodies
(cytoplasmic
proteinaceous inclusions)



skein-like inclusions

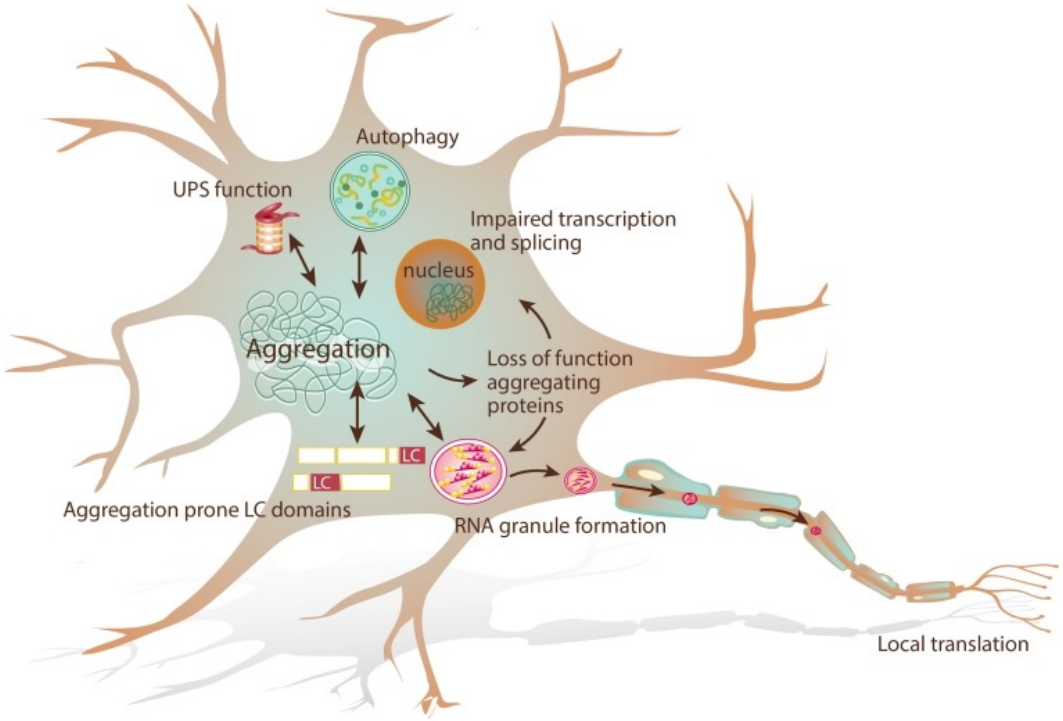


axonal swelling

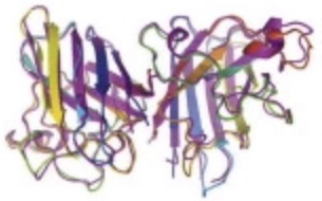
Astrogliosis
Microglial activation

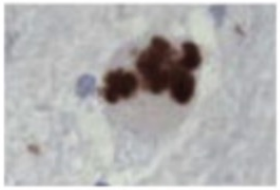
Motor neuron diseases: ALS

What are the types of proteins that mainly compose these aggregates?

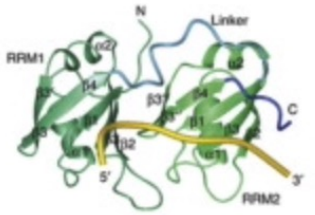


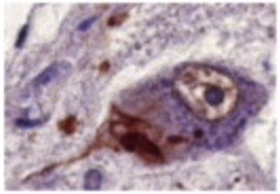
SOD1



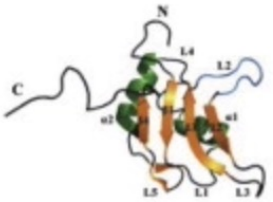


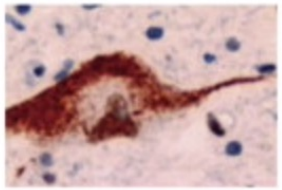
TDP-43



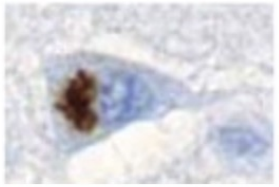


FUS





C9ORF72 *not done*



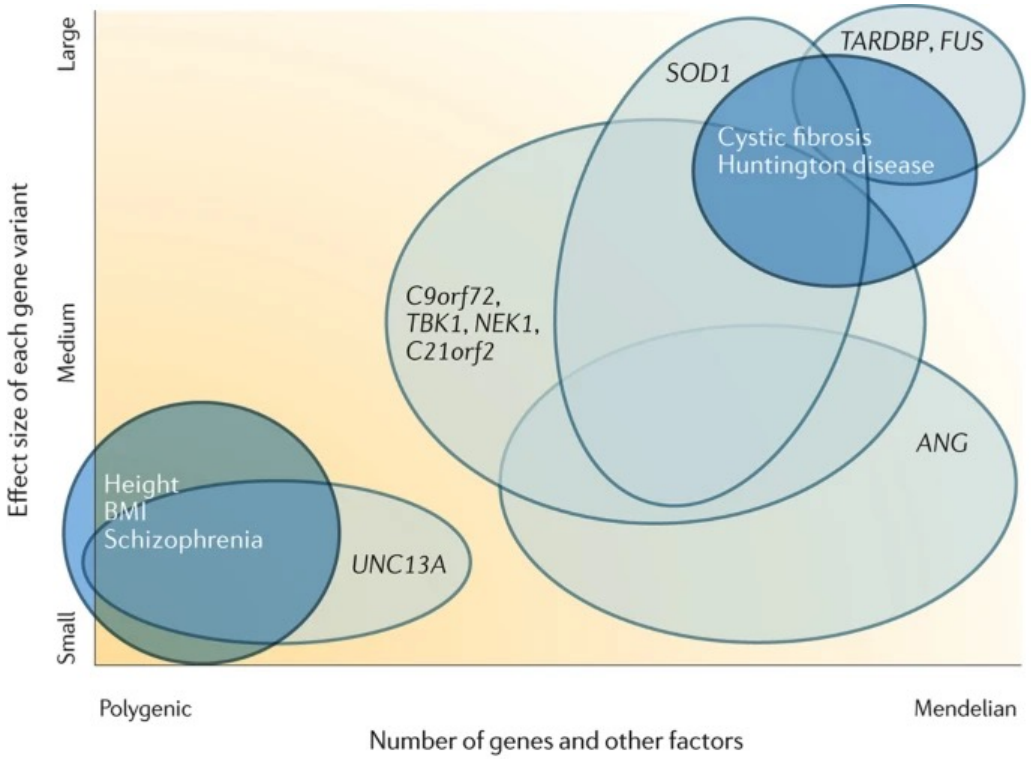
Motor neuron diseases: ALS

Etiology: genetic versus sporadic forms

Two forms, clinically indistinguishable

- Sporadic (sALS): 90% of all cases
- Familial (fALS): 10% of cases

Mean age of onset: ***sALS > fALS***

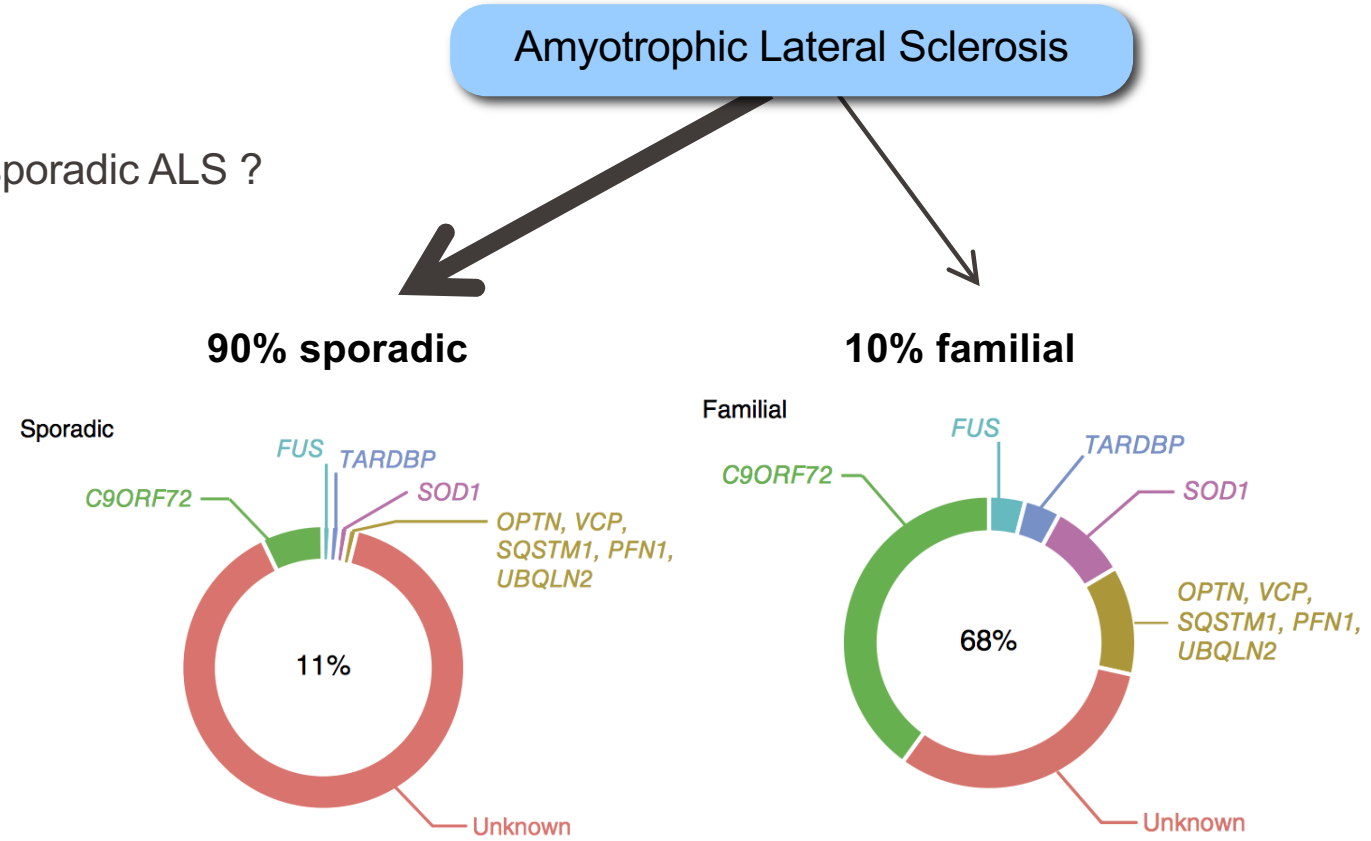


- *Nature Reviews Neurology volume 13, pages 96–104 (2017)*

Motor neuron diseases: ALS

- genetic cause identified in >70% of familial ALS cases.
- genes include C9ORF72, SOD1, TDP43, FUS.
- **autosomal dominant inheritance.**
- **gain of a toxic function ?**
- similar factors might be involved in sporadic ALS ?

Genetics of ALS



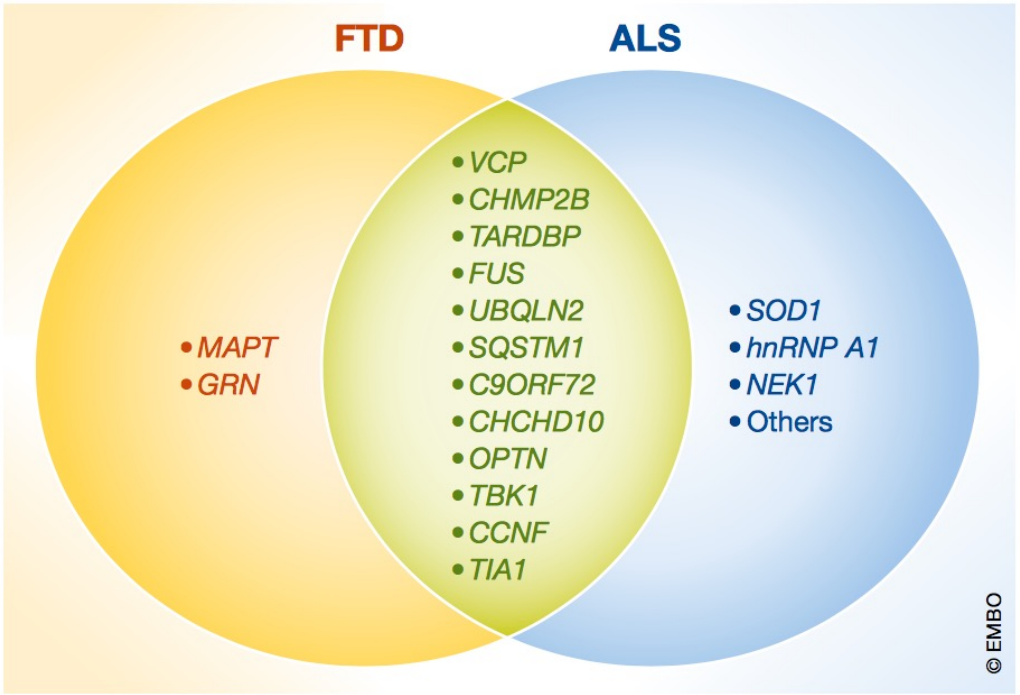
- Renton AE et al, Nat Neurosci 2014

fALS – non exhaustive list of genes that predispose to ALS

ALS disease type	Gene	Chromosome	Inheritance	Clinical features
Mendelian genes				
ALS1	***SOD1***	21q22.1	**AD**	Typical ALS
ALS2	*ALS2 (Alsin)*	2q33	AR	Juvenile onset, slowly progressive, predominantly corticospinal
ALS4	*SETX*	9q34	**AD**	Adult onset, slowly progressive
ALS6	***FUS***	16q12	**AD**	ALS, frontotemporal dementia
ALS8	*VAPB*	20q13.3	**AD**	Atypical ALS, slowly progressive with tremors
ALS9	*ANG*			
ALS10	***TDP43***	1p36.22	**AD**	Spinal onset, low motor neurons, frontotemporal dementia
ALS11	FIG4	6q21	**AD**	
ALS	OPTN	10	AR	
ALS-X	UBQLN2	X (centromere)	**XD**	Typical ALS
ALS-FTDP	Tau	17q21	**AD**	ALS, frontotemporal dementia + parkinsonism
ALS-FTD	***C9ORF72***	9p-21	**AD**	ALS, frontotemporal dementia
ALS(?)	*Dynactin*	2p13	**AD**	Adult onset, slowly progressive
Mendelian loci				
ALS3	?	18q21	**AD**	Typical ALS
ALS5	?	15q15-21	AR	Juvenile onset, slowly progressive
ALS7	?	20p13	**AD**	Typical ALS
ALS-FTD	?	9q21-22	**AD**	ALS, frontotemporal dementia
Mitochondrial genes				
ALS-M	COX1	mtDNA	Maternal	Single case, predominantly corticospinal
ALS-M	IARS2	mtDNA	Maternal	Single case, predominantly lower motor neuron

Motor neuron diseases: ALS

Genetics: ALS and frontotemporal dementia



- Gao FB et al, EMBO J 2017

Motor neuron diseases: ALS

Sporadic ALS: environmental factors

Risk factors:

- only established environmental risk factor: age

Putative risk factors:

- smoking

?

- heavy metal exposure (mercury, lead, aluminium)
- higher incidence in the first US Gulf War veterans
- service in US military
- being an Italian soccer player
- exposure to infectious agents
  - Mycoplasma (up to 80% of patients of ALS patients)
  - Borrelia burgdorferi

Motor neuron diseases: ALS

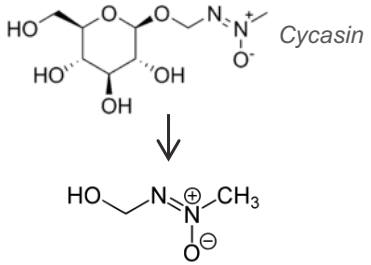
Sporadic ALS: environmental factors

Geographical clustering

- Guam (Pacific ocean; US) – Chamorro people: epidemic in 1950s
- Kii peninsula (Japan)
- West New Guinea
- North Africa (juvenile ALS)
- Madras (India)

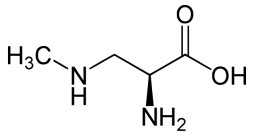
Lytico-bodig disease: characteristics of ALS and PD

Cycas circinalis is used to produce flour



Cycasin

[(Z)-methyl-ONN-azoxy]methanol (MAM)



β-Methylamino-L-alanine (BMAA)

Mariana fruit bat (flying fox): accumulates BMAA in fat

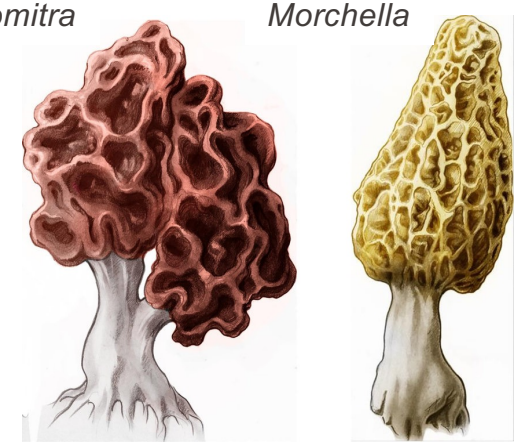
Exposure to cycas-derived toxins is considered a risk factor for ALS

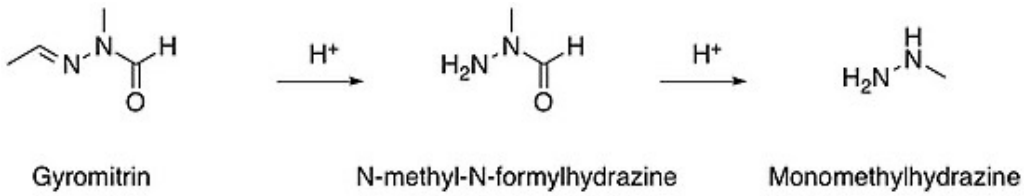
Motor neuron diseases: ALS

Sporadic ALS: environmental factors

- Cluster of ALS cases caused by ‘False Morel’ consumption
- Hotspot of ALS in Savoie, in the Tarentaise Valley
- Incidence of ALS cases higher than normal.
- 14 ALS cases between 1991 and 2013
- Genotoxic fungi

Gyromitra *Morchella*





MMH metabolite similar to MAA
- affects GABA production
- ROS production
- DNA damage

E. Lagrange, et al. J Neurol Sci, 427 (2021); https://doi.org/10.1016/j.jns.2021.117558.

Motor neuron diseases: ALS

Sporadic ALS: environmental factors

Is frequent and prolonged exercise a ‘second hit’ in ALS?

Rob Burrow in 2013

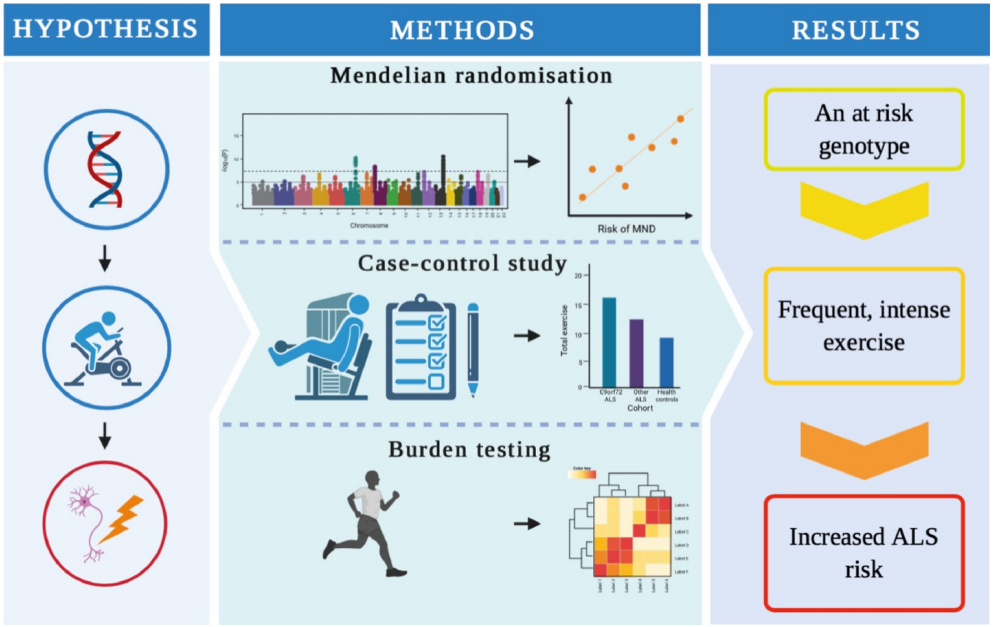
Rob Burrow died in 2024 at the age of 41 after 4.5 yrs MND

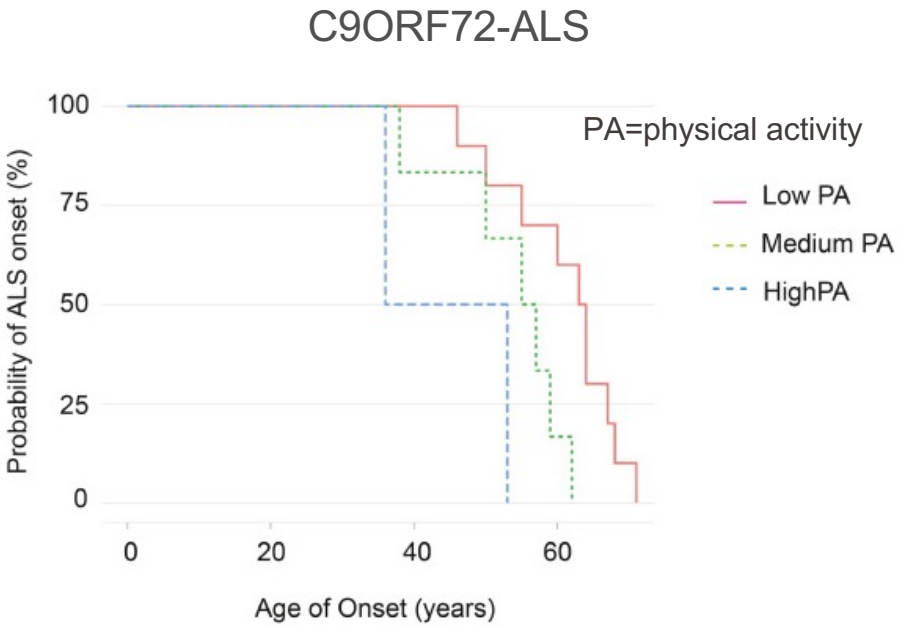
Lewis Moody diagnosed with ALS in 2025 at the age of 47.

Motor neuron diseases: ALS

Sporadic ALS: environmental factors

Is frequent and prolonged exercise a ‘second hit’ in ALS?





- Physical exercise is a risk factor for amyotrophic lateral sclerosis: Convergent evidence from Mendelian randomisation, transcriptomics and risk genotypes. TH Julian *et al*, Lancet 2021

Motor neuron diseases: ALS

Sporadic ALS: environmental factors

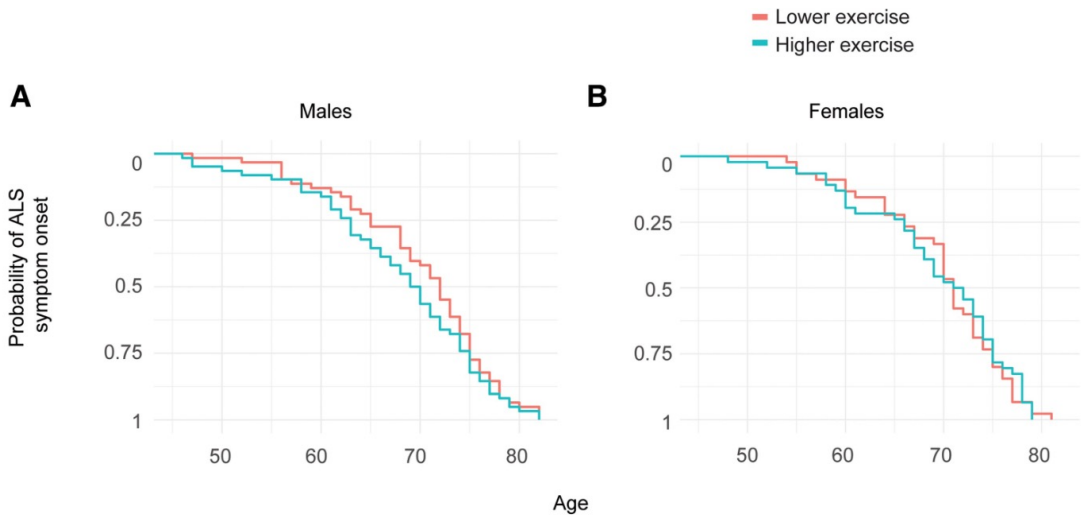
Is frequent and prolonged exercise a ‘second hit’ in ALS?

AIM

Who is at risk of exercise-associated ALS?

HYPOTHESIS

1. The effect of extremely frequent or intense exercise may be distinct from other forms of exercise
2. The effect of exercise may vary between males and females



RESULTS

In **males** there is a causal relationship between genetic liability to frequent or strenuous leisure-time exercise and risk of ALS; in **females** there is no significant relationship

In **males** there is a correlation between physical activity and the timing of ALS symptom onset; in **females** there is no significant relationship. The relationship is strongest for extreme exercisers

In **males** who perform extreme exercise there is a genetic inhibition of mTOR signalling which could link motor neuron firing to neurotoxicity.

Brain, Volume 148, Issue 10, October 2025, Pages 3652–3664, https://doi.org/10.1093/brain/awaf235

Lecture plan

1. **Motor system**
   - Overview
2. **Motor Neuron Diseases**
   - Clinical presentation
   - Molecular pathology
3. **Amyotrophic Lateral Sclerosis**
   - Clinical presentation, epidemiology, etiology
   - SOD1 and non-cell autonomous mechanisms
   - RNA-binding proteins, RNA metabolism

ALS: genetic causes

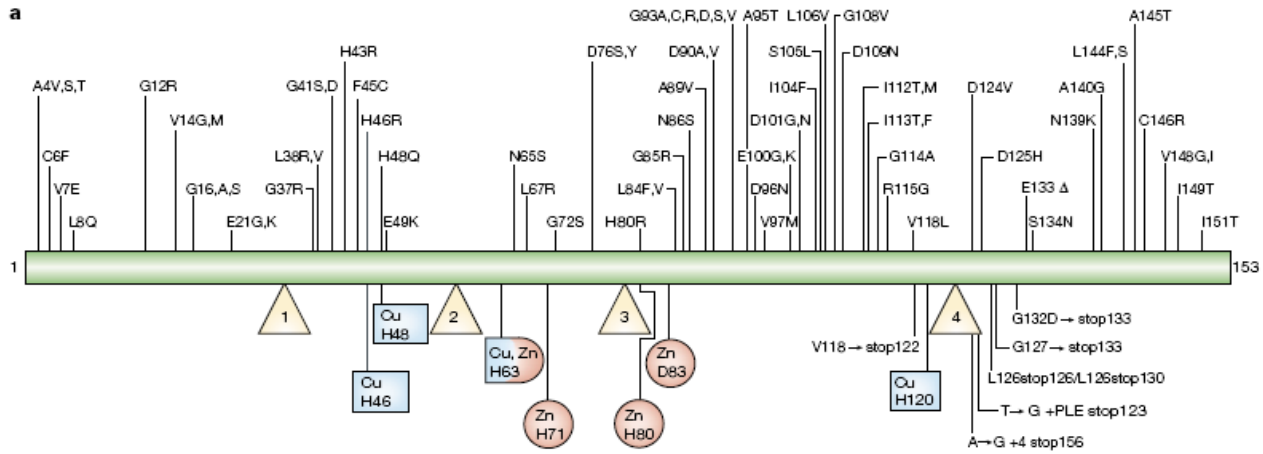
SOD1 – Cu/Zn Superoxide dismutase

Cu/Zn SOD1 mutations

≈ 2% of all ALS cases

> 140 SOD1 mutations

Toxic gain of function



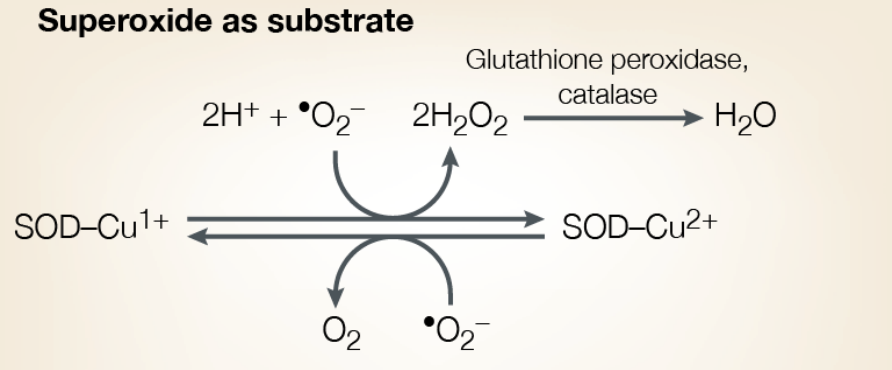
- ALS-causing mutations are located throughout the SOD1 polypeptide

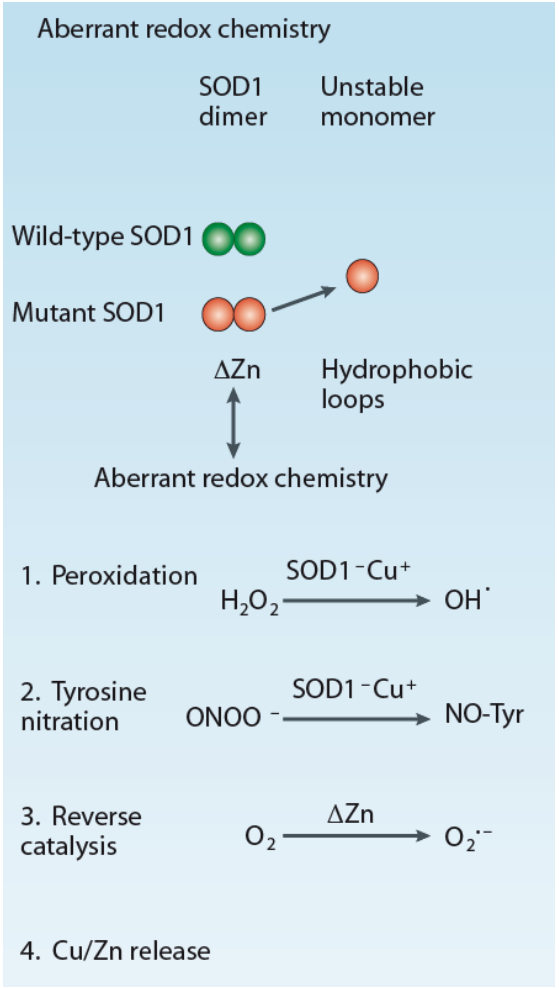
- Cleveland & Rothstein, Nat Rev Neurosci 2001

ALS: SOD1 pathology

SOD1: oxidative stress

Normal SOD1 dismutase activity → *Aberrant SOD1 activity in ALS ?*





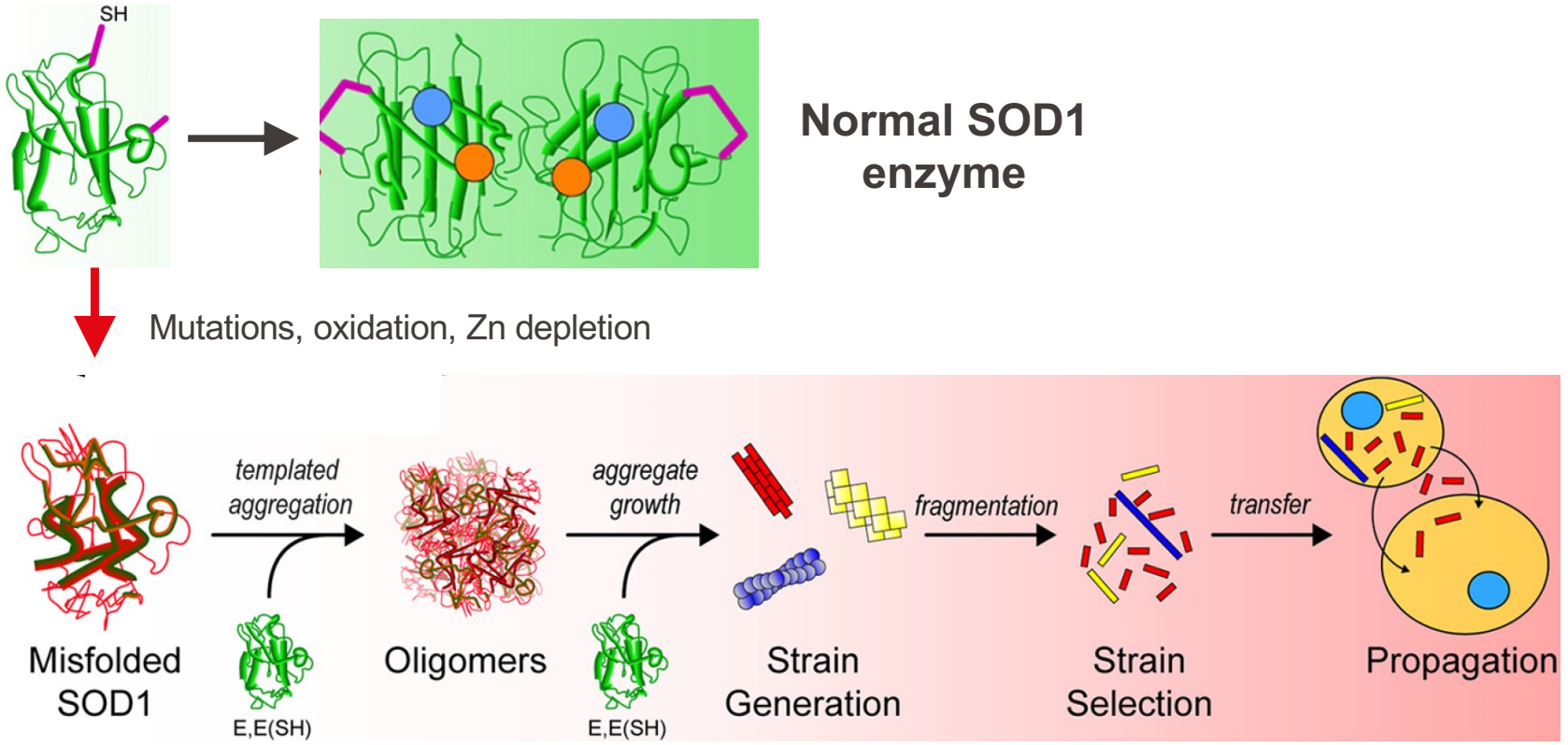
SOD1 mutations can lead to…

- aberrant chemistry of the active copper and zinc sites
- greater access of abnormal substrates to the active site (e.g. leading to peroxidation)
- clumsy handling of Cu and Zn ions (e.g. leading to reverse catalysis)
- however, these changes in activity are not consistent across SOD1 mutations.

- Pasinelli P & Brown RH, Nat Rev in Neurosci 2006

ALS: SOD1 pathology

SOD1 pathology is characterized by protein misfolding and aggregation

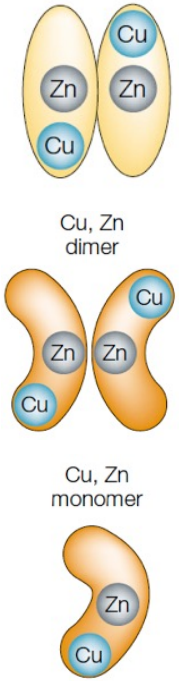


"Aggregation - Gain of toxic function"
→ pathology develops in multiple CNS cell types

- *McAlary L et al, Front Mol Neurosci. 2019; 12, 262*

ALS: SOD1 pathology

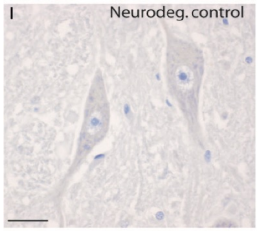
Mechanisms leading to SOD1 misfolding and aggregation



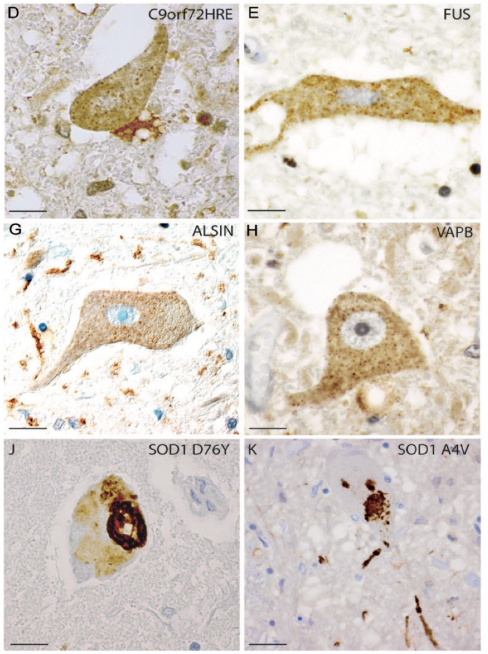
- SOD1 is an extremely stable protein (disulfide bridges, metals)
- **Mutations** disrupt the structure
- **Dimer dissociation**, **loss of metals**
- **Apomonomers are aggregation-prone**
- Inclusions found in both FALS and SALS (humans and rodent models)
- Outstanding question:
  are aggregates: toxic or (transiently) protective ?

Misfolded SOD1 deposition in ALS

Non-ALS



Spinal cord motoneurons:
Various ALS cases show misfolded SOD1



Forsberg K et al, J Neurol Neurosurg Psychiatry. 2019;90(8):861-869

ALS: SOD1 pathology

Mutated SOD1 fALS
Loss of function or gain of a toxic role ?

Gain of a toxic function

- Autosomal dominant inheritance
- SOD1 knock-out mice do not develop neurodegeneration
- Abnormal folding
- Experimental evidence for toxic activities

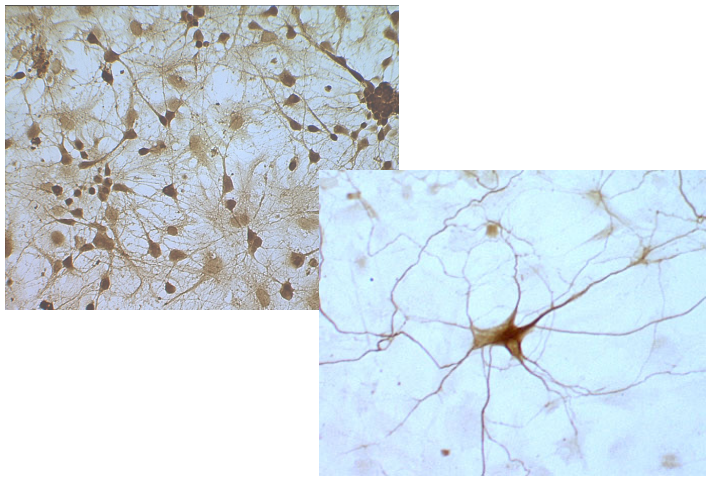
Loss-of-function

- Enzyme involved in reactive oxygen species detoxification

ALS: experimental models

Modeling ALS in the lab

In vitro



Primary motoneuron cultures

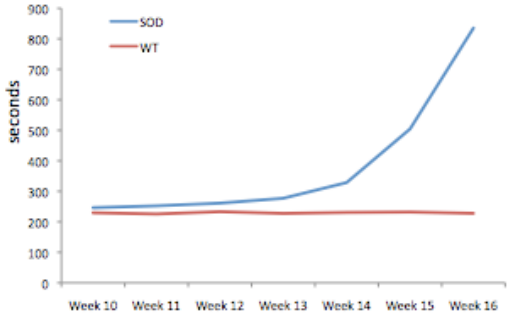
iPS-derived cell cultures

- Gurney ME, et al. Science 1994 264(5166)

In vivo

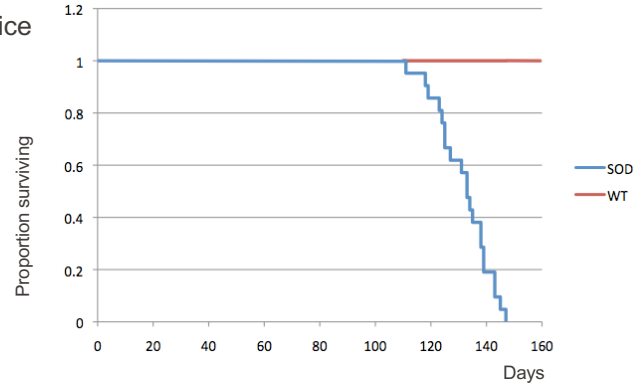
Mutated hSOD1 over-expression

SOD1^{G93A} mice
Swimming performance



Mutated hSOD1
(G93A, G37R, G85R)
(SOD1 promoter, ubiquitous expression)

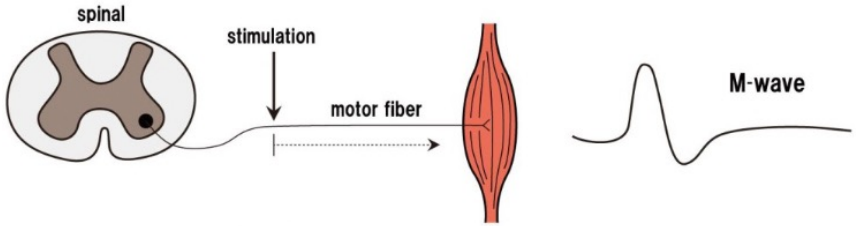
SOD1^{G93A} mice
Survival

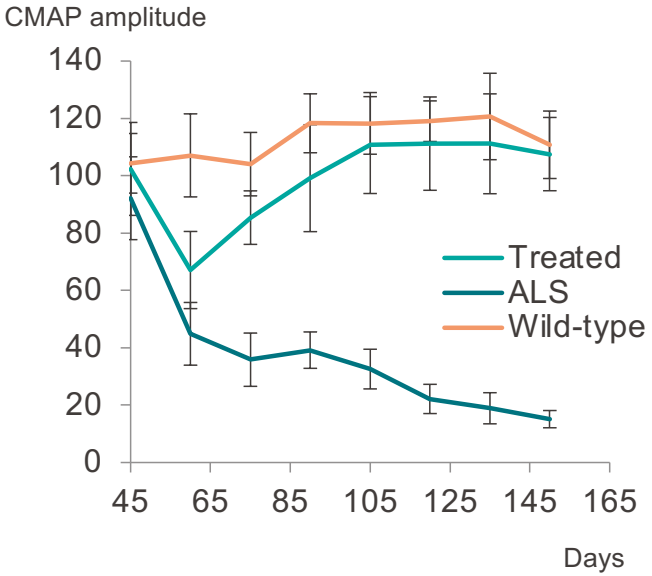


Motor neuron diseases: question 6

The following graph shows the monitoring of the compound muscle action potential (EMG) in a mouse model of amyotrophic lateral sclerosis.

How would you interpret the result in the treated group?





A. The treatment is fully protecting the neuromuscular function
B. There is no loss of motoneurons in the spinal cord, because the EMG is rescued
C. After a first loss of neuromuscular junctions, the remaining motoneurons reinnervate the muscle
D. The decrease in action potential amplitude reflects a transient atrophy of the muscle

ALS: SOD1 pathology

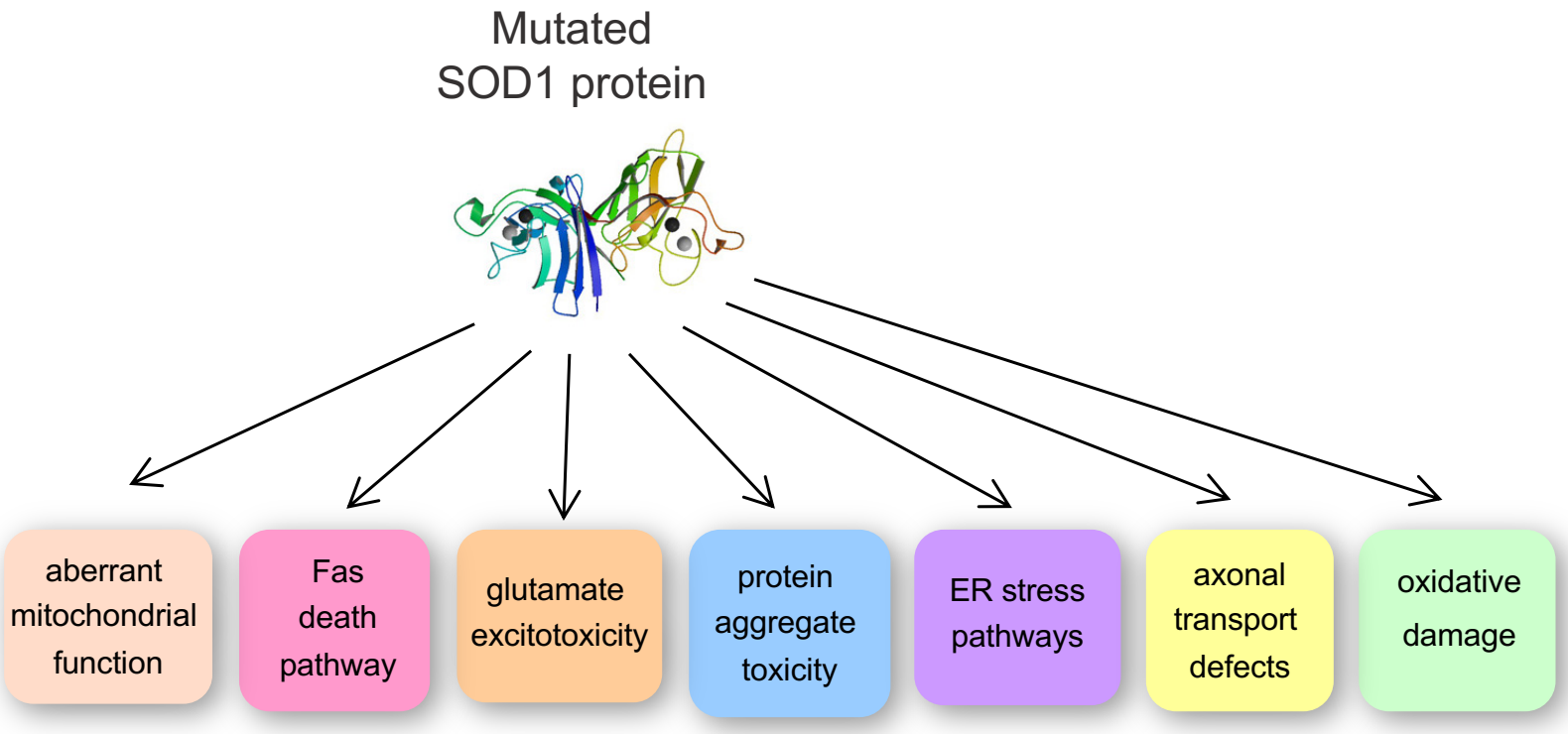
What did we learn from the ALS models based on mutated SOD1?

(some bad news…)

- Broad range of cellular toxicity processes induced by mutated SOD1.
- Non-cell autonomous disease mechanisms.

ALS: SOD1 pathology

SOD1 neurotoxicity: the gain of a complex toxic function



Motor neuron diseases: question 10

In $SOD1^{G93A}$ mice (with 16 to 24 copies of the human SOD1 gene), blocking individual pathways leading to cell death only increases animal survival by 5-10%.

What is your interpretation (answers are ranked)?

A. This animal model is not relevant for testing treatment efficacy - 5

B. Multiple pathways act in parallel to cause neuronal degeneration - 1

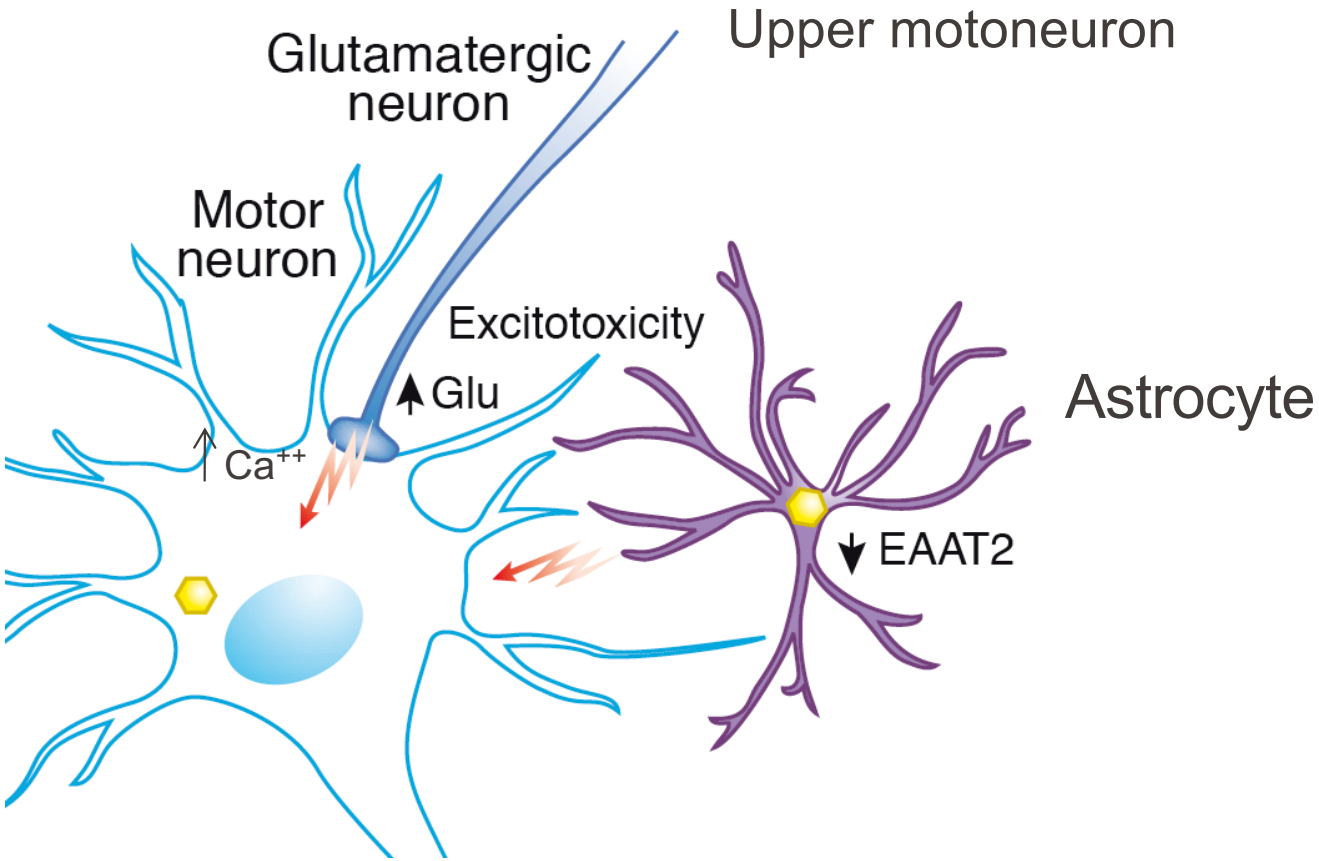
C. This indicates that it is poorly efficient to act downstream in the pathologic cascade - 2

D. The exact cause of the pathology has not been identified yet - 4

E. The level of expression of mutated SOD1 in transgenic mice is too high to reliably test therapeutic approaches - 3

EPFL ALS: mechanisms

Excitotoxicity



ALS: non-cell autonomous disease

SOD1 fALS: challenging the notion of ***cell autonomous pathogenesis***

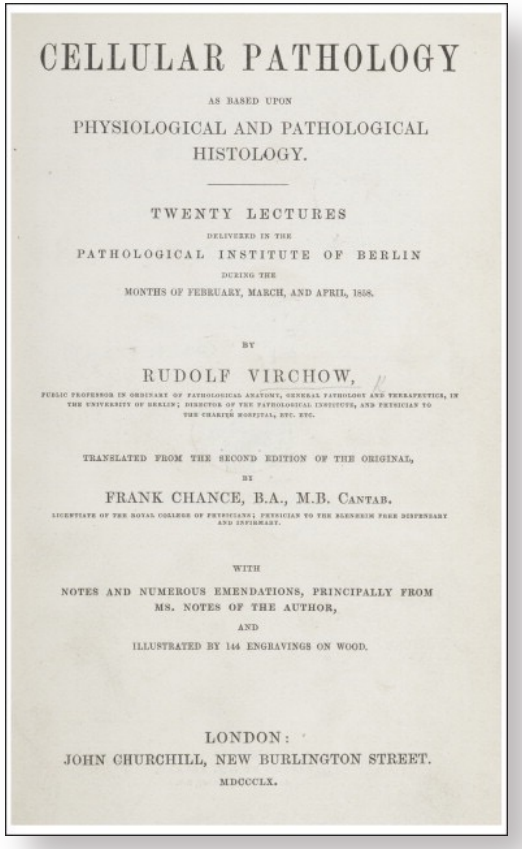
« theory of the cell state »

Metazoans = complex colonies of protozoan-like cells with a highly evolved division of labor

Rudolf Virchow (German physician, 1821-1902)
Claude Bernard (French physiologist, 1813-1878)

Neurodegenerative disorders were often initially considered « cell autonomous », mechanistically resulting from damage within individual cell types, mainly neurons.

⇒ wrong assumption!



CELLULAR PATHOLOGY

AS BASED UPON

PHYSIOLOGICAL AND PATHOLOGICAL HISTOLOGY.

TWENTY LECTURES

DELIVERED IN THE

PATHOLOGICAL INSTITUTE OF BERLIN

DURING THE

MONTHS OF FEBRUARY, MARCH, AND APRIL, 1858.

BY

RUDOLF VIRCHOW,

PUBLIC PROFESSOR IN ORDINARY OF PATHOLOGICAL ANATOMY, GENERAL PATHOLOGY AND THERAPEUTICS, IN THE UNIVERSITY OF BERLIN; DIRECTOR OF THE PATHOLOGICAL INSTITUTE, AND PHYSICIAN TO THE CHARITÉ HOSPITAL, ETC. ETC.

TRANSLATED FROM THE SECOND EDITION OF THE ORIGINAL,

BY

FRANK CHANCE, B.A., M.B. CANTAB.

LICENTIATE OF THE ROYAL COLLEGE OF PHYSICIANS; PHYSICIAN TO THE BLENHEIM FREE DISPENSARY AND INFIRMARY.

WITH

NOTES AND NUMEROUS EMENDATIONS, PRINCIPALLY FROM MS. NOTES OF THE AUTHOR,

AND

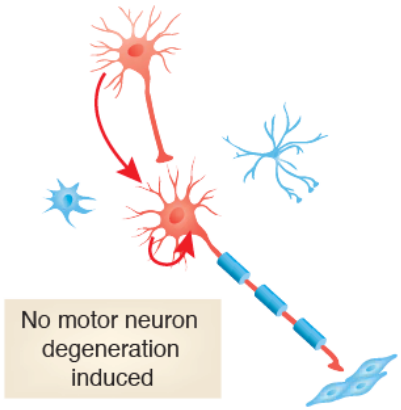
ILLUSTRATED BY 144 ENGRAVINGS ON WOOD.

LONDON:
JOHN CHURCHILL, NEW BURLINGTON STREET.
MDCCCLX.

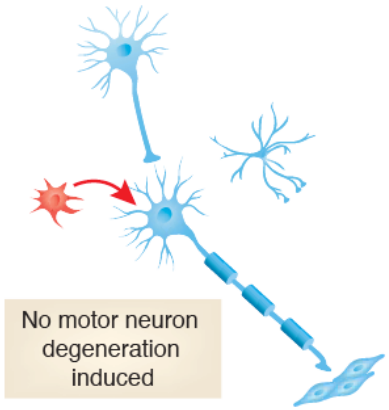
ALS: non-cell autonomous disease

Transgenic mouse models:
mutated SOD1 has to be ubiquitously expressed to generate ALS pathology

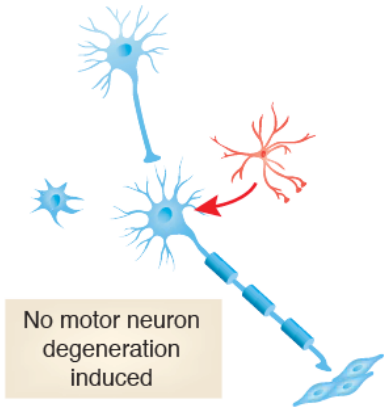
Mutant SOD1 only in neurons
(*Thy1* or *Nefl* promoter)



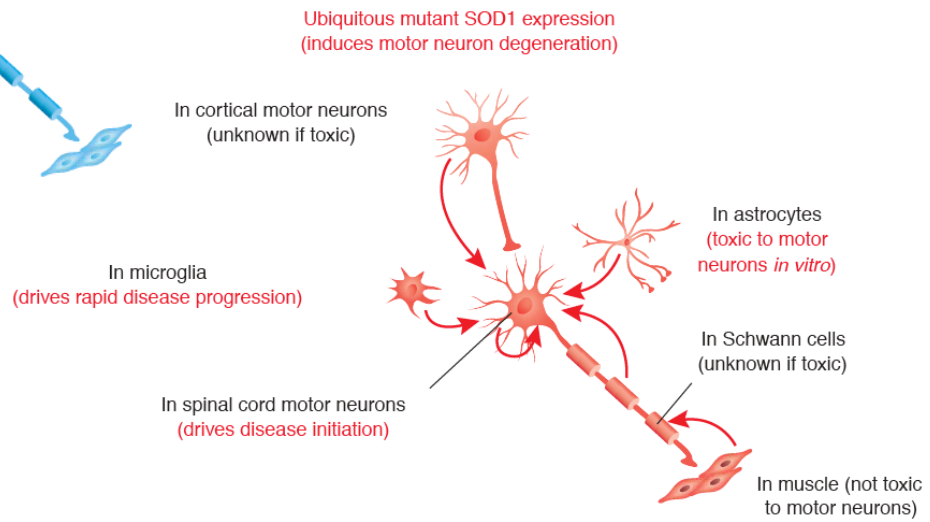
Mutant SOD1 only in microglia
(transplanting the myeloid lineage)



Mutant SOD1 only in astrocytes
(*Gfa2* promoter)



Ubiquitous mutant SOD1 expression
(induces motor neuron degeneration)

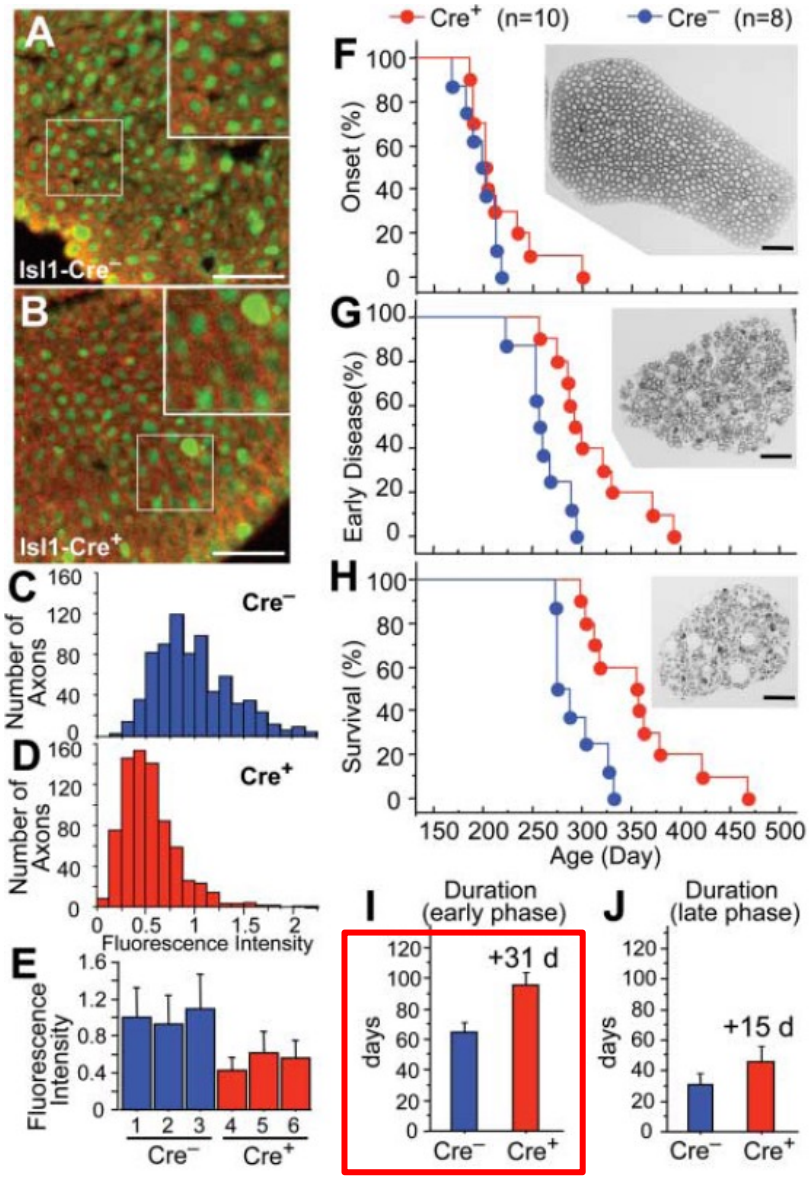


- Lobsiger CS, Cleveland DW, Nat Neurosci 10(11), 2007

ALS: non-cell autonomous disease

Mutated SOD1 expression in motor neurons determines ALS onset.

- Cre-lox system to inactivate mutated SOD1 overexpression in specific cell types.
- Reduction of mutated SOD1 in motor neurons only (Islet1-Cre) retards disease progression in the early phase of the disease.

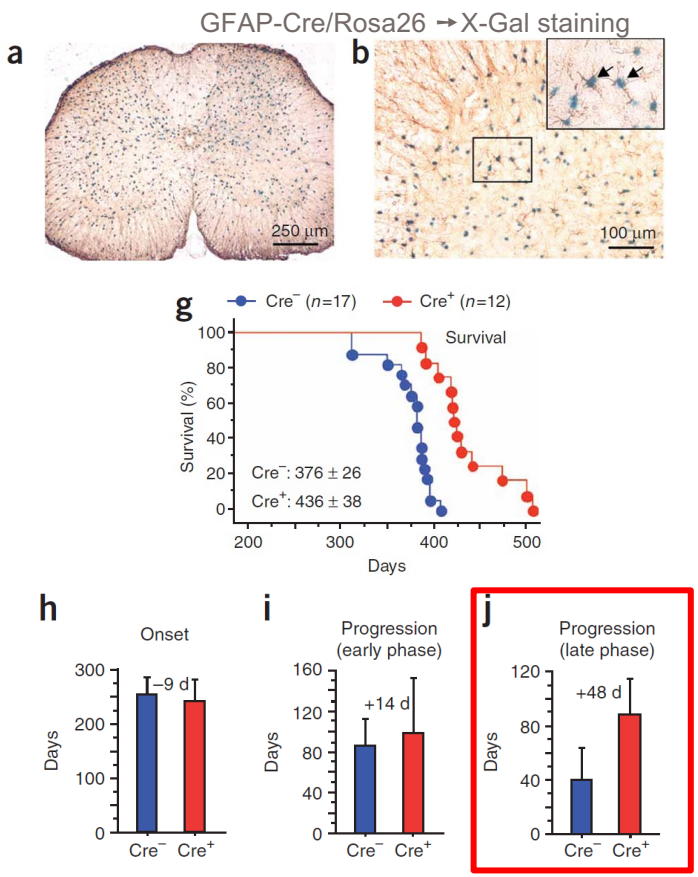


- S. Boillée et al., Science 312, 1389 (2006)

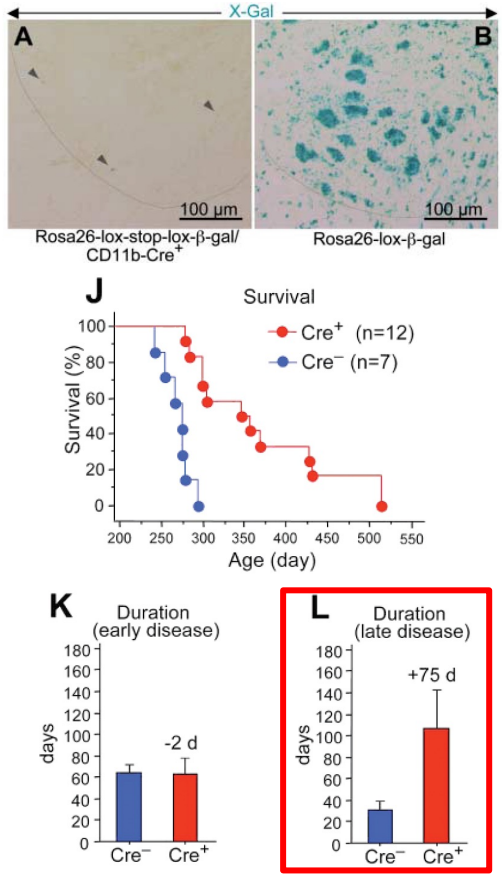
ALS: non-cell autonomous disease

Expression of mutated SOD1 in glial cells determines late disease progression

Astrocytes



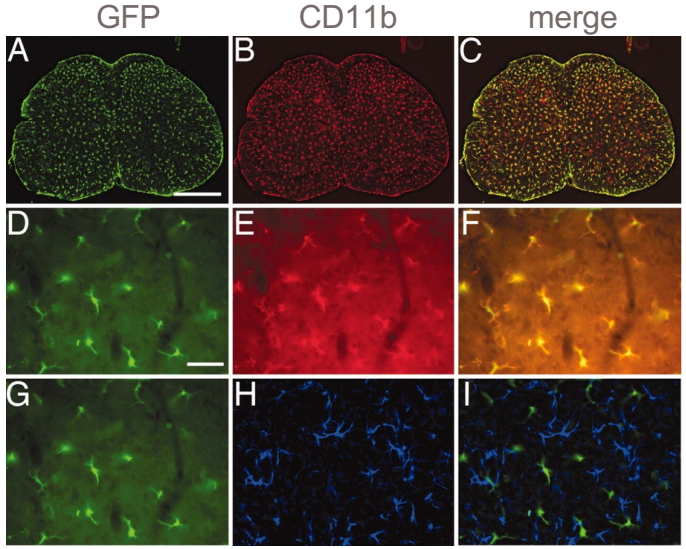
Microglial cells



ALS: non-cell autonomous disease

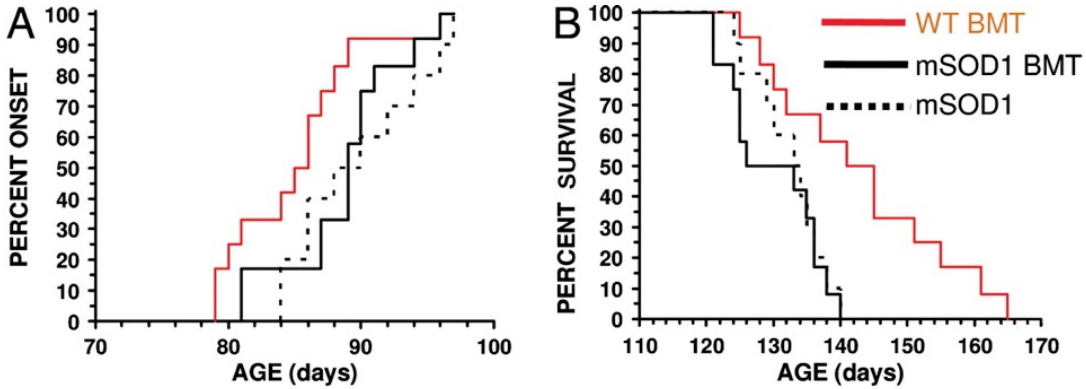
Wild-type microglial cells from bone marrow slow down disease progression in fALS mice

Tx of GFP-expressing bone marrow cells



- PU.1-/- mice: lack mΦ, neutrophils, T and B cells, **CNS microglia**
- Need bone marrow Tx to survive
- Crossed with fALS SOD1^{G93A} mice

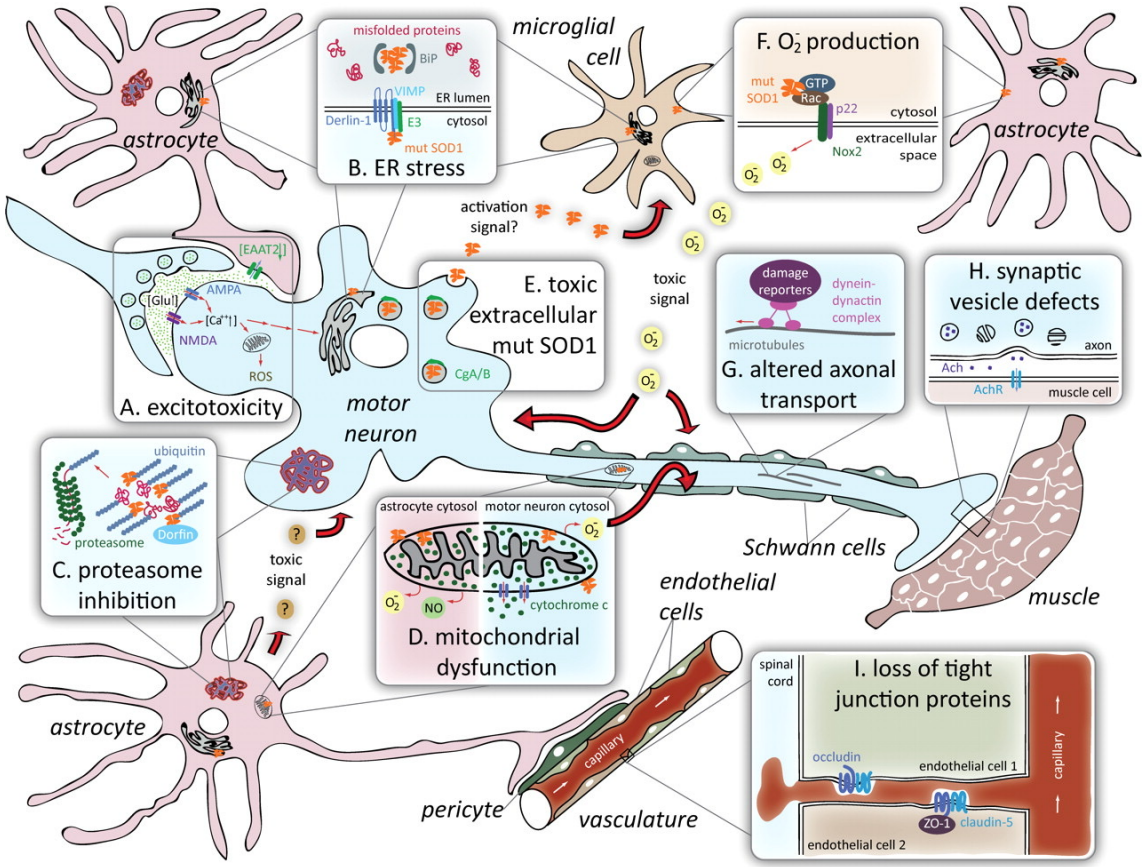
- Tx of wild-type bone marrow cells
  Tx of SOD1^{G93A} bone marrow cells



- Beers D.R. et al., PNAS 103(43), 2006

ALS: non-cell autonomous disease

Proposed non cell autonomous disease mechanisms in SOD1-mediated ALS



- Ilieva H et al. J Cell Biol 2009;187:761-772

JCB

Motor neuron diseases: question 11

From the previous experiments on the role of mutated SOD1 in various cell types, which one(s) are the correct conclusion(s)?

A. It will be impossible to achieve therapeutic efficacy by suppressing SOD1 toxicity only in motoneurons.

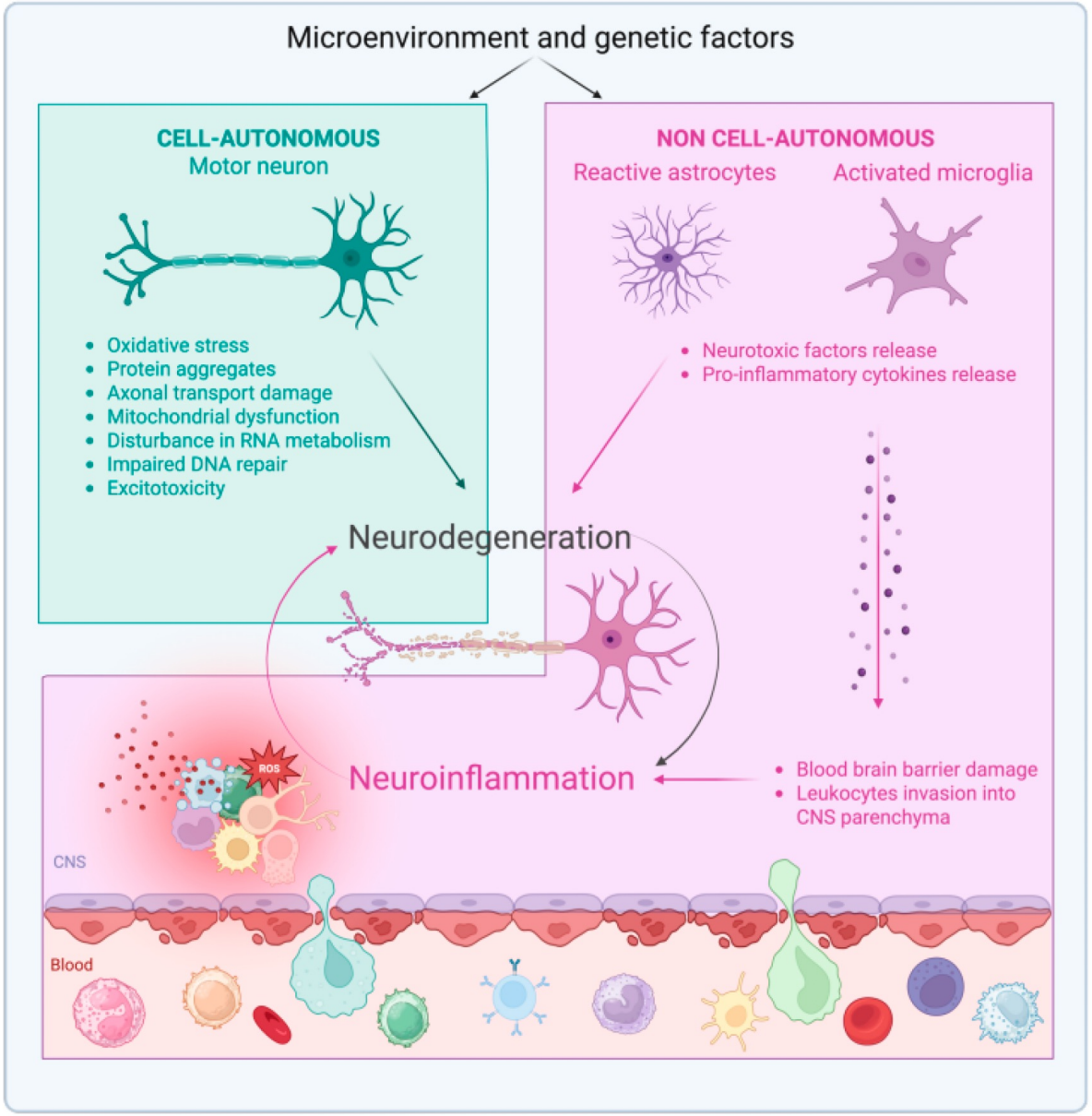
B. Strategies that prevent astroglial and microglial cell activation are more effective than the ones targeting motoneurons.

C. At the time the disease is diagnosed, mainly strategies targeting glial/microglial pathology should be applied.

D. Mutated SOD1 leads to a pathogenic crosstalk between neuronal and glial cells.

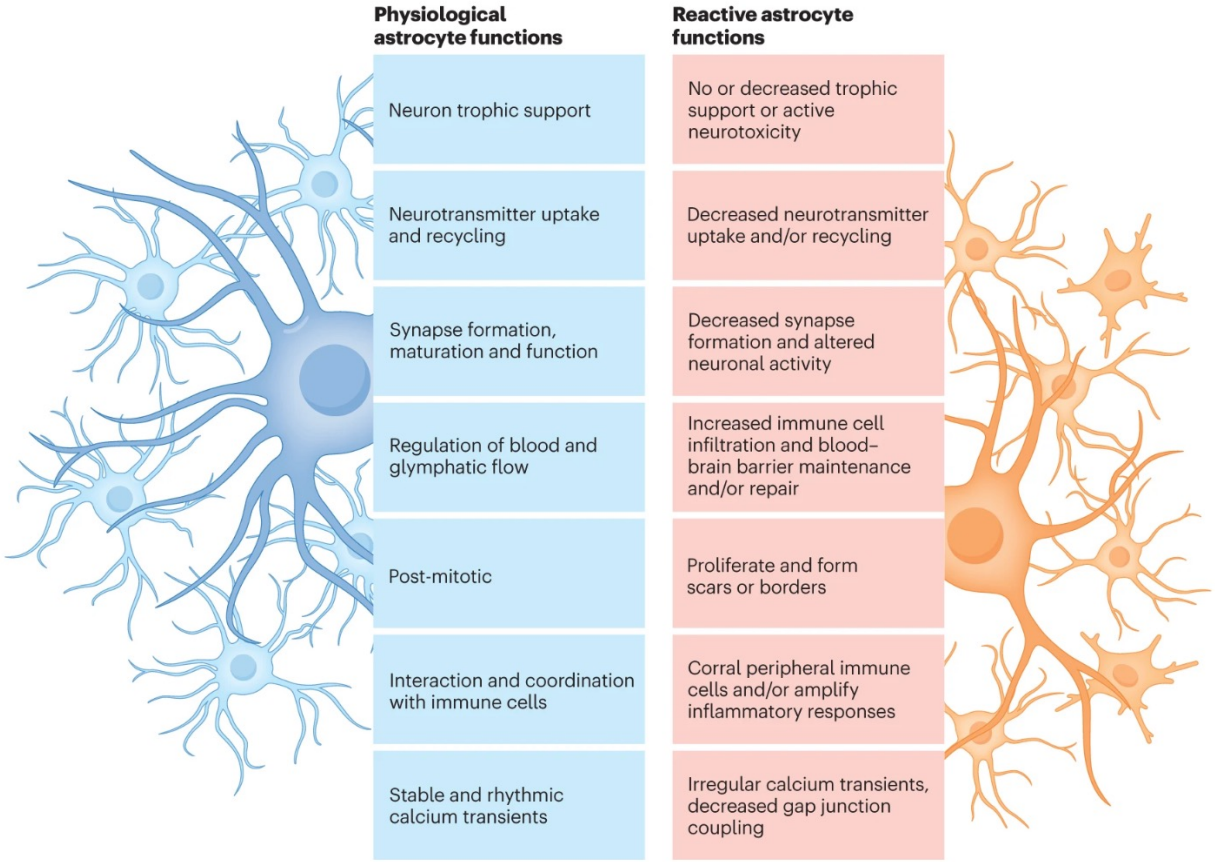
ALS:

a disease model for ‘non-cell autonomous’ mechanisms in neurodegeneration



ALS: non-cell autonomous disease

Reactive astrocytes: from support activity to pathological roles



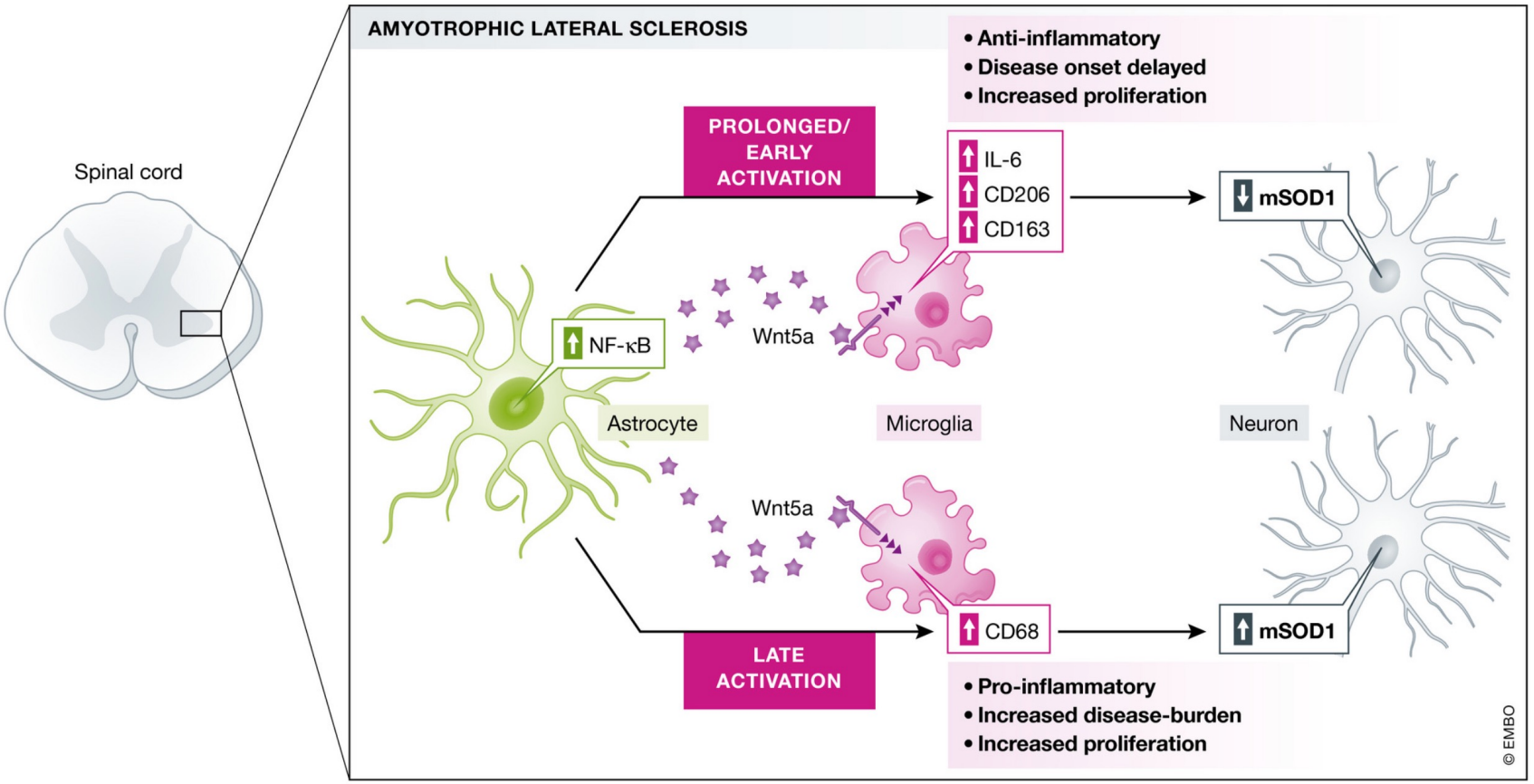
Chronic degenerative disease:

the same cell type can change from a supporting role to a deleterious role during disease progression.

- *Nature Reviews Neurology volume 19, pages 395–409 (2023)*

ALS: non-cell autonomous disease

Example of crosstalk between glial cells: changes with disease progression



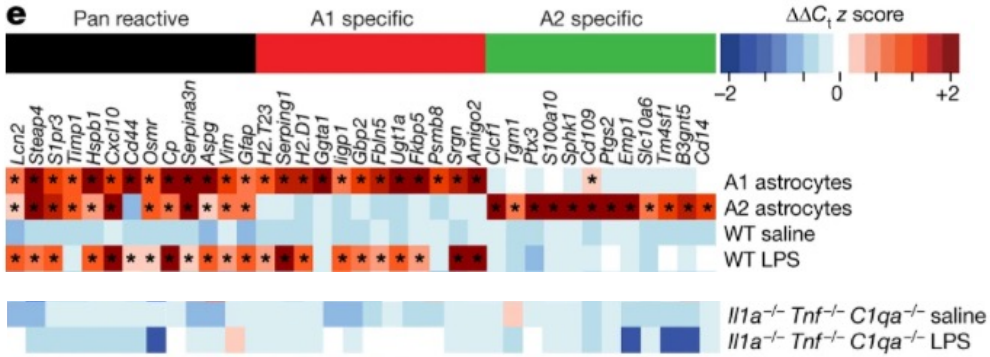
- *The EMBO Journal (2018)37:e100130https://doi.org/10.15252/embj.2018100130*

ALS: non-cell autonomous disease

Crosstalk between microglial cells and astrocytes

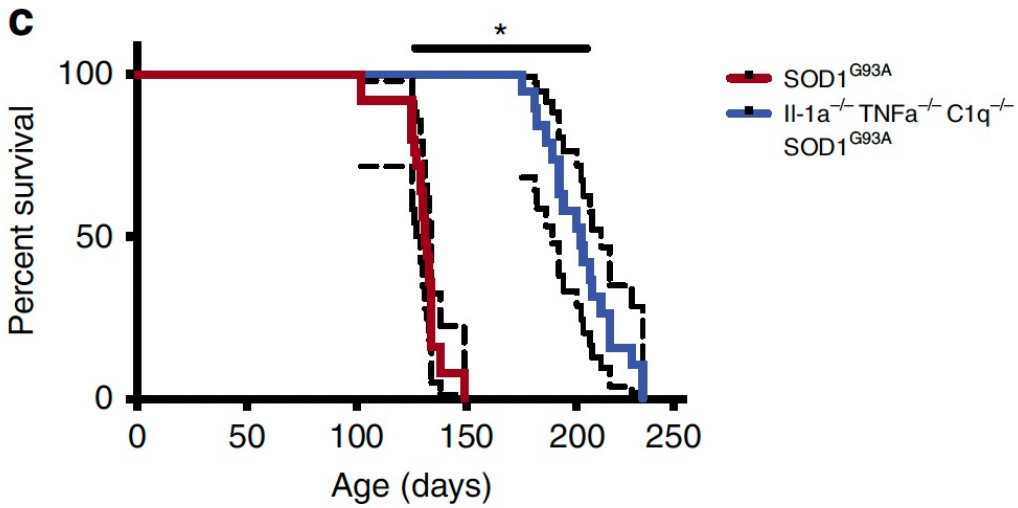
Activated microglia induce A1 astrocytes by secreting Il-1α, TNF and C1q, to induce 'A1' astrocytes. A1 astrocytes lose the ability to promote neuronal survival, outgrowth, synaptogenesis and phagocytosis.

Different subtypes of reactive astrocytes



Triple-knockout (Il1a−/−Tnf−/−C1qa−/−) mice fail to produce A1 astrocytes following LPS injection.

KO of factors to suppress 'A1' astrocyte (IL-1a, TNFα, C1q) prolongs the survival of ALS mice

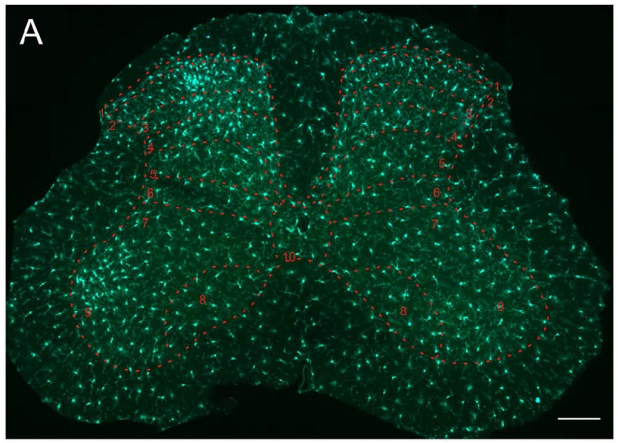


- *Nat Commun. 2020 Jul 27;11(1):3753. doi: 10.1038/s41467-020-17514-9*
  Nature, 2017;541(7638):481-487. doi: 10.1038/nature21029.

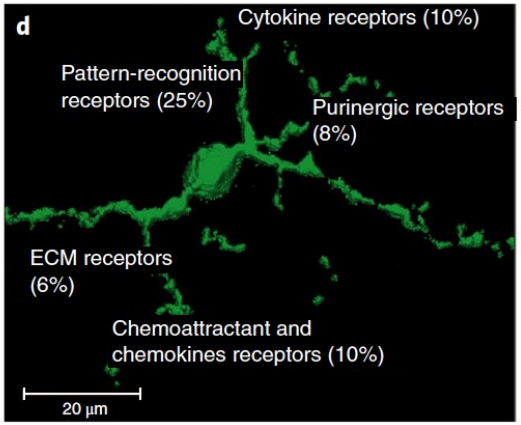
ALS: non-cell autonomous disease

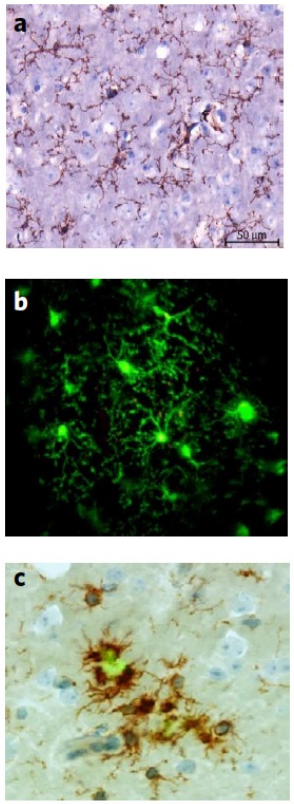
Microglia: a versatile cell type

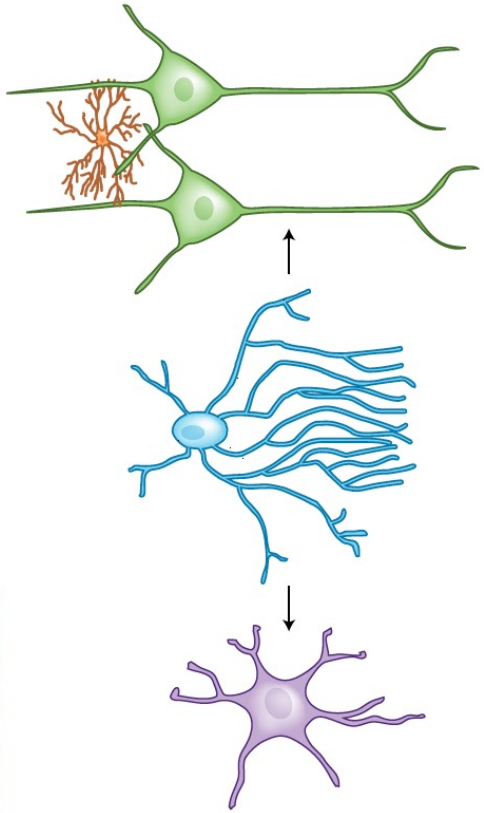
Mouse spinal cord



Ontology of the sensome







Nurturer:
TGFβr (homeostasis)
Chemokine receptors (migration)
C1q (synaptic remodeling)
Trem2 (apoptotic neuron removal)

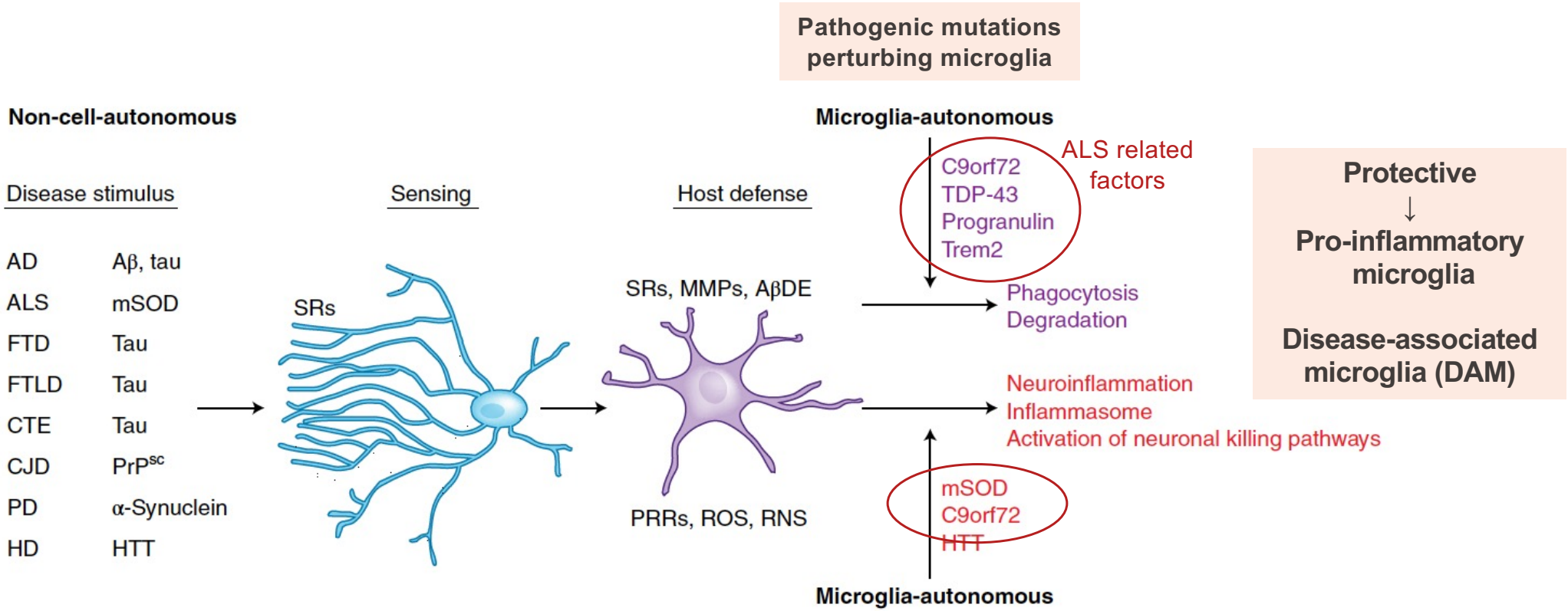
Sentinel:
Sensome

Warrior:
Phagocytic receptors
Antimicrobial peptides
Pattern-recognition receptors
RNS, ROS production

- Hickmann S et al, Nature Neuroscience 21, 1359–1369, 2018

ALS: non-cell autonomous disease

Microglia: a versatile cell type



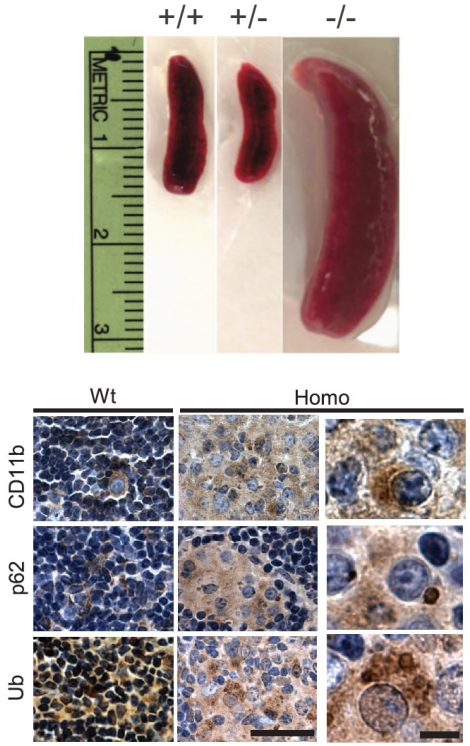
- Hickmann S et al, Nature Neuroscience 21, 1359–1369, 2018

- SR: 'sensing' receptors
- PRR: pattern recognition receptors

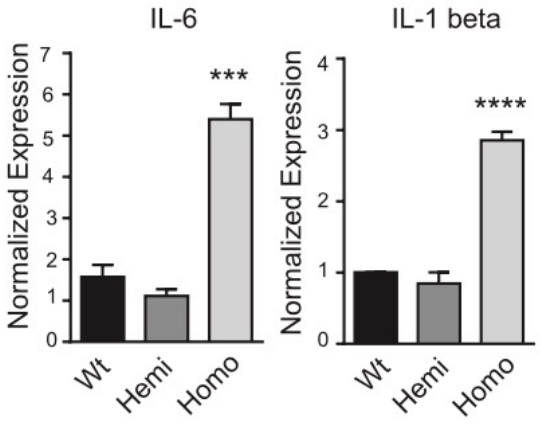
ALS: non-cell autonomous disease

Decreased C9orf72 expression in **C9orf72−/− mice** and C9-ALS patients leads to altered microglial function and neuroinflammation

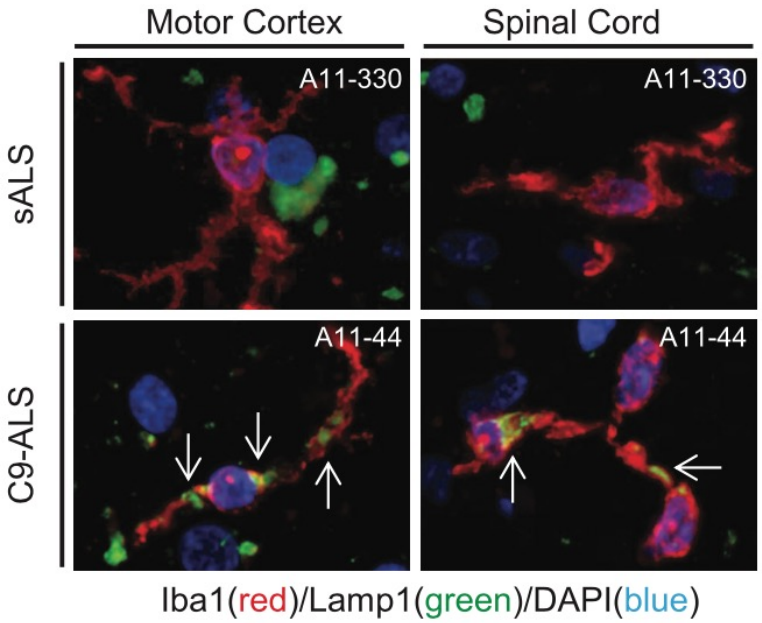
Splenomegaly: accumulation of engorged macrophage-like cells



Expression of pro-inflammatory cytokines in microglia isolated from C9orf72−/− mice



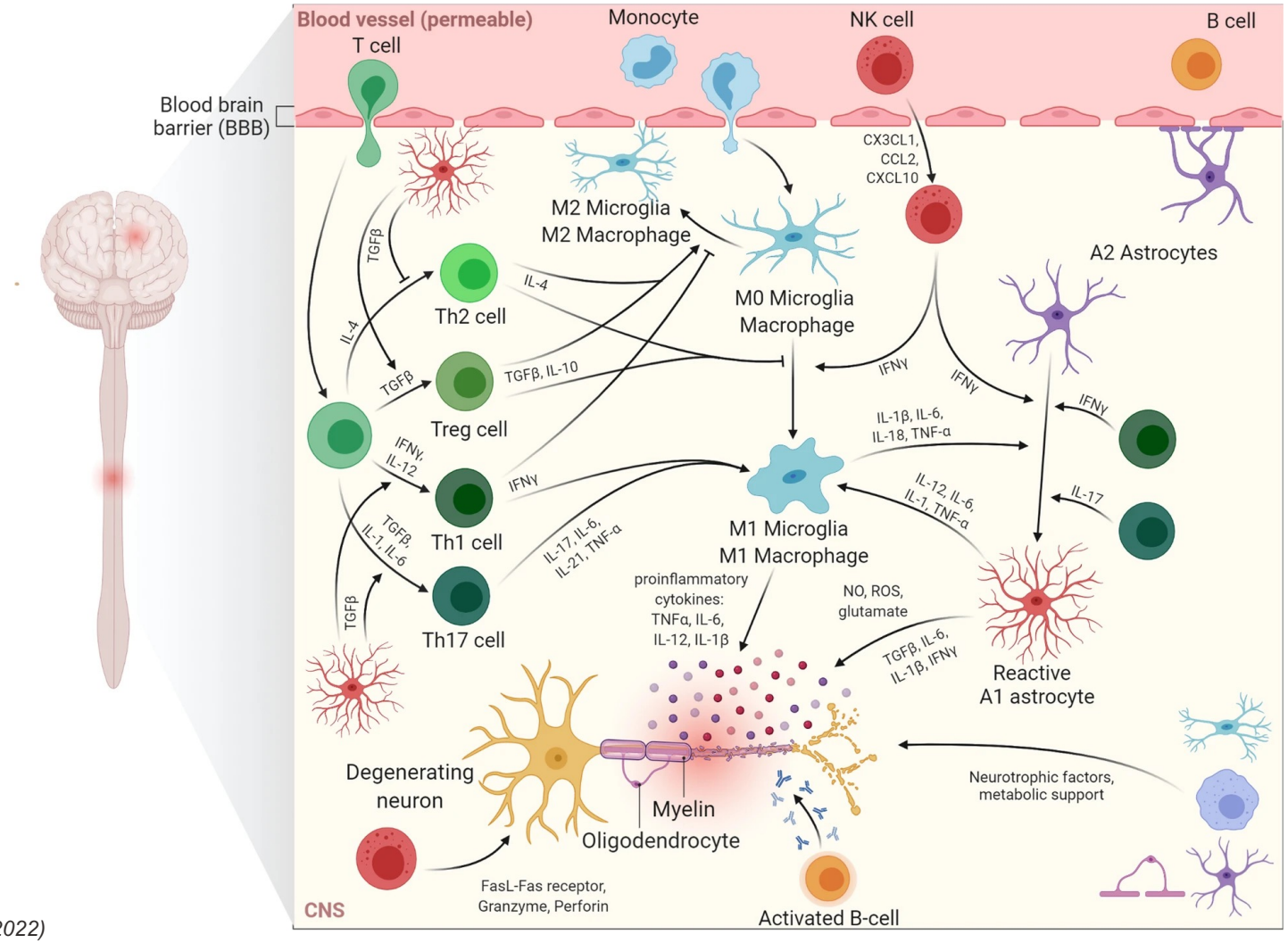
Accumulation of phagocytic material (Lamp1) in C9-ALS microglia



- O'Rourke et al, Science 351 (6279), 2016

ALS: non-cell autonomous disease

The role of the immune system in ALS



- *Molecular Neurodegeneration vol 17: 22 (2022)*