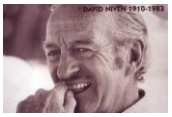
Imperial College London

Motor Neurone Disease/ Amyotrophic Lateral Sclerosis I

Overview
Clinical features
Neuropathology
Pathogenesis

Motor disorder resulting in progressive paralysis and death normally from respiratory failure

Amyotrophic lateral sclerosis (ALS) is a chronic neurodegenerative condition leading to muscle wasting, paralysis and death usually within 3-5 years



- Motor Neurone Disease
- Lou Gehrig's disease
- Charcot's disease

ALS causes muscle atrophy, wasting and spasticity

- Progressive muscle weakness of limbs, trunk, tongue and respiratory muscles.
- Onset is insidious usually confined to distal muscles of a single limb and progresses to become widespread
- Impaired swallowing and speech ('bulbar signs')
- Respiratory failure
- No impairment of bladder, bowel or sexual function.
- Cognitive, oculomotor, sensory & autonomic function spared.

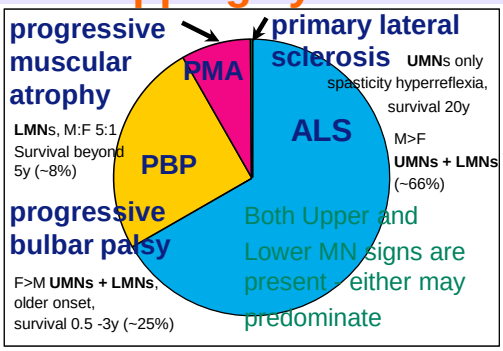
ALS is distinguished from other ***motor neurone diseases**** by the presence of BOTH upper & lower motor neurone signs

Lower motor neurone (LMN): muscle weakness, wasting, fasciculations, cramps.

Upper motor neurone (UMN): stiffness and slowness of movement, slow and clumsy speech. Babinski signs are often present.

*e.g. spinal muscular atrophy and hereditary motor neuropathies

Overlapping syndromes



Incidence - prevalence - survival

Prevalence: 5 -8 /100,000

Annual incidence: 1 - 2.5 /100,000

90-95% of cases are sporadic (SALS)
5-10% are familial (FALS) - autosomal dominant inheritance with the typical clinical picture of SALS

Onset of disease is usually after 40 and incidence increases from 3rd to 8th decade

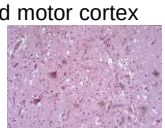
Average survival 2-5 years

High incidence foci in Guam, Kii peninsula of Japan, New Guinea (ALS-PD-AD).

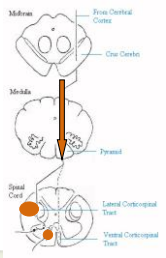
ALS is characterised by:

- Motor neurone loss in spinal cord, brain stem and motor cortex

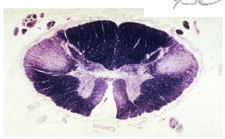
Control lumbar cord



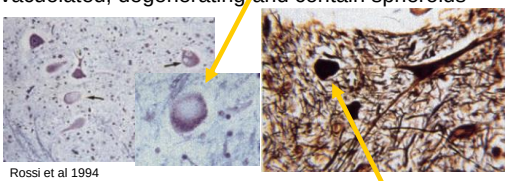
ALS lumbar cord



- Corticospinal tract degeneration (crossed and uncrossed fibres)

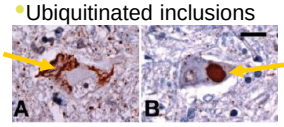


- Motor neurones are chromatolytic, swollen, vacuolated, degenerating and contain spheroids



Rossi et al 1994

- Ubiquitinated inclusions



Mackenzie et al 2007

neurofilament aggregations

Ubiquitin immunoreactive LMNs with filamentous (A) or compact inclusions (B)

What causes ALS?

Environmental factors

Neurofilaments

- Selective vulnerability of MNs high metabolic and functional activity
- Adult-onset
- Gender effect M>F

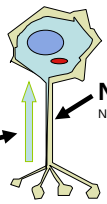
Genetic factors

Oxidative stress

Excitotoxicity

Excitotoxicity/ Ca^{2+} overload

High oxidative activity/ oxidative stress



Neurofilaments

NF-L, NF-M, NF-H

- ONLY large calibre neurofilament-rich axons, (a major determinant of conduction velocity) degenerate in ALS
- NF accumulations occur in proximal axons in ALS
- Overexpression of NF-L in mice causes muscle atrophy and death at ~ 28d

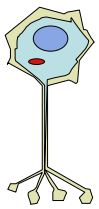
Molecular motors Dynein Dynactin

- Retrograde axonal transport of NFs, NTFs, misfolded proteins.
- Dynactin Mutations in atypical ALS family causes low affinity for microtubules (Puls et al 2003).

The major antioxidant in MNs, superoxide-dismutase (SOD1) is abnormal in 20% of familial cases of ALS (see next lecture).

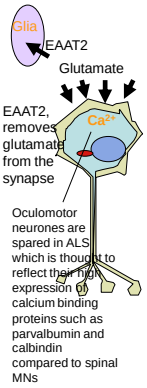
Oxidative damage ?

- High oxidative activity of MNs could lead to the build up of toxic reactive free radical molecules, $O_2^{\cdot -}$, $OH^{\cdot}$, $ONOO^{-}$ (peroxynitrite) generated during oxidation are normally removed by cellular antioxidants (SOD, GSH)
- Free radical damage affects lipids, proteins and DNA and has been detected in ALS (plasma, csf, autopsy samples): protein carbonyls, 3-nitrotyrosine and DNA metabolites e.g. 8-hydroxydeoxyguanosine.



Oxidative damage hypothesis: what are the implications for treatment ?

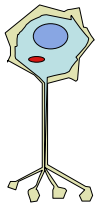
- However initial antioxidant therapy (vitamins C and E) to date has not been proved of benefit.
- Too late ?



Excitotoxicity hypothesis:

excess excitation by GLUTAMATE is toxic to MNs and causes degeneration in ALS.

- CSF glutamate elevated (40% of cases)
- Glutamate & Glial glutamate transporter, EAAT-2, decreased in spinal cord in ~ 60% of SALS cases
- EAAT2 is responsible for removal of ~90% of extracellular glutamate
- GluR2 AMPA receptor down-regulated in ALS increasing Ca^{2+} permeability
- Glutamate analogues cause neurodegeneration (e.g.beta methyl amino alanine BMAA and beta oxazole amino alanine BOAA taken in the diet)



Excitotoxicity hypothesis:

what are the implications for treatment ?

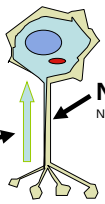
- Riluzole, the currently approved medication for ALS patients which has a modest benefit in ALS (breathing capacity) promoting survival, blocks voltage-gated sodium channels and reduces glutamate release
- Upregulation of EAAT2 expression? Screening of FDA approved drugs for this property yielded candidates e.g. beta lactam antibiotic, ceftriaxone, which is now going through Phase I-III trials in man.

Excitotoxicity/Ca^{2+}

- Glial glutamate transporter, EAAT-2, decreased in SALS
- Oculomotor neurones are spared in ALS (parvalbumin and calbindin)

High oxidative activity

The major antioxidant in MNs, superoxide-dismutase (SOD1) is abnormal in 20% of familial cases of ALS



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Dynein
Dynactin

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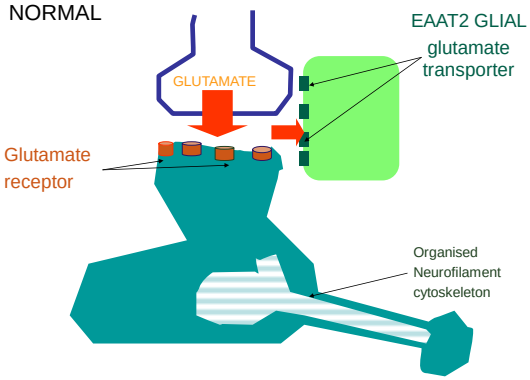
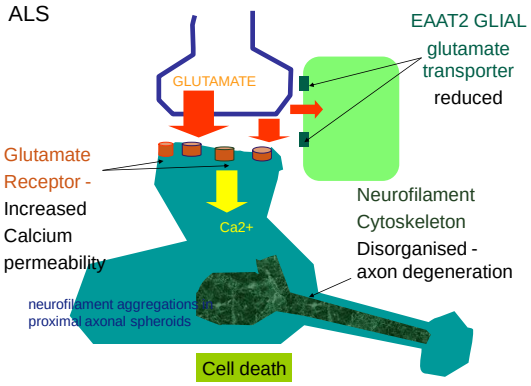




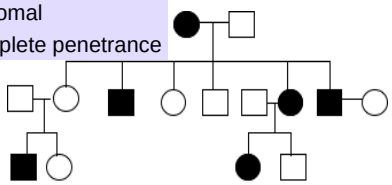
Familial Motor Neurone Disease/ Amyotrophic Lateral Sclerosis (FALS) II

10% of cases of ALS are inherited

Identification of genes causing familial disease provides information about disease mechanisms in sporadic cases

FALS

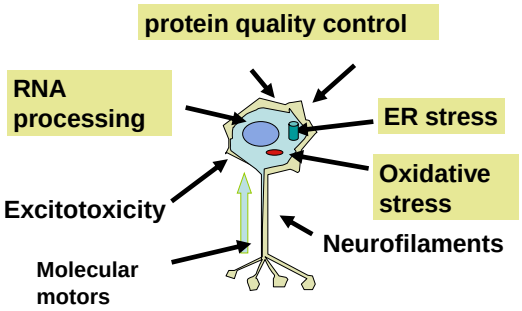
- Dominant
- Autosomal
- Incomplete penetrance



FALS Case History

- Difficulty climbing stairs
- Weakness of R thumb
- Brisk reflexes
- Progressive weakness and wasting
- Wheelchair-bound
- Spasticity of upper limbs
- Fasciculations in the tongue and brisk jaw jerk
- Died of pneumonia

Pathways targeted by FALS mutations



Familial ALS: classical ALS, upper and lower motor neuron signs, rapid progression, dominant

c9ORF72	ALS/FTD	~35%
SOD-1	ALS1	20%
FUS/TLS	ALS6	4%
TARDBP	ALS10	3%
VAPB	ALS8	< 1%
D-amino acid oxidase		< 1%
Ubiquilin 2	X-linked	< 1%
Angiogenin	ALS9	< 1%
Optineurin	ALS12(AR/AD)	< 1%
FIG4	ALS11(CMT4J)	< 1%

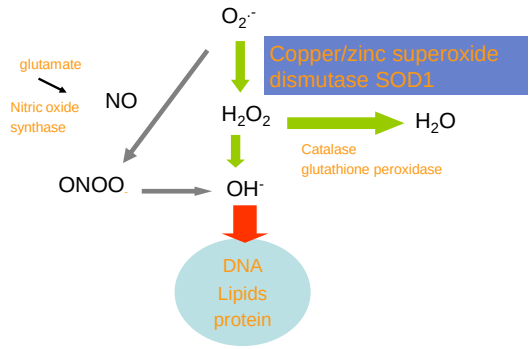
Rare forms of ALS/ motor neurone disease, atypical presentation, juvenile onset, recessive, normal lifespan

Alsin	ALS2	Juvenile, PLS, AR
Senataxin	ALS4	Juvenile, CMT, dHM, AD

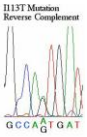
Pathways targeted by FALS mutations

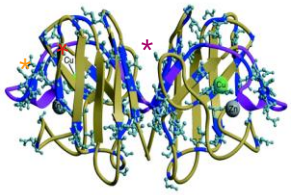
Copper/ zinc dependent superoxide dismutase (SOD1)

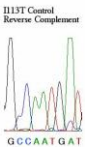
SOD1 function



SOD1





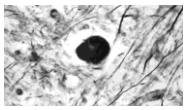


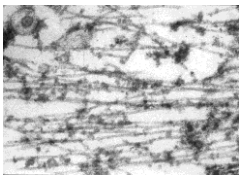
- Over 100 mutations
- Most are missense mutations
- Insertions, deletions & splice site variations some result in truncated proteins
- Mutations occur in most domains:
  - copper binding histidines *
  - substrate binding sites *
  - dimerisation interface *

SOD1 enzyme activity

- Enzyme activity can be detected in several peripheral tissues e.g. erythrocytes, where enzyme activity is ~ 50% control values
- With some mutations enzyme activity is only slightly reduced or normal
- In general, there is no correlation between disease severity and reduction of enzyme activity except with a single mutation (G93R) where enzyme activity is reduced by > 70%

SOD1 mutations associated with neurofilament-rich inclusions

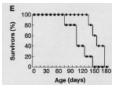


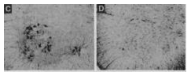


How do SOD1 mutations disrupt the cytoskeleton?

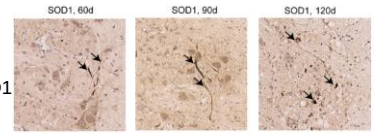
Overexpression of SOD1 mutations (G93A) in transgenic mice causes loss of motor neurones, denervation and paralysis

(Gurney et al 1994)





- Early changes include dendritic vacuolisation and mitochondrial swelling
- Extensive ubiquitination occurs with disease in SOD1 mice and ALS



SOD1 mutations promote cell death in cell culture: implications for drug treatment

- SOD1 mutations are pro-apoptotic in neuronal cell lines and activate caspase-mediated cell death (Patel et al 2002)
- Overexpression of Bcl-2 delays FALS in "mnd mice" (Kostic et al 1998)
- Caspase inactivation doubles survival in "mnd mice" (Friedlander et al 1998)

SOD1: mechanisms of pathogenesis

Overexpression of mutant SOD1 provides a model of MND but SOD1 knock-out mice do NOT develop MND

The SOD1 mutation produces disease though a GAIN OF FUNCTION effect

Effect of mutation alters protein function

Effect of mutant protein

SOD1 mutation

▼

Decreased stability
Abnormal metal binding
Toxic oxidative properties
Aggregation propensity

▼

Mutant protein overload
Impaired intracellular transport
Protein aggregation

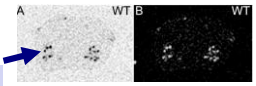
▼

Reduced availability of molecular chaperone

▼

MN degeneration

Molecular chaperones e.g. heat shock proteins bind to misfolded proteins and target for proteolysis (HSP27, 70, 40, αBcrystalin)



HSP27 is enriched in MNs*

*HSP27/22 mutations cause hereditary neuropathy

SOD1-HSP aggregates found in ALS & SOD1 mouse in mitochondria and cytoplasm (Okado-Matsumoto and Fridovich 2002

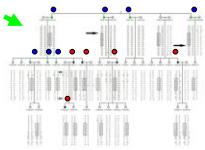
Pathways targeted by FALS mutations

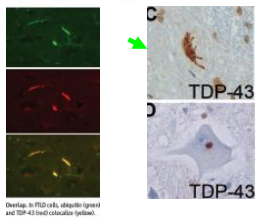
RNA processing

RNA processing genes in ALS

- Chr 9 locus in a family with both ALS and FTD present (Morita et al 2005)
- TDP-43 identified as a major component of ubiquitinated inclusions in FTD (Arai et al 2006: Neumann et al 2006) and later found in chr 9 linked families and SALS (Cairns et al 2007)

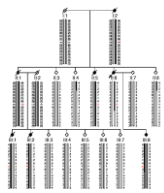
Fronto-temporal dementia (FTD) is a clinically diverse behavioural disorder with semantic dementia and progressive non-fluent aphasia





RNA processing genes in ALS

- FALS cases found with mutations in TARDBP, the gene encoding the TDP-43 protein (Sreedharan et al 2008), mainly ALS not FTLD



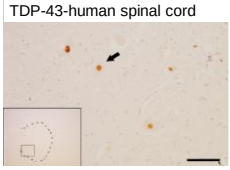
Onset 47y duration 4.5y M337V

TDP-43 positive ubiquitinated aggregates are found in multiple neurodegenerative diseases "TDP-43 proteinopathies"

- **SALS**, **FALS** with mutations in TDP-43 and chr 9p-linked ALS/FTLD but **NOT** SOD1
- **Fronto-temporal lobar degeneration (FTLD)** sporadic , **FTLD** with mutations in progranulin, valosin-containing protein **NOT** Tau
- **AD** ~ 30%
- **PD** ± dementia partially co-localised with tau/ alpha-synuclein aggregates.
- **SCA3**, **HD and Myopathies**

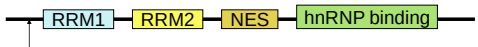
TDP43

TDP-43-human spinal cord



- TDP-43 binds TAR DNA sequences in DNA/RNA acting as a transcriptional repressor, inhibits splicing and regulates mRNA transport/ local translation

Site of most mutations FALS and SALS (29/30)



caspase cleavage

RNA recognition motifs
RRM1 essential
~5UGs+

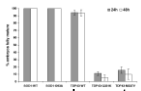
Nuclear export signal

Glycine-rich, interacts with ribonucleoprotein complexes associated with mRNA generation

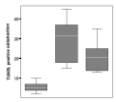
Effect of TDP-43 mutations

- TDP-43 is cleaved and relocates from nucleus to cytoplasm.
- Hyperphosphorylated, ubiquitinated aggregates present in cytoplasm (nuclear), glia, dystrophic neurites

Developmental delay in chick embryos



Increased apoptosis



Sreedharan et al 2008

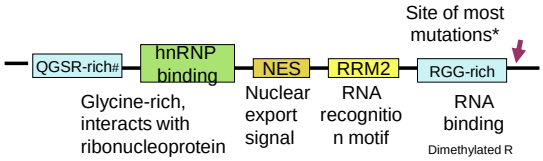
- Drosophila and rodent wt-TDP-43 transgenics produce neurodegeneration and paralysis
- TDP-43 mutations exhibit higher toxicity than wt in chick embryo

Many unanswered questions

- What are the RNA targets?
- Loss of function or gain function?

FUS identified: another RNA/DNA binding protein involved in RNA processing

FUS (fused in sarcoma)/
TLS (translocation in liposarcoma #)



Kwiatkowski et al., 2009 and Vance et al., 2009

translocated to C-terminal of transcription factors (activation)

FUS agggregates are found in FALS and familial FTLD with FUS mutations, SCA 1-3 and HD

TDP-43 and FUS in RNA processing

TDP-43		FUS
REPRESSOR- binds to TAR DNA HIV-1	**Transcriptional regulation**	ACTIVATOR- nuclear hormone receptors, transcription factors, NFkB Associates with RNA pol II/TFIID complex
Binds to CFTR pre mRNA UG intronic tract promoting exonic skipping	**Splicing**	Part of spliceosome machinery In response to DNA damage inhibits CREB and p300 HAT (represses cyclin D Transcription)

TDP-43 and FUS in RNA processing

TDP-43		FUS
REPRESSOR	Transcription	ACTIVATOR
Exon skipping	Splicing	Spliceosomes

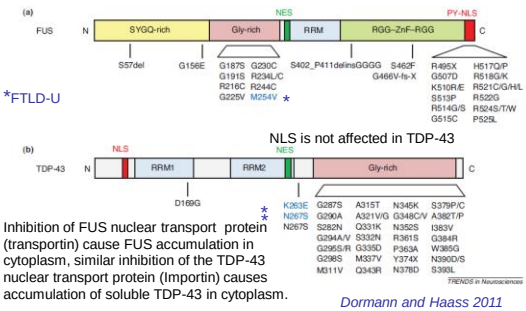
MicroRNA processing

Both associate with Drosha nuclear RNAse III protein involved in miRNA maturation

Subcellular localisation, translation, RNA granules

Both found in RNA transporting granules. TDP-43 loss reduces dendritic branching/synapse formation and FUS KO reduces spine formation. TDP-43 in stress granules promotes transport of NFL to axons for repair.

Nuclear localisation signal (NLS) is the site of the most frequent FUS mutations: impair nuclear import



NLS is not affected in TDP-43

Inhibition of FUS nuclear transport protein (transportin) cause FUS accumulation in cytoplasm, similar inhibition of the TDP-43 nuclear transport protein (Importin) causes accumulation of soluble TDP-43 in cytoplasm.

Dormann and Haass 2011

Effects on nuclear transport are relevant to SALS as these proteins decrease in abundance with age but what triggers the formation of inclusions?

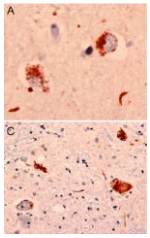
Cell stress, oxidative stress, heat shock, ER stress,

Formation of stress granules in the cytoplasm that contain FUS and TDP-43 containing housekeeping mRNAs do not require translation during stress

Do these stress granules act as precursors of the large inclusions?

9p21 ALS/FTD locus (3 genes):

Chromosome 9 open reading frame 72 (**c9orf72**) mutation identified Sept 2011 (DeJesus Hemandez et al 2011: Renton et al 2011)



TDP-43 +ve inclusions in cytoplasm
Cerebral cortex and spinal cord

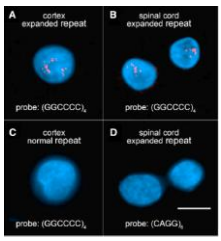
DeJesus Hemandez et al 2011 Expanded repeats in C9ORF72 in FTD and ALS

Prevalence of c9ORF72 expansions

Familial FTD (171)	12%	(DeJesusHernandez et al 2011) 8%GRN/6%MAPT
Sporadic FTD (203)	3%	(DeJesusHernandez et al 2011) 3%GRN/2%MAPT
Familial ALS (268)	38%	(Renton et al 2011) US/GER/ITA
	36%	UK/Europe
Sporadic ALS (195)	4%	(DeJesusHernandez et al 2011) 1%TARDBP/2%FUS

c9ORF72

- Function unknown
- Hexanucleotide expansion is located in an intron and affects mRNA levels and nuclear RNA granules accumulate: a potential "non-coding repeat expansion disorder" e.g. Myotonic dystrophies



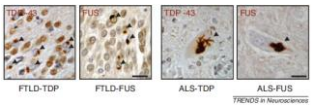
Fluorescently labelled (GGCCCC)4 probe
DeJesus Hernandez et al 2011

Misfolded TDP-43 and FUS are pathological hallmarks of :

ALS Most sporadic cases
Majority of FALS (~ 60% due to mutations in TARDBP, FUS, C9orf72, VCP)

FTLD-U All cases with ubiquitin positive inclusions which are mostly TDP-43 positive (90%) or FUS positive (10%), 50% sporadic and 50% familial (due to mutations in Granulin, TARDBP, FUS (single case), C9orf72, VCP)

Cytosolic inclusions of nuclear proteins



ALS occurs with FTD
FTD cases may have MN degeneration

Pathways targeted by FALS mutations

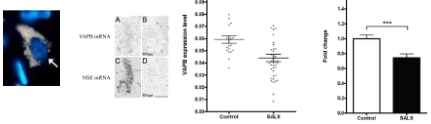
Accumulation of misfolded proteins, ubiqitinated protein aggregates and inclusions (SOD1/ TDP-43/FUS/C9orf72)

UPR is activated in ALS

VAPB (Vesicle associated protein B)

- A VAPB mutation was first described in a large Brazilian family linked to 20q13 with a predominantly lower MN disorder (Nishimura et al 2004).
- A second FALS-associated mutation was found in a UK family in a conserved region (Chen et al 2010)
- VAPB is localised in motor neurons and is significantly decreased in sporadic ALS spinal cord

VAPB mRNA



Anagnostou et al 2008

VAPB is found in the ER: site of protein folding, synthesis of secreted proteins, disulphide bridges formation.

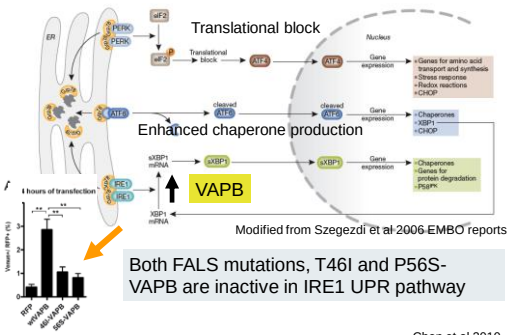
MSP = major sperm protein, CC = coiled coil, TM = transmembrane

FFAT binding domain: used to target FFAT motif in lipid-associated proteins facilitating transport to Golgi and plasma membrane

VAPB is involved in promoting the unfolded protein response (UPR) of the ER to protein overload and folding-deficient mutant proteins

Hypoxia / ischaemia/ Ca^{2+} imbalance/ ATP depletion/ ALS /PD

Unfolded proteins activate the unfolded protein response (UPR)

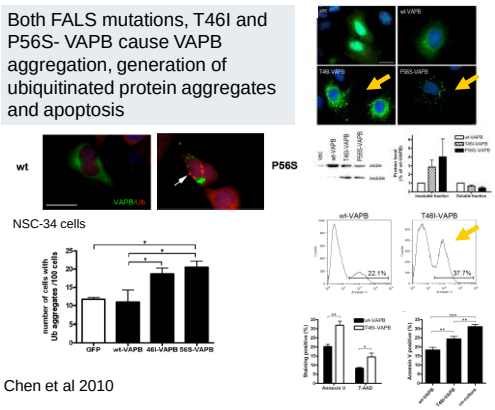


Modified from Szegezdi et al 2006 EMBO reports

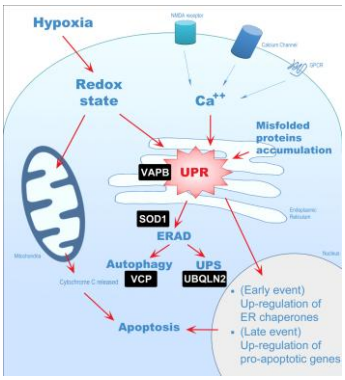
Both FALS mutations, T46I and P56S-VAPB are inactive in IRE1 UPR pathway

Chen et al 2010

Both FALS mutations, T46I and P56S- VAPB cause VAPB aggregation, generation of ubiquitinated protein aggregates and apoptosis



Chen et al 2010



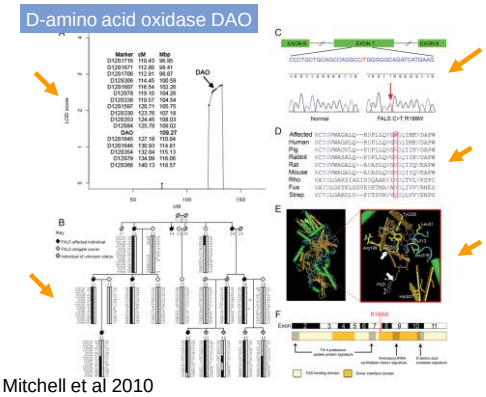
Chen and de Belleroche 2011

Pathways targeted by FALS mutations

Oxidative metabolism and neuronal and glial interactions:

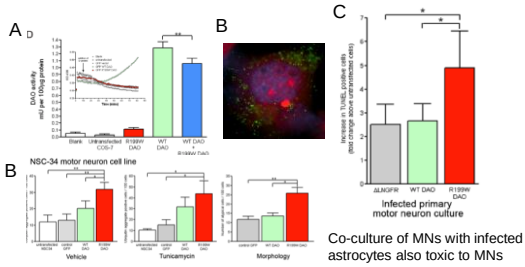
D-amino acid oxidase (DAO) - implicated in ALS and involved in neuronal glial cross talk

D-amino acid oxidase DAO



Mitchell et al 2010

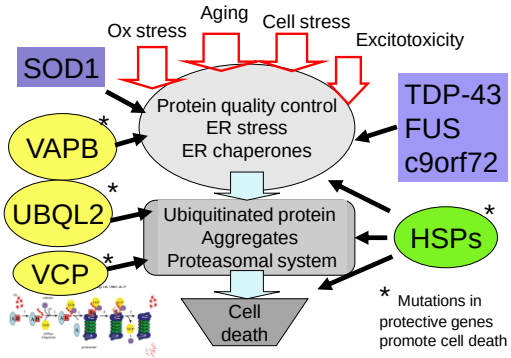
R199W DAO has no enzyme activity (A), can dimerise with the wild-type protein and causes the formation of ubiquitinated aggregates (B) and leads to cell death (C)



Co-culture of MNs with infected astrocytes also toxic to MNs

Mitchell et al 2010

Central role of protein aggregation in ALS



Learning objectives

I Motor Neurone Disease/ amyotrophic lateral sclerosis (ALS): Overview, prevalence, neuropathology and pathogenesis

- Describe the key features of ALS: clinical presentation, prevalence and neuropathology
- Define the main structural and molecular components that characterise the motor neurone (neurofilaments, molecular motors) and describe how they are affected in ALS.
- Discuss the evidence for the role of oxidative stress and excitotoxicity in the pathogenesis of ALS.

Learning objectives: II Familial ALS (FALS)

- Describe the clinical features and prevalence of FALS
- Define the known genes that are causal in classical cases of FALS: copper/zinc-dependent ALS (SOD1), TDP-43, FUS and vesicle associated protein B (VAPB) and their prevalence in the FALS population.
- Describe the nature of SOD1 mutations, their diversity, clinical heterogeneity and functional heterogeneity.
- Discuss the experimental evidence for the pathogenic mechanisms mediating ALS in FALS cases with SOD1 mutations (neuropathology, biochemical, cell culture and transgenic studies)
- Define the other known FALS loci that are associated with classical ALS (chromosome 9 ALS with FTD) and atypical forms of ALS (Alsin, Senataxin) and discuss their relevance to understanding ALS pathogenesis.

Reviews

- From Charcot to Lou Gehrig: deciphering selective motor neuron death in ALS. Cleveland and Rothstein Nature Reviews Neuroscience, 2, 806-819 (2001)
- Molecular biology of amyotrophic lateral sclerosis: insights from genetics. Pasinelli and Brown Nature Reviews in Neuroscience 7, 710-724 (2006) .
- Mini-review: Rethinking ALS: The FUS about TDP-43(2009) Lagier-Tourenne and Cleveland Cell 136, 1001-4.