RNA binding proteins and protein binding RNAs in ALS and FTD

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What is amyotrophic lateral sclerosis?
(AKA, motor neuron disease, Lou Gehrig’s disease)

Progressive muscle wasting disease due to the degeneration of motor neurons.

People become progressively paralysed unable to walk, talk, feed or toilet themselves.

Spares sensation and eye movements, bladder and bowel function, and cognition.

People die because they are unable to breathe, death occurs ~20 months after diagnosis.

There is no effective treatment for this disease.
Epidemiology and prognosis

Average age of onset mid-50’s

Mode of transmission
- Sporadic – 90-95%
- Familial – 5-10% (autosomal dominant)

Male : Female – 3:2
Incidence 1-2.5 / 100,000

Prognosis – difficult to predict in an individual patient
- 50% live 3-4 or more years
- 20% live 5 or more years
- 10% live 10 or more years
- Occasional patients live 20 years or more.
Frontotemporal dementia

- Prominent frontal and temporal lobe atrophy.
- Deterioration of personality and cognition.
- Mirror image of AD with pronounced behaviour problems initially and memory problems later.
- Accounts for up to 3-20% of dementias.
- Common cause of dementia in younger population - in 45-64 age group at 15 per 100,000 (same as AD).
- Mean age of onset 52.8
Genetic overlap of ALS and FTD

Ito Neurology 2011
Progress of genetic findings related to ALS etiology and pathogenesis

# Mutations in Slovenian ALS patients

## Clinical data of Slovenian ALS patients with detected genetic changes

<table>
<thead>
<tr>
<th>Gene</th>
<th>Nucleotide change</th>
<th>AA change</th>
<th>Frequency (%)</th>
<th>Gender</th>
<th>Onset; age of onset; disease duration; associated symptom</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD1</td>
<td>c.43G&gt;A</td>
<td>p.Val14Met</td>
<td>2.3</td>
<td>F</td>
<td>Spinal; 67 y; 4+ y</td>
<td>Deng et al., 1995</td>
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<tr>
<td>SOD1</td>
<td>c.280G&gt;T</td>
<td>p.Gly93Cys</td>
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<td>Spinal; 51 y; 5+ y</td>
<td>Rosen, 1993</td>
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<tr>
<td>TARDBP</td>
<td>c.990A&gt;G</td>
<td>p.Leu330Leu</td>
<td>1.2</td>
<td>F</td>
<td></td>
<td>This study</td>
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<tr>
<td>FUS</td>
<td>c.1566G&gt;A</td>
<td>p.Arg522Arg</td>
<td>1.2</td>
<td>M</td>
<td>Spinal; 84 y; 1 y</td>
<td>Ticozzi et al., 2009</td>
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<tr>
<td>C9ORF72</td>
<td>Expansion GGGGCC</td>
<td></td>
<td>5.9</td>
<td>M</td>
<td>Spinal; 60 y; 6+ yrs</td>
<td>Renton et al., 2011</td>
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<td></td>
<td></td>
<td></td>
<td>F</td>
<td>Bulbar; 55 y; 2 y</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>M</td>
<td>Spinal; 70 y; 1 y</td>
<td></td>
</tr>
</tbody>
</table>
Molecular pathology of TDP-43 in FTD

Toxicity could be loss of nuclear function or effect of cytoplasmic aggregates.

Neumann, Science 2006
Molecular pathology of TDP-43 in ALS

TDP-43 +ve inclusions, lost from nucleus, insoluble phos+ 25kDa fragments.

Neumann, Science 2006
TDP-43 mutations in ALS
TARDBP mutations

A

B

C

Sreedharan et al. Science 2008
TDP-43 mutations in familial and sporadic ALS

Mutations present in 1-3% of familial and sporadic ALS,
•~40 mutations in ALS all but one are in the G-rich C-terminal domain
•~40% affect a potential phosphorylation site

Dormann, *TINS* 2011
TDP-43 is toxic to neural tissues

Electroporation with mt TDP-43 but not wild-type causes death of motor neurons.

Sreedharan et al. Science 2008
FUS mutations in ALS
Three Mutations in FUS in 8 kindreds

1561 C>T  R521C
1562 G>A  R521H
1540 A>G  R514G

SYQG  Gly Rich  RGG  RRM  RGG  Zn finger  RGG

Human  Mouse  Chick  Xenopus  Zebrafish
RGGDRGGFRGGRGGGDRGGFGPGKMDSDRGDGHRQDRRERPY
RGGDRGGFRGGRGGGDRGGFGPGKMDSDRGDGHRQDRRERPY
RGGDRGNFRGGRGGERGGFGPGKMDSDRGDGHRQDRRERPY
RGGDRGGFRGGRGGDRGGFGPGKMDSDRGDGHRQDRRERPY
RGGDRGGFRGGRGGDRGGFGPGKMDSDRGDGHRQDRRERPY

Vance/Rogelj, Science 2009
FUS mutations in familial ALS

- 40 mutations to date.
- 2/3 of the mutations are at the C terminus of the protein.
- 1/3 of the mutations in the Glycine-rich region.

Dormann, TiNS 2011
FUS inclusions in patients carrying mutations

A  FUS-R521H  #63
B  FUS-R521H  #63
C  FUS-R521H  #63
D  FUS-R521H  #63
E  FUS-R521C  #18
F  FUS-R521C  #86

G  Control  #223
H  Control  #150
I  SOD1 FALS  #34
J  SOD1 FALS  #101
K  Sporadic ALS  #69
L  Sporadic ALS  #72

M  Control  #223
N  Sporadic ALS  #72
O  FUS-R521H  #63

Vance/Rogelj, Science 2009
FUS mutations affect subcellular localisation

CV1 cell line

Rat embryonic neurons

Endogenous FUS

HA-Wild type FUS

HA-R521C FUS

HA-R521H FUS

Vance/Rogelj, Science 2009
C terminus of FUS contains an NLS

Mutant FUS does not colocalize with P bodies, nuclear speckles, RNA transport granules
Mutant FUS colocalizes with stress granules

**C9orf72 mutation**

- Expansion recently associated with approximately 40% of familial ALS, 25% of familial FTLD, and 6%–7% of sporadic cases.

- 500 to >5000 repeats in patients with ALS or FTLD. Individuals without disease possess 2–19 repeats (average ∼2).

- The intronic location of G4C2 repeat strongly suggests a disease mechanism directly involving RNA.

- TDP-43 proteinopathy.

*Vatovec, Neurobiol Ageing 2014*
RNA/DNA structures

- Repeat may cause highly complex DNA and/or RNA structures.
- Sense and antisense transcripts.
dG4C2 forms parallel GQs

**Figure 1:** NMR and CD spectra of d(G₄C₂)

(a)

(b)

Imino regions of ¹H NMR 600 MHz spectra of d(G₄C₂) immediately after addition of 100 mM KCl (a) and one day later (b).

(c) CD spectra of d(G₄C₂) in 100 mM KCl.
Uniform structure breaks down with higher repeat number d(G4C2)4

Figure 4: NMR and CD spectra of d(G4C2)4

(a) Imino regions of 1H NMR spectra of d(G4C2)4 (a) without KCl and (b) after two weeks after addition of 100 mM KCl.

(c) CD specter of d(G4C2)4 in 100 mM KCl.
Mechanism?

Three possible mechanisms:

1. Haploinsufficiency.
2. Repeat-associated RNA toxicity.
3. RAN translation resulting in DPRs.
RNA toxicity hypothesis

G4C2 repeat expansion is transcribed

G4C2 RNA forms foci

Sequesters RNA binding proteins

SF2, SC35, hnRNP-H

Causes aberrant RNA splicing

Neuronal Cell Death

ALS and/or FTD

Lee, Cell Reports 2013
RNA pulldown

- In vitro transcription of constructs with an S1 aptamer on 3’ end.

- 48x(G4C2)-S1; Controls: RFP(1-300)-S1 and S1 only

- Incubate in fresh nuclear or cytoplasmic brain extract from rats.
G4C2-RNA pull down from rat brain

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<tr>
<th>Sample</th>
<th>Protein code*</th>
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<tr>
<td>1, 4</td>
<td>SFPQ</td>
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<tr>
<td>2</td>
<td>NONO</td>
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<td>3, 6</td>
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<td>EEF1A2</td>
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<td>hnRNP H</td>
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Targeted screening of hnRNPs and other RBPs

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<th>RNA binding proteins</th>
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<th>hnRNPs</th>
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<td>GENE</td>
<td>G4C2-Foci</td>
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<td>TDP-43</td>
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<td>U2AF65</td>
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<td>hnRNP-U</td>
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<tr>
<td></td>
<td>SF2</td>
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<tr>
<td></td>
<td>SC35</td>
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</table>

Lee, Cell Reports 2013
RNA foci are dependent on repeat length

Lee, Cell Reports 2013
SC35, SF2, and hnRNP-H colocalize with G4C2 nuclear foci

Lee, Cell Reports 2013
RNA foci in C9ORF72 brain tissues colocalize with hnRNP-H

Lee, Cell Reports 2013
SFPQ Splicing factor, proline- and glutamine-rich

pcDNA3.2/GW/TOPO 72xG4C2

HEK293T

pcDNA3.2/GW/TOPO 72xG4C2

HeLa

pcDNA3.2/GW/TOPO 72xG4C2
NONO  Non-POU domain-containing octamer-binding protein

pcDNA3.2/GW/TOPO
72xG4C2

HEK293T

pcDNA3.2/GW/TOPO
72xG4C2

HeLa

pcDNA3.2/GW/TOPO
72xG4C2
Colleagues and funding

**IJS-ALS group**

Current: Anja Kovanda, Simona Darovic, Sonja Prpar Mihevc, Anja Pucer Janež, Ana Bajc Česnik, Vera Župunski (FKKT), Tomaž Bratkovič (FFA)

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