Lecture 4
ALS – Part III: Potential Causes, Mechanisms and Treatment

Jan 13, 2017

- Assigned readings:

Office hours, Wed. 18th at 3:30-4:30
Included in these slides are uncompleted slides from Jan 11. Updated Jan 13
In ALS patients, approx. 5-10% have familial disease, called familial ALS (F-ALS). We do not know if the cause or causes of FALS is the same as for patients without genetic disease (called ‘sporadic’, because it occurs ‘sporadically’ throughout the population. We believe that insights about FALS will help understand sporadic ALS.

In FALS, linkage analysis indicated many families have mutations in the gene for Cu/Zn superoxide dismutase on chromosome 21.

Cu/Zn SOD1 is a dimer and is responsible for the catalysis of superoxide (O2-) to H2O2; which is then catalyzed to H2O by glutathione peroxidase in nervous system tissue.

Why are motoneurons targeted in ALS? Not known, but the motoneuron axon is very long and requires considerable axon transport to transport material to the synapse.
# Genetics of ALS

<table>
<thead>
<tr>
<th>Locus</th>
<th>Gene</th>
<th>Protein</th>
<th>Protein function</th>
<th>Mutations</th>
<th>Proportion of ALS</th>
<th>Date of discovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>21q22.1</td>
<td>SOD1</td>
<td>Cu-Zn superoxide dismutase</td>
<td>Superoxide dismutase</td>
<td>&gt;150</td>
<td>20%</td>
<td>1993 (ref. 2)</td>
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<tr>
<td>2p13</td>
<td>DCTN1</td>
<td>Dynactin subunit 1</td>
<td>Component of dynein motor complex</td>
<td>10</td>
<td>1%</td>
<td>2003 (ref. 52)</td>
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<tr>
<td>14q11</td>
<td>ANG</td>
<td>Angiogenin</td>
<td>Ribonuclease</td>
<td>&gt;10</td>
<td>&lt;1%</td>
<td>2006 (ref. 141)</td>
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<tr>
<td>q36</td>
<td>TARDBP</td>
<td>TDP-43</td>
<td>RNA-binding protein</td>
<td>&gt;40</td>
<td>&lt;1%</td>
<td>2008 (refs 67 and 142)</td>
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<tr>
<td>16p11.2</td>
<td>FUS</td>
<td>FUS</td>
<td>RNA-binding protein</td>
<td>&gt;40</td>
<td>&lt;1%</td>
<td>2009 (refs 68 and 69)</td>
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<tr>
<td>9p13.3</td>
<td>VCP</td>
<td>Transitional endoplasmic reticulum ATPase</td>
<td>Ubiquitin segregase</td>
<td>5</td>
<td>1-2%</td>
<td>2010 (ref. 44)</td>
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<td>10p15-p14</td>
<td>OPTN</td>
<td>Optineurin</td>
<td>Autophagy adaptor</td>
<td>1</td>
<td>4%</td>
<td>2010 (ref. 42)</td>
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<tr>
<td>9p21-22</td>
<td>C9orf72</td>
<td>C9orf72</td>
<td>Possible guanine nucleotide exchange factor</td>
<td>Intrinsic GGGGCC repeat</td>
<td>25%</td>
<td>10%</td>
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<td>Xp11.23-Xp13.1</td>
<td>UBQLN2</td>
<td>Ubiquilin 2</td>
<td>Autophagy adaptor</td>
<td>5</td>
<td>&lt;1%</td>
<td>2011 (ref. 40)</td>
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<td>5q35</td>
<td>SQSTM1</td>
<td>Sequestosome 1</td>
<td>Autophagy adaptor</td>
<td>10</td>
<td>&lt;1%</td>
<td>2011 (refs 41 and 143)</td>
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<td>17p13.2</td>
<td>PFN1</td>
<td>Profilin-1</td>
<td>Actin-binding protein</td>
<td>5</td>
<td>&lt;1%</td>
<td>2012 (ref. 144)</td>
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<tr>
<td>12q13.1</td>
<td>HNRNPA1</td>
<td>hnRNPA1</td>
<td>RNA-binding protein</td>
<td>3</td>
<td>&lt;1%</td>
<td>2013 (refs 70 and 71)</td>
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<tr>
<td>5q31.2</td>
<td>MATR3</td>
<td>Matrin 3</td>
<td>RNA-binding protein</td>
<td>3</td>
<td>&lt;1%</td>
<td>2014 (ref. 76)</td>
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<tr>
<td>2q36.1</td>
<td>TUBA4A</td>
<td>Tubulin α-4A chain</td>
<td>Microtubule subunit</td>
<td>7</td>
<td>&lt;1%</td>
<td>2014 (ref. 145)</td>
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<tr>
<td>22q11.23</td>
<td>CHCHD10</td>
<td>Coiled-coil-helix-coiled-helix domain-containing protein 10</td>
<td>Mitochondrial protein of unknown function</td>
<td>2</td>
<td>&lt;1%</td>
<td>2014 (ref. 146)</td>
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<tr>
<td>12q14.1</td>
<td>TBK1</td>
<td>Serine/threonine-protein kinase TBK1</td>
<td>Regulates autophagy and inflammation</td>
<td>10</td>
<td>?</td>
<td>2015 (ref. 147)</td>
</tr>
</tbody>
</table>

In Readings: Taylor et al. 2016: To know: SOD1, TARDBP, C9orf72
MUTATIONS IN SUPEROXIDE DISMUTASE (SOD) Cu/ Zn SOD:

Cause of some FALS cases

- Analysis of familial ALS (FALS) revealed the following:
  - Many ALS families have mutations in the gene for Cu, Zn superoxide dismutase (SOD1) — a homodimer enzyme responsible for the conversion of superoxide (O2-) to H2O2 (hydrogen peroxide) and molecular oxygen

\[
2\text{O}_2^- + 2\text{H}^+ \xrightarrow{\text{SOD}} \text{H}_2\text{O}_2 + \text{O}_2
\]
Why does mSOD cause damage?

• Not known: called ‘toxic’ gain of function
• But: numerous possibilities

See Next Slide + readings (Taylor et al., 2016)

1) Aggregation: the mSOD may form toxic aggregates
2) mSOD may permit more excitotoxic-mediated damage (described later in course)
3) The mSOD molecule may be more unstable than normal, or be harder to degrade
4) Degradation of proteins take place within the proteosome and the proteosomal function could be impaired
5) The mSOD could interfere with the axonal transport of substances
The cause of the neuron death in ALS is unknown: Here are some possibilities:
Newer version: more interest in RNA metabolism in 2016

From Taylor et al., 2016
Cause

- SOD1 mutations by type:

  - Substitution = 95
  - Polymorphism = 5
  - Insertion = 3
  - Deletion = 4
  - Compound = 1

Note: a mutation in the Cu/Zn SOD was completely unexpected.

mention: founder effect (founder mutation)
Cause

• Conclusions regarding mSOD:
  – There is a likely causative association between FALS and mSOD
    • Mechanism of mSOD action unclear, though it likely represents a gain (rather than loss) of function
    • Increased aggregation (so what?)
    • Increased promiscuity with production of ONOO
The mutant SOD over-expressing mouse model is very useful in investigating ALS.
Selected over-expression studies in mice have suggested that when mSOD is expresses in neurons only- the disease does not occur, Therefore the disease has been thought to not be ‘cell autonomous’ to the neuron

Non- Cell autonomous  
Effect of mutant SOD

Results of over-expression of Mutant SOD; Ileva et al., JCB 2009
ALS: Cause

- Possible **pathogenesis** of motoneuron death in ALS:
  - Toxic gain of function exerted by mutant superoxide dismutase (mSOD)
  - Oxidative damage by caused free radicals
  - Aberrant protein aggregates
  - (NMDA) receptor (NMDAR)-mediated excitotoxicity
  - Abnormal protein phosphorylation of cellular substrates
  - Misfolded SOD might associate with NADPH oxidase and increase superoxide
TDP-43

• TDP-43 highly conserved, nuclear localization, can regulate RNA, control RNA splicing, RNA transport, and other functions

• Abnormal accumulations in most affected ALS CNS tissue. Usually is found in cytoplasm (where it not usually found in normals), there are inclusions, and hyperphosphorylation of TDP-43 (also N-terminal truncation of the protein)
TDP-43 and FUS/TLS

Figure 1. TDP-43 and FUS/TLS Mutations in ALS

(A) Thirty dominant mutations in TDP-43 have been identified in sporadic (red) and familial (black) ALS patients, with most lying in the C-terminal glycine-rich region of TDP-43. All are missense mutations, except for the truncating mutation TDP-43Gly74Trp.

(B) Fourteen mutations in FUS/TLS have been identified in familial ALS cases, with most lying in the final 13 amino acids of this protein (R514S and G515S are found in cis). (Data compiled from Kwiatkowski et al., 2009; Vance et al., 2009; domains from http://www.uniprot.org and http://www.cbs.dtu.dk/services/NetNES.)
TDP-43 in ALS

TDP-43 and mutations in TDP-43 in ALS

In ALS: mutations of TDP-43 are found in about 3% of patients with familial ALS. If so, why is it such a ‘big deal’?
- It is also found in about 1.5% of patients with non-familial (sporadic disease). These patients have clinically and pathologically ‘typical ALS’.
- Patients with TDP mutations are also found in some patients with fronto-temporal dementia (FTD, aka fronto-temporal lobar degeneration; FTLD)
- Pathologically, TDP-43 is found in ‘inclusions’ in ALS (and other diseases). Thus, TDP-43 may be the ‘pathogenic protein’ in this protein disorder (also called a ‘proteinopathy’)

TDP-43

What is it?
- TDP-43 (TAR DNA binding protein) is a DNA- and RNA-binding protein that was a regulator of transcription. It was initially was originally found as this regulator represses transcription of the virus HIV-1 (the AIDS virus). Since then found to regulate other genes such as CFTR gene and apo-EII gene.

This figure shows a schematic of TDP-43 as a Transcriptional repressor

Figure shows that mutations in TDP-43 are frequent in the glycine-rich C-terminal tail
TDP-43 is usually intranuclear
(in ALS and other conditions – cytoplasmic)

In normal mice (and humans)
TDP-43 is found in the nucleus D (cellular marker) and E (immunoreactivity to TDP-43)

In mice that over-express SOD1; TDP-43 is present in the cytoplasm, especially associated with ubiquitin (ubiq) (m, n-s) Shan et al., 2012
C9ORF 72

- Previously were some cases of FALS in chromosome 9;
- In 2011, several groups including UBC members found region in 9p21 associated with ALS (and FTLD)
- Now thought to be a hexanucleotide expansion of a gene of unknown function (called C9ORF72), open reading frame 72;
- Not clear how expanded hexanucleotide needs to be to be pathological (20< normal; >30 phenotype)
- Is a big deal because it now appears that C9ORF 72 responsible for 20-25% of FALS and some (5%) of sporadic ALS
- C9ORF72 also responsible for 15% of FTLD
C9ORF has bulbar involvement

Table 2  Motor features at time of initial assessment

<table>
<thead>
<tr>
<th>Site of symptom onset</th>
<th>C9ORF72</th>
<th>SOD1</th>
<th>9p/SOD-neg</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extremity (%)</td>
<td>12/23 (52.2)</td>
<td>19/19 (100)</td>
<td>117/174 (67.2)</td>
<td>0.003</td>
</tr>
<tr>
<td>Bulbar (%)</td>
<td>10/23 (43.5)</td>
<td>0/19 (0)</td>
<td>46/174 (26.4)</td>
<td>0.005</td>
</tr>
<tr>
<td>Axial (%)</td>
<td>0/23 (0)</td>
<td>0/19 (0)</td>
<td>6/174 (3.4)</td>
<td>NS</td>
</tr>
<tr>
<td>Generalized (%)</td>
<td>1/23 (4.3)</td>
<td>0/19 (0)</td>
<td>5/174 (2.9)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Vancouver data
H. Stewart, I. Mackenzie,
C9ORF mutation in 25% of FALS (Vancouver data)

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Mutation frequency, demographics and clinical course of study subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C9ORF72 (n, %)</td>
</tr>
<tr>
<td>Proportion of total cohort (%)</td>
<td>23/231 (10.0)(^d)</td>
</tr>
<tr>
<td>Proportion fALS (%)</td>
<td>17/62 (27.4)(^d)</td>
</tr>
<tr>
<td>Proportion sALS (%)</td>
<td>6/169 (3.6)(^d)</td>
</tr>
<tr>
<td>Sex (male, %)</td>
<td>11/23 (47.8)</td>
</tr>
<tr>
<td>Mean age at ALS onset (years)</td>
<td>58.2 ± 9.9</td>
</tr>
<tr>
<td>Range of ALS onset age (years)</td>
<td>39.5–82.7</td>
</tr>
<tr>
<td>Mean survival (months)(^a)</td>
<td>34.3 ± 22.8</td>
</tr>
<tr>
<td>Median survival (months)(^a)</td>
<td>29.4</td>
</tr>
<tr>
<td>Range of survival (months)(^a)</td>
<td>12.2–96.3</td>
</tr>
<tr>
<td>Dementia(^c) in proband (%)</td>
<td>7/22 (31.8)</td>
</tr>
<tr>
<td>FTD in proband (%)</td>
<td>5/22 (22.7)</td>
</tr>
<tr>
<td>Dementia(^c) in relative (%)</td>
<td>9/22 (40.9)</td>
</tr>
<tr>
<td>Dementia(^c) in family(^b) (%)</td>
<td>11/22 (50.0)</td>
</tr>
</tbody>
</table>

Denominator for dementia frequencies based on available clinical information

\(^{a}\) ALS familial amyotrophic lateral sclerosis, FTD frontotemporal dementia, NS not significant, sALS sporadic amyotrophic lateral sclerosis

\(^{b}\) Excludes patients maintained on permanent ventilatory assistance via tracheostomy

\(^{c}\) Proband or other family member

\(^{d}\) Any dementia subtype, including FTD

\(^{d}\) Comparison between C9ORF72 and SOD1 groups only

C9ORF = 25% of FALS; SOD = 25% of FALS:
C9ORF = 3-5% of SALS; much higher incidence of dementia in patients and family
Note that the hex mutation is in the intron. Therefore, belief is that RNA may be toxic. Transcribed RNA will have Expanded hex sequence. Expanded repeat might ‘sequester’ RNA-binding Proteins (of which there are many) – Alternatively, it is thought that Dipeptides can be produced Directly from the expanded Repeat “ non-AUG” Translation “RAN translation”; Repeat-associated non-AUG translation (‘RAN’) From Taylor et al., 2016
The GGGG GCCs Guanines may restrict DNA transcription
GGGGG impair transcription

Figure 5 | A model for the molecular cascade resulting from the C9orf72 HRE structural polymorphism. The DNA and RNA•DNA structures formed in the GGGGCC repeat region impede RNA polymerase transcription, which results in transcriptional pausing and abortion. This leads to a loss of full-length products and an accumulation of abortive transcripts. Abortive transcripts that contain the hexanucleotide repeats form G-quadruplexes and hairpins and bind essential proteins in a conformation-dependent manner. Sequestration of these proteins leads to nucleolar stress and other downstream defects. The repeat-containing transcripts can also escape the nucleus and be bound by ribosomal complexes, thereby increasing repeat-associated non-ATG-dependent translation that results in aggregative polydiipeptides.
How is disease caused with repeat expansion disease?

1) Haploinsufficiency: Maybe gene with expansion does not function correctly; and so only single functional gene does not allow for normal amount of protein (unlikely in FTD and F-ALS)

2) RNA expansion causes abnormal RNA configuration (‘hairpin’) and then get protein-and RNA focus; toxicity and apoptosis (in some diseases – not FTD or F-ALS)

3) RNA expansion interferes with RNA translation (non-ATG initiated translation; aka RAN translation – get peptides that cause toxicity)
Another slide of the same mechanisms
Aggregates and inclusions in tissue from ALS patients. Note that within the cytoplasm of neurons are material that labels with antibodies to various proteins (upper). Lower panel (left) is RNA foci. Lower panels (right) show antibodies to a dipeptide in tissue from a patient with a C9 mutation (GGGGCC).

Figure 1 | Components of the nervous system that are affected by ALS. a, ALS mainly affects the descending corticospinal motor neurons (upper motor neurons) that project from the motor cortex into synapses in the brainstem and spinal cord, and the bulbar or spinal motor neurons (lower motor neurons) that project into skeletal muscles. b, Subtypes of ALS show typical pathological features: SOD1 aggregates (arrows) in spinal motor neurons in SOD1-related familial ALS (top left); TDP-43 redistribution to cytoplasmic inclusions (arrows) in spinal motor neurons in sporadic ALS (top right); RNA foci in the nucleus (arrows) and the cytoplasm (arrowhead) of a cortical neuron affected by C9 ALS–FTD (bottom left); GA (bottom centre) and GR (bottom right) dipeptide-repeat pathology in the dentate nucleus of a brain affected by C9 ALS–FTD (bottom right).

From Lecture 2

Figure from ALS Readings: Taylor et al. 2016
A reminder about RNA

**Figure 1.** Neuronal expression of protein-coding genes. Diagram highlighting mRNA biogenesis and processing, nuclear export, axonal transport and mRNA translation. (1) Chromatin remodelling; (2) RNA polymerase II (RNA Pol. II) dependent transcription; (3) co-transcriptional processing: 5'-end capping, splicing/alternative splicing, 3'-end cleavage and poly-adenylation; (4) nuclear export of mRNAs; (5) axonal transport of mRNAs; and (6) translation of mRNAs for the biosynthesis of proteins.
Note: Possible involvement of corticospinal axons and motoneurons in ALS
Cause – An integrative Perspective

Note: The motorneuron resides in a tough neighborhood
Treatment

- Broadly speaking, there are three classes of treatment options that have been tried in ALS
  - Growth Factors: a) systemic, b) muscle, c) retrograde transport to nerve
  - Stem cell: bone marrow-derived stem cell, CNS-derived stem cell
  - Pharmacological: Riluzole
Treatment – Riluzole

- Only available therapy to slow the progression of the disease is Riluzole
  - prolongs survival by 3-6 months
  - Inhibits glutamate release
Treatment – Riluzole

- Riluzole has several known mechanisms of action

Inhibiting glutamatergic neurotransmission:
- Potential interactions with the NMDA glutamate receptors (pre- and post-synaptic)
- Potential interactions with AMPA/kainate glutamate receptors
- Enhance glutamate uptake from synaptic cleft
- Inhibit glutamate and aspartate release

Ca\(^{2+}\) channel blockade

Na\(^{+}\) channel blockade:
- Site of action: \(\alpha\)-sub-unit
- Antagonist of persistent Na\(^{+}\) current
- Blockade of transient Na\(^{+}\) channel

GABAergic mechanisms:
- Reduced uptake of GABA from neuronal synapse
- Potentiation of GABA\(_A\) receptor affinity for GABA
- Demonstration of general anaesthetic properties at high doses
- Restoration of cortical inhibitory patterns in ALS patients

Miscellaneous actions:
- Non-competitive antagonism of protein kinase C
- Inhibition of pertussis toxin-sensitive and cholera toxin-sensitive G-proteins
- Antagonism of neuronal nitric oxide synthase
Treatment – Growth Factors

• Gene delivery of Insulin Growth Factor (IGF) therapy has been attempted

Kaspar et al. (2003)

• Injected AAV-IGF-1
  – found that IGF-1 delays the onset of behavioral symptoms and delays death in the G93A mouse
  – Increase in pAkt is thought to be responsible
  – retrograde delivery is key
    • lentivirus-IGF-1 had a minimal effect
Possible Causative mechanisms

Fig. (1). The pathogenic processes that trigger motor neuron degeneration in ALS. At the core of motor neuron degeneration is overactivation of glutamatergic receptors located on the post-synaptic neuron. Pathological activation of post-synaptic glutamate receptors may be attributed to decreased glutamate uptake by astrocytic processes. The passage of excessive Ca$^{2+}$ through ionotropic receptors increases intracellular Ca$^{2+}$ levels, leading to the activation of degradative enzymes, including phospholipase A$_2$, proteases and nitric oxide synthase. Elevations in intracellular Ca$^{2+}$ also cause perturbations in mitochondrial function, which results in the production of free radicals, as well as impaired production of adenosine triphosphate (i.e. ATP). Combined with ATP depletion, the production of nitric oxide and other free radical species results in the inactivation of the Na$^+$/K$^+$ pump, raising intracellular Na$^+$ concentrations, which leads to neuronal depolarisation (i.e. ‘hyperexcitability’). As a consequence, reverse operation of the Na$^+$/Ca$^{2+}$ exchanger may occur in an attempt to normalise intracellular Na$^+$ levels, but the increased entry of Ca$^{2+}$ may exacerbate the already elevated intracellular Ca$^{2+}$ levels. Axonal transport may become impaired secondary to excitotoxicity. Furthermore, the formation of cytoplasmic aggregates (TDP-43 in sporadic ALS and a minority of familial cases; and SOD-1 and FUS in their respective familial counterparts) may incite neurodegeneration through undefined mechanisms. Microglial infiltration marks the development of neuroinflammatory processes, which may exacerbate excitotoxicity through the production of inflammatory cytokines.
Gene therapy

Fig. 4 Gene therapy mechanism of action. Schematic representation of possible gene therapy approaches in ALS treatment. All of these approaches can be effective by intrathecal, intraventricular, or peripheral injection of AAV or lentivirus targeting motor neurons or glial cells. 

a. Antisense oligonucleotide (ASO) are short synthetic oligonucleotides (15-25 nucleotides) which bind to targeted mRNA. ASO reduces the expression of a specific protein by two main mechanisms: ASO induces the mRNA degradation by endogenous RNase H or blocks the mRNA translation. This is a potential therapeutic avenue in ALS by reducing the protein level of TDP-43, SOD1 of FUS protein level or by targeting of C9orf72 RNA foci. 

b. siRNAs are double-stranded RNAs which operated through RNA interference pathway. After strand unwinding, one siRNA strand binds argonaute proteins as part of the RNA-induced silencing complex (RISC) and is recruited to a target mRNA which is then cleaved. Antibodies are another potential therapeutics avenue in ALS [111]. Antibodies can target misfolded proteins and reduce the amount of toxic aggregates. It is suggested that they can reduces the disease propagation between cells. They can also be exploited to block the pathological interaction between proteins by binding to the specific interaction sites. 

c. Gene delivery is another potential therapeutic avenue for loss-of-function mutations. Virus can provide a functional replacement of a missing gene by mRNA or cDNA delivery. This approach was particularly tested in spinal muscular atrophy and revealed great outcomes but is not yet extensively tested in ALS [231].