ALS Genetics: Overview and New Perspectives

NEALS Webinar
June 18, 2013
• Overview: ALS Genes – Models and Summary
• Recent novel genes
• Perspectives on treatments
ALS reflects an interplay between genetic architecture, environment, behavior and chance.

**Genetic architecture**: (1) the genes involved in a disease, (2) their variation in the population, and (3) their impact on phenotype.
~30 ALS genes have been identified.

<table>
<thead>
<tr>
<th>Familial (26)</th>
<th>Sporadic (6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALS1 21q</td>
<td>SOD1</td>
</tr>
<tr>
<td>ALS2 2q</td>
<td>alsin</td>
</tr>
<tr>
<td>ALS4 9q</td>
<td>senataxin</td>
</tr>
<tr>
<td>ALS6 16q</td>
<td>FUS</td>
</tr>
<tr>
<td>ALS8 20q</td>
<td>VAPB</td>
</tr>
<tr>
<td>ALS9 14q</td>
<td>ANG</td>
</tr>
<tr>
<td>ALS10 1p</td>
<td>TDP43</td>
</tr>
<tr>
<td>ALS11 6q</td>
<td>FIG4</td>
</tr>
<tr>
<td>ALS12 10p</td>
<td>OPTN</td>
</tr>
<tr>
<td>ALS13 12q</td>
<td>Ataxin-2</td>
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<tr>
<td>ALS14 9p</td>
<td>VCP</td>
</tr>
<tr>
<td>ALS15 17p</td>
<td>PFN1</td>
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<tr>
<td>ALS 12q</td>
<td>DAO</td>
</tr>
<tr>
<td>ALS 17q</td>
<td>TAF15</td>
</tr>
<tr>
<td>ALS 19p</td>
<td>NTE</td>
</tr>
<tr>
<td>ALS 2p</td>
<td>dynactin</td>
</tr>
<tr>
<td>ALS 5q</td>
<td>SQSTM1/p62</td>
</tr>
<tr>
<td>ALS 7q</td>
<td>PON1-3</td>
</tr>
<tr>
<td>ALS 9p</td>
<td>C9orf72</td>
</tr>
<tr>
<td>ALS 9p</td>
<td>sigma-R1</td>
</tr>
<tr>
<td>ALS 9q</td>
<td>Ubiquilin 1</td>
</tr>
<tr>
<td>ALS X</td>
<td>Ubiquilin 2</td>
</tr>
<tr>
<td>ALS 12q</td>
<td>HNRNPA1</td>
</tr>
<tr>
<td>ALS 7p</td>
<td>HNRNPA2B1</td>
</tr>
</tbody>
</table>
The rate of gene discovery in ALS is increasing:
~ 30 ALS genes
~30 ALS genes have been identified.

> 50% of FALS is attributable to known variants.
Five recent themes in ALS pathogenesis

1. Some gene defects disturb **protein stability**, turn-over and function.
   - SOD1, VCP, p62
   - Ubiquilin-1, 2

2. **RNA and chromatin biology** are abnormal in ALS.
   - TDP43, FUS/TLS, C9orf72, ELP3, SETX, ANG, TAF15, Ataxin-2, HNRNPA1, -A2B1

3. **Prion domains** promote aggregation.
   - TDP43, FUS/TLS, AF15, HNRNPA1, -A2B1, CREST

4. **Cytoskeletal functions** (axonal transport, vesicle trafficking, ? sprouting) are disturbed.
   - Dynactin, VAPB, KIFAP3, PFN1, EphA4

5. Susceptibility to **environmental toxins** may be enhanced in ALS.
   - PON1-3, NTE

Multiple complex pathways are implicated in neuronal death.
1. Some gene defects disturb protein stability, turn-over and function.

2. RNA and chromatin biology are abnormal in ALS.

3. Prion domains promote aggregation.

4. Cytoskeletal functions (axonal transport, vesicle trafficking, ? sprouting) are disturbed.

5. Susceptibility to environmental toxins may be enhanced in ALS.

Multiple complex pathways are implicated in neuronal death.
25% of familial ALS (3% of all ALS) arises from mutations in the gene encoding Cu/Zn superoxide dismutase (SOD1).

>150 mutations (11 → truncation, others missense e.g. A4V)
Early in ALS, mutant SOD1 provokes multiple intrinsic toxicities.

- Abnormal neurite development
- Binds mSOD1, ↓ ATP, Ca++, → apoptosis
- Excessive bursting, Excitotoxicity
- ER stress, Autophagy
- Abnormal axonal transport
- Early axonal retraction
As ALS evolves, SOD1\textsuperscript{mut} evokes diverse influences from non-neuronal cells.
Five recent themes in ALS pathogenesis

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   - Ubiquilin-1, 2

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*Multiple complex pathways* are implicated in neuronal death.
Model
Cytosolic relocalization of FUS and TDP43 is a component of the normal neuronal injury response that becomes irreversible, and lethal, if FUS or TDP43 are mutated.

Stressor (??)
(mutation, vaccination, injury, chemical toxicity)

Viable

Compromised

Non-viable

Acquired toxic function of mislocalized, aggregated FUS?
Loss of nuclear function?
The ALS genes TDP43 and FUS/TLS share domain structures and some functions.

TDP-43 and FUS/TLS figure in almost every step in gene expression.
FUS proteins with ALS-related mutations are mislocalized to the cytosol (at autopsy and in vitro).

Kwiatkowski et al. R521G
Expansions in an intronic hexanucleotide repeat within the C9orf72 gene cause ALS-FTD.

**Expanded GGGGCC Hexanucleotide Repeat in Noncoding Region of C9ORF72 Causes Chromosome 9p-Linked FTD and ALS**

DeJesus M, Rademakers R et al. Neuron 72, 1–12, October 20, 2011 ©2011

**A Hexanucleotide Repeat Expansion in C9ORF72 Is the Cause of Chromosome 9p21-Linked ALS-FTD**

Renton AE, Traynor B et al. Neuron 72, 1–12, October 20, 2011 ©2011
In ALS-FTD and ALS, GGGGCC expansions are identified in intron 1 of C9orf72.

Normal gene → 3 to ~ 20 repeats
ALS gene → 100’s of repeats
The C9orf72 expansion is toxic to neurons.

There are multiple neuromuscular degenerative diseases caused by non-coding, intronic expansions.

<table>
<thead>
<tr>
<th>Disease</th>
<th>Repeat</th>
<th>Gene</th>
<th>Region</th>
<th>Normal Repeat</th>
<th>Disease Repeat</th>
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<tbody>
<tr>
<td>ALS</td>
<td>GGGCC</td>
<td>C9orf72</td>
<td>Int 1</td>
<td>&lt;20</td>
<td>25-500</td>
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<tr>
<td>DM1</td>
<td>CTG</td>
<td>DMPK</td>
<td>3' UTR</td>
<td>5-38</td>
<td>50-1500</td>
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<tr>
<td>DM2</td>
<td>CCTG</td>
<td>ZNF9</td>
<td>Int 1</td>
<td>&lt;30</td>
<td>75-11000</td>
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<td>FXTAS</td>
<td>CGG</td>
<td>FMR1</td>
<td>5' UTR</td>
<td>20-45</td>
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<td>HDL2</td>
<td>CTG</td>
<td>JPH3</td>
<td>3' UTR</td>
<td>6-28</td>
<td>&gt;41</td>
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<td>SCA3</td>
<td>CAG</td>
<td>ATXN3</td>
<td>Ex 10</td>
<td>&lt;44</td>
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<td>SCA8</td>
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<td>KLH1</td>
<td>5' UTR</td>
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<td>ATTCT</td>
<td>E46L</td>
<td>3' UTR</td>
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<td>SCA12</td>
<td>CAG</td>
<td>PPP2R2B</td>
<td>5' UTR</td>
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<tr>
<td>SCA31</td>
<td>TGGAA*</td>
<td>BEAN-TK2</td>
<td>3' UTR</td>
<td>&lt;5</td>
<td>2000</td>
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<tr>
<td>SCA36</td>
<td>GGCCTG</td>
<td>NOP56</td>
<td>Int 1</td>
<td>&lt;10</td>
<td>&gt;1670</td>
</tr>
</tbody>
</table>

* and diverse other pentapeptide repeats

RNA expansions are toxic through several mechanisms:

(1) impair transcriptional regulation
(2) alter mRNA splicing and metabolism
(3) re-distribute, reduce levels of RNA-binding proteins
(4) generate anti-sense transcript
(5) RAN translation – toxic peptides

The C9orf72 expansions are associated with diverse clinical phenotypes.

- ALS – FTD
- ALS or FTD
- Parkinsonism
- PSP
- Adult-onset psychosis
Overview: ALS Genes 2012

Recent novel genes
- A gene that affects nerve terminal extension

Perspectives on treatments
From Israel, a new gene whose mutations predispose to ALS

Vivian Drory
Sergiu Blumen

Mutations in the profilin 1 gene cause familial amyotrophic lateral sclerosis

Chi-Hong Wu¹, Claudia Fallini², Nicola Ticozzi³, Pamela J. Keagle¹, Peter C. Sapp¹,4, Katarzyna Piotrowska¹, Patrick Lowe¹, Max Koppers⁵, Diane McKenna-Yasek¹, Destree M. Baron¹, Jason E. Kost¹, Paloma Gonzalez-Perez¹, Andrew D. Fox¹, Jenni Adams¹, Franco Taroni⁶, Cinzia Tilocca³,⁷, Ashley Lyn Leclerc¹, Shawn C. Chafe⁸, Dev Mangroo⁸, Melissa J. Moore⁹, Jill A. Zitzewitz¹⁰, Zuo-Shang Xu¹⁰, Leonard H. van den Berg⁵, Jonathan D. Glass¹¹, Gabriele Sikiliano¹², Elizabeth T. Cirulli¹³, David B. Goldstein¹³, Francois Salachas¹⁴, Vincent Meininger¹⁴, Wilfried Rossoll², Antonia Ratti³,¹⁵, Cinzia Gellera⁶, Daryl A. Bosco¹, Gary J. Bassell²,¹¹, Vincenzo Silani³,¹⁵, Vivian E. Drory₁⁶, Robert H. Brown Jr¹ & John E. Landers¹ Nature 488(7412):499-503, 2012.
ALS Genetics: Overview and New Perspectives

• Overview: ALS Genes 2012
• Recent novel genes
  – A gene that affects survival in ALS
• Perspectives on treatments
From Belgium, a gene variant that slows ALS

Wim Robberecht

Overexpression of mutant superoxide dismutase 1 causes a motor axonopathy in the zebrafish

Robin Lemmens¹,²,∗†, Annelies Van Hoecke¹,‡, Nicole Hersmus¹, Veerle Geelen¹, Isabel D’Hollander², Vincent Thijs², Ludo Van Den Bosch¹, Peter Carmeliet³,⁴ and Wim Robberecht¹,²
ALS Genetics: Overview and New Perspectives

- Overview: ALS Genes 2012
- Recent novel genes
- Perspectives on treatments
### Current and Recent NEALS Trials and Studies

<table>
<thead>
<tr>
<th>Study</th>
<th>PI</th>
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<tbody>
<tr>
<td>Ceftriaxone</td>
<td>Merit Cudkowicz</td>
</tr>
<tr>
<td>Arimoclomol</td>
<td>Michael Benatar</td>
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<tr>
<td>Creatine - Tamoxifen</td>
<td>Nasem Atassi</td>
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<tr>
<td>Pramipexole</td>
<td>Merit Cudkowicz</td>
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<td>Anti-SOD1 ASO</td>
<td>Tim Miller</td>
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<td>Neural Stem</td>
<td>Jonathan Glass</td>
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<td>Cytokinetics</td>
<td>Jeremy Shefner</td>
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<td>Pre-FALS</td>
<td>James Berry</td>
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<td>Biomarkers</td>
<td>Robert Bowser</td>
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<td>Biopsy study</td>
<td>Jeff Rothstein</td>
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<tr>
<td>Nutritional study</td>
<td>Anne-Marie Wills</td>
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<tr>
<td>Exercise study</td>
<td>Nick Maragakis</td>
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</tbody>
</table>
Approaches to Gene Silencing in ALS

• Protein inactivation
  Antibodies

• Allele inactivation
  • Small Molecules
    Simple drugs
  • Antisense Oligonucleotides
    Single stranded DNA
  • RNAi
    RNA duplexes
    MicroRNA
RNAi-mediated SOD1 silencing prolongs survival in ALS mice.

RNAi-mediated SOD1 silencing prolongs survival in ALS mice.

The list of known ALS genes and the associated insights into new pathways and treatment targets is expanding.

Mendelian genetics will continue to define novel ALS genes.

Future studies will begin to have enough power to assess
- epistatic - “oligogenic” causes
- epigenetic factors
Conclusions and Questions

• The GGGGCC expansion predisposes to bulbar ALS → the biology of ALS phenotypes may be illuminated.

• Is there a role for non-coding variants in neurodegeneration?

• What is the role of disturbances in development of the motor system in adult-onset neurodegeneration?

• Insights from genetics have accelerated the search for ALS treatments.
Thank You !!