Amyotrophic Lateral Sclerosis: Perspectives from Genetics

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University of Massachusetts Medical Center

for
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Department of Neurology
Massachusetts General Hospital
Harvard Medical School

August 7, 2008
Boston, MA
Mutations
  *Mendelian and Complex Genetics*

Mechanisms
  *Recent lessons in pathophysiology*

Medicines
  *Therapeutic advances*
Acknowledgements

ALS Genetics

Boston

R. Horvitz  P. Sapp  D. Yasek

Outside Boston

T. Siddique  J. Haines  M. Vance

S. Purcell  J. Landers

A. Al-Chalabi  J. Glass

W. Broom  T. Kwiatkowski  I. Rodriguez-Leyva

P. Andersen  C. Shaw  W. Robberecht

Cell Death

P. Pasinelli

Clinical Trials

M. Cudkowicz

Neuronal Physiology

M. Paton  B. Van Zundert

Microglia

M. Carroll  I. Chiu

Squid Axon

S. Brady  G. Morfini  H. Brown
ALS is a devastating orphan disease

Mean age of onset = 55 years

...... uniformly fatal, in 4-5 years.

<table>
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<th>Incidence</th>
<th>Total</th>
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<td>70,000 World</td>
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<td>~20,000 US</td>
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<td>500,000 World</td>
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The hallmark of ALS is muscle *denervation and atrophy* due to loss of spinal motor neurons; this begins *focally*.

Degeneration of brain motor neurons causes *spasticity*.
Cytoskeletal pathology and ubiquitinated protein aggregates are seen in motor neurons.
Ubiquitinated proteins in most ALS motor neurons include TDP-43.

- nuclear protein
- transcription repressor
- part of splicing apparatus
- in early ALS and FTD
  - cleaved
  - hyperphosphorylated
  - ubiquitinated

There is neuroinflammation in ALS spinal cord.

Activated microglia in anterior gray matter of ALS spinal cord.

The development of ALS probably reflects an interplay: environment, genetic factors, and chance, aging.
The development of ALS probably reflects an interplay: environment, genetic factors, and chance, aging.

**Genotype**

**Behavior** → **Disease** ← **Chance**

**Environment**

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<td>-- Lyme</td>
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<td><strong>Diet</strong></td>
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<td>-- high fat</td>
<td>3.8</td>
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<tr>
<td>-- high glutamate</td>
<td>3.2</td>
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<tr>
<td>-- high fiber</td>
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The development of ALS probably reflects an interplay: environment, genetic factors, and chance, aging.

Genotype

↓↓↓↓↓↓

Behavior ⟷ Disease ⟷ Chance

↑↑↑↑↑↑

Environment

10% of ALS: autosomal dominant
Amyotrophic Lateral Sclerosis: 13 Mendelian loci, 7 genes

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<td>X cen</td>
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Amyotrophic Lateral Sclerosis: 
13 Mendelian loci, 7 genes
→ 5 have ALS or ALS-like phenotype

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Mutations

Mendelian Genetics - FALS

Mechanisms

Recent lessons in pathophysiology

Medicines

Therapeutic advances
10% of ALS cases are inherited as a dominant, highly penetrant (>90%) trait.

William Osler  1890  First description of familial ALS
25% of familial ALS (3% of all ALS) arises from mutations in the gene encoding Cu/Zn superoxide dismutase (SOD1).

135 mutations (124 → missense, 11 → truncation)
SOD1$^{A4V}$ cases are common in North America but rare in Europe; extremely rapidly progressive MND.

In northern Sweden, SOD1$^{D90A+/+}$ are asymptomatic; SOD$^{D90A/D90A}$ → slowly progressive MND.
The A4V variant of SOD1 arose in Asia and was carried across the Bering Strait about 10,000 years ago.
Amyotrophic Lateral Sclerosis: Mendelian loci

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Tunisian family

- corticospinal, corticobulbar signs
- lower motor neuron signs

Kuwaiti family

- exclusively corticospinal, corticobulbar signs
- no lower motor neuron findings


ALSIN deficiency in juvenile ALS causes defective microtubule assembly, motility and trafficking of cellular constituents.

- **184 kd**
- *Prominently expressed in large neurons*
- *Implicated in vesicle trafficking*

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Dynactin subunit mutations are implicated in MND.

- Onset average 34 (23-44)
- Dominant inheritance
- Predominantly lower MNs

- Early stridor and shortness of breath
  - vocal fold paralysis
- Weakness in hands > arms and legs
- Thenar weakness early
- Steppage gait late
- Chronic reinnervation by EMG

Dynactin subunit mutations are implicated in MND.

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</table>
- Juvenile onset
- Corticospinal and lower motor neurons involved
- No known exposure to organophosphates
Mendelian Genes

→ other loci: chr 9p, 16p

Complex Genetics

Other considerations
Mendelian Genes

→ unusual phenotypes

Complex Genetics

Other considerations
Novel Motor Neuron Disease Phenotype
Madras Motor Neuron Disease:
Juvenile onset of motor weakness and deafness
Rapidly progressive, severely disabling

Drs. Gourie-Devi, Nalini, Nithin
NIMHANS, Bangalore

Dr. C. Thangaraj
CCMB, Hyderabad
Overview:
More than 60 gene defects cause human motor neuron diseases (ALS, HSP, SMA, X-SBMA, PN)

These gene defects implicate common elements in the pathogenesis of these diseases:

- Axonal motors and filaments (7)
- Vesicle trafficking (4)
- DNA/RNA metabolism (6)
- Cell signaling (5)
- Heat shock proteins (3)
Mutations

*Complex Genetics - SALS*

(1) *Candidate genes in SALS*

Mechanisms

*Recent lessons in pathophysiology*

Medicines

*Therapeutic advances*
Candidate genes in sporadic ALS*

- VEGF
- Angiogenin
- Survival Motor Neuron
- Neurofilaments
- 35 other MN disease genes

* implicated in some but not all populations studied
Angiogenin

- 14 kda
- Multifunctional
  - Promotes angiogenesis
  - Ribonuclease
  - Permissive for VEGF
- 7 variants implicated in SALS.
  - Scotland, Ireland
  - Not US, UK

Paraoxonases (PON) may be risk factors for SALS

- Paraoxonases PON1, PON2, and PON3
- Gene cluster, chr 7

- Hydrolases

- PON1 and PON3
  - expressed in the liver
  - associated with HDL

- PON2
  - intracellular, also associated with a plasma membrane
  - ubiquitous

- Functions:
  - antioxidative: attenuate lipid peroxidation by LDL
  - anti-atherogenic
  - cytoprotective: inactivate wide range of toxins
Properties of PON1

- 41 kd - monomer
- conserved in evolution
- central hydrophobic channel
- 6 bladed propeller
- Ca in active site
- 3 helices $\rightarrow$ closed channel
- Insert into HDL particle membranes
- Highly polymorphic
- Multiple substrates

Paraoxonases (PON) may be risk factors for SALS


**HYPOTHESIS** – diminished toxin degradation or anti-oxidant capacity predisposes to ALS.
Mutations

*Complex Genetics*

(2) SNP-based genome analyses in SALS

Mechanisms

*Recent lessons in pathophysiology*

Medicines

*Therapeutic advances*
Summary
Whole Genome Analyses in ALS
2007-2008

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<th>PI</th>
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<td>Cronin</td>
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- ITPR2 – highly expressed in motor neurons
- Controls release of calcium from ER – calibrating intracellular calcium levels in motor neurons
- Release is mediated by inositol tri-phosphate
- Excessive Ca\(^{2+}\) is potentially toxic via multiple mechanisms
Mutations

*Mendelian and Complex Genetics*

Mechanisms

*Recent lessons in pathophysiology*

Medicines

*Therapeutic advances*
Over-expression of mutant SOD1 generates animal models of ALS.

- **G93A mouse** *(Day Lab)*
- **H46R rat** *(Day Lab)*
- **A4V fly** *(Mel Feaney, HMS)*
- **ALS worm** *(Rick Morimoto)*
- **ALS zebrafish**
Mouse experiments reveal that mutant SOD1 protein is cytotoxic.

<table>
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<th>Mouse Type</th>
<th>Mouse SOD1</th>
<th>Human SOD1 (transgene)</th>
<th>Phenotype</th>
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<td>Tg–Mut SOD1</td>
<td>Mouse level</td>
<td>Mutant human level</td>
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3% of ALS arises from mutations in the gene encoding cytosolic Cu/Zn superoxide dismutase (SOD1).

SOD1 catalyzes the conversion of superoxide anion to oxygen and hydrogen peroxide.
Transgenic expression of mutant SOD1 apoenzyme, devoid of Cu, → mouse ALS.

Hypothesis: the critical factor in SOD1\textsuperscript{mutant} ALS is conformational instability of this abundant protein.
Instability of the mutant SOD1 protein contributes to its toxicity.
Early events in ALS are intrinsic to the motor neurons.

- Binds mSOD1
- Decreased ATP, Ca++ → apoptosis
- Excessive bursting
- Abnormal axonal transport
- Early axonal retraction
Squid *Loligo pealii*

Drs. Scott Brady, Gerardo Morfini, University of Illinois, Wood Hole
Hannah Brown, Harvard Medical School, Woods Hole
• isolated axon

• not influenced by
  - cell body
  - gene transcription

• direct impact of SOD1 on transport motor apparatus
Rate of transport

Cell body  NM Junction

Anterograde

Retrograde

Rate of transport (μm/sec)

minutes

n = 5
Normal SOD1 has no effect on transport.
SOD1 mutants slow anterograde transport.

SOD1 mutants impair the function of axonal kinesin, probably by altering phosphorylation.
Mutant SOD1 binds chromogranin and is secreted.

Late events in ALS involve non-neuronal cells, with diverse influences on motor neurons.
Activated astrocytes adversely affect motor neurons in vitro.

Co-culture of stem-cell derived MNs and astrocytes

DiGeorgio, Carrasco, Siao, Maniatis, Eggan
Activated microglia in ALS can be both toxic and beneficial \textit{in vivo}. 

Activated microglia

Resting microglia

Activated astrocytes

Resting astrocytes

mSOD1
Reduction of oxidative bursting by microglia (NADPH / Nox2 knock-out) enhances survival in SOD1^{G93A} mice.

Does this relate to sporadic ALS?

Does neuroinflammation alter disease course?

Survival

Retrospective analysis of 4 studies, n=596:
- topiramate placebo, celebrex,
- creatine, MGH longitudinal
- 2/15 meds affected survival: ASA, NSAIDs

Merit Cudkowicz (MGH)
Does this relate to sporadic ALS?

WT SOD1 is neurotoxic when oxidized.

Ezzi SA, Urushitani M, Julien J-P. *Wild-type superoxide dismutase acquires binding and toxic properties of ALS-linked mutant forms through oxidation.* epub 2007
Mab C4F6 recognizes a toxic form of mutant SOD1 protein.

Mab C4F6 reacts with a sub-fraction of WT-SOD1 in SALS spinal cords.

Julien J-P personnal communication
Hypothesis:
Some cases of sporadic ALS are mediated by modified WT SOD1, with toxicity similar to that of mutant SOD1.
Mutations
*Mendelian and Complex Genetics*

Mechanisms
*Recent lessons in pathophysiology*

Medicines
*Therapeutic advances*
Only one drug is FDA approved for ALS (Riluzole) but...
the ALS pipeline has many drugs in trials

**Current**
- Minocycline
- IGF-1 (#3)
- Ceftriaxone
- Sodium Phenylbutrate
- Coenzyme Q10
- Thalidomide
- Ritonavir/hydroxyurea
- Valproic acid
- Copaxone
- Memantine
- Celebrex/Creatine vs Minocycline/Creatine

**Planned**
- Arimoclomol (phase IIB)
- Talampanal
- Trophos
- R+ Pramipexole
Understanding molecular pathology in ALS has aided therapy development

- Stem Cells
- Drug discovery
- Gene therapy
- Protein delivery
- Allele inactivation
- Protein inactivation
Understanding molecular pathology in ALS has aided therapy development

- Stem Cells
- **Drug discovery**
- Gene therapy
- Protein delivery
- Allele inactivation
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Microwell screening can be done with high throughput:
>200,000 drugs tested/day

- 400 cases
  - $10 M / 3 years
- 40 mice
  - $50 K / 6 mo’s
- 2 wells
  - $5 / 24 hr

$2 \times 10^6$ less expensive
$10^3$ faster
Instability of the mutant SOD1 protein contributes to its toxicity.

Stabilization of the dimer may be beneficial.
Computational Docking: A fast and effective way to screen big libraries of compounds

1.5 million compounds

Parallel processing on Beowulf cluster speed up computation.

3000 molecules are predicted to stabilize SOD1

S. Ray
Computational Docking:  
A fast and effective way to screen big libraries of compounds

1.5 million compounds

3000 molecules predicted to bind SOD1

S. Ray
Multiple molecules identified by cyber-pharmacology do stabilize SOD1.

Ray SS, Nowak RJ, Brown, RH Jr., Lansbury PT. Inhibition of aggregation of familial amyotrophic lateral sclerosis-linked superoxide dismutase 1 mutants by drug-like structural stabilizers PNAS,102(10),3639-3644, 2005
Understanding molecular pathology in ALS has aided therapy development

- Stem Cells
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Viral vectors may allow delivery of genes to the nervous system – e.g. by retrograde transport from muscles.

AAV$_2$-IGF1 gene therapy potently increases ALS survival.

![Survival Graph](Image)

<table>
<thead>
<tr>
<th>Group</th>
<th>Onset</th>
<th>Death</th>
</tr>
</thead>
<tbody>
<tr>
<td>GFP</td>
<td>91</td>
<td>123</td>
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<td>IGF-1</td>
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<td>160</td>
</tr>
</tbody>
</table>

AAV-IGF1 delivered- 60 days old
AAV$_2$-IGF1 gene therapy potently increases ALS survival.

![Survival graph](image)

**Survival**

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AAV-IGF1 delivered- 60 days old

40 days
Understanding molecular pathology in ALS has aided therapy development

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- **Protein delivery**
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- Protein inactivation
Understanding molecular pathology in ALS has aided therapy development.

- Stem Cells
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Silencing mutant SOD1 using RNAi protects against neurodegeneration and extends survival in an ALS model.


Rabies-G-pseudotyped EIAV lentivirus
Many muscle injections at age 7 days
(face, tongue, diaphragm, intercostal muscles, hindlimb)

At end-stage: more MN persisted in treated than in untreated mice

100 days
Trials of allele inactivation therapy for SOD1-mediated ALS are planned (Isis)

Intraventricular infusion of anti-SOD1 anti-sense oligonucleotides prolongs survival in transgenic SOD1$^{G93A}$ rats.

Understanding molecular pathology in ALS has aided therapy development.

- Stem Cells
- Drug discovery
- Gene therapy
- Protein delivery
- Allele inactivation
- Protein inactivation
Will vaccination therapy inactivate toxic proteins in ALS?

Vaccination with SOD1 prolongs survival in ALS mice and reduces titer of toxic form of SOD1.

Conclusions

• Four gene defects cause familial ALS; PON gene variants may be robustly associated with SALS.

• In SOD1\textsuperscript{mutant} ALS, pathological processes
  – multi-factorial and begin early.
  – biphasic
    • initially intrinsic to motor neurons
    • then involve non-neural cells (neuroinflammation).

• WT SOD1 may be implicated in some cases of SALS.

• These molecular insights are yielding new therapeutic strategies in ALS.
Thank You!