Precision mouse models of childhood ALS caused by excessive sphingolipid synthesis

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Precision mouse models of disease

Traumatic injury

Cell type specificity

Axon injury responses in human neurons
Causes of neurodegeneration are still poorly understood

Etiologies of neurodegenerative disease

Genes

Environment

Loss of neurons/neuronal function
Amyotrophic lateral sclerosis

Placeholder for background on ALS
Mutations in SPTLC1 cause juvenile ALS

(serine palmitoyltransferase 1)
All sphingolipid biosynthesis is initiated by SPT.

Essential lipids of membranes in mammalian cells
Wide spectrum of functions
Sphingolipid biosynthesis pathway

- Serine + palmitoyl-CoA → 3-ketosphinganine → sphinganine → dihydroceramide → ceramide
- CerS

Key steps:

- **SPT**
- **3-KR**
- **DES**

Related components:

- Sphingomyelins
- Myelin sheath
- Glycosphingolipids
- Sphingosine-1-phosphate (S1P) cell survival, signaling
Location of disease-causing mutations in SPTLC1

- Previously known SPTLC1 mutations cause HSAN1 (Hereditary Sensory and Autonomic Neuropathy type 1)

<table>
<thead>
<tr>
<th>TMD1</th>
<th>Catalytic domain</th>
<th>473 aa</th>
</tr>
</thead>
<tbody>
<tr>
<td>C133Y</td>
<td>S331F</td>
<td></td>
</tr>
<tr>
<td>C133W</td>
<td>A352V</td>
<td></td>
</tr>
<tr>
<td>V144D</td>
<td></td>
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C133Y, C133W, V144D, S331F, A352V
Metabolomic alterations resulting from HSAN1 mutations in SPTLC1
Location of disease-causing mutations in SPTLC1

• Previously known SPTLC1 mutations cause HSAN1

C133Y
C133W
V144D

S331F
A352V

HSAN1

TMD1

SPTLC1

473 aa
Location of disease-causing mutations in SPTLC1

- Previously known SPTLC1 mutations cause HSAN1
- The childhood ALS-linked mutations cluster in a different region: transmembrane domain 1 (TMD1)

A20S  Y23F  Δ39  Δ40-41
C133Y  C133W  V144D
S331Y  S331F  A352V

HSAN1  Childhood ALS

TMD1  SPTLC1
473 aa
Metabolomic alterations resulting from ALS vs HSAN1 mutations in SPTLC1

Sphingolipidomic analysis of patient serum

Mohassel et al., Nat Med 2021
Location of disease-causing mutations in SPTLC1

- A20S
- Y23F
- Δ39
- Δ40-41
- C133Y
- C133W
- V144D
- S331Y
- S331F
- A352V

HSAN1
Childhood ALS

TMD1
Catalytic domain
473 aa
Location of ALS mutations within SPTLC1 structure

Wang et al., Nat Struc & Mol Bio 2021
Mohassel et al., Nat Med 2021
Metabolomic alterations resulting from **ALS vs HSAN1** mutations in SPTLC1

Hypotheses - mice engineered with these ALS mutations will exhibit:
1. elevated sphingolipid levels
2. ALS-like neurodegeneration
SPT-ORMDL3 interaction occurs at TMD1

Wang et al., Nat Struc & Mol Bio 2021
Mohassel et al., Nat Med 2021
High conservation of TMD1 (encoded by exon 2)
Gene editing approach in mouse to knock in the mutation

<table>
<thead>
<tr>
<th>AA number</th>
<th>20</th>
<th>23</th>
<th>38 39 40 41</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homo sapiens</td>
<td>LYE</td>
<td>A</td>
<td>PA Y H L I L E G I L I L W I I R</td>
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</tr>
<tr>
<td>Mus musculus</td>
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<td>PA Y H L I L E G I L I L W I I R</td>
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<tr>
<td>Gallus gallus</td>
<td>FYE</td>
<td>A</td>
<td>PA Y H L I L E G I L I L W I I R</td>
</tr>
<tr>
<td>Takifugu rubripes</td>
<td>FYE</td>
<td>A</td>
<td>PA Y H L I L E G I L I L W I FR</td>
</tr>
<tr>
<td>Danio rerio</td>
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<td>A</td>
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<tr>
<td>Xenopus tropicalis</td>
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Zoe Piccus et al., unpublished
High conservation of TMD1 (encoded by exon 2)

Gene editing approach

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**Exon 2**

A20S

GCTCCAGCATACCATCTTTTATTTGGAAGGAATTCATAATACCTTTGGATAATCAGACCTCGTTTTCTCTAAAACTTACAAGTTGAGGAGCTGTCTGATCTTACAGCCAAG

Zoe Piccus et al., unpublished
**Characterization of Sptlc1^{A20S} knock-in mouse line**

<table>
<thead>
<tr>
<th>A20S</th>
<th>del 40-41</th>
</tr>
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<tbody>
<tr>
<td>Normal lifespan</td>
<td>Homozygotes runted and die early (5-6 weeks old)</td>
</tr>
<tr>
<td>Mild motor symptoms</td>
<td>Tremors, muscle atrophy</td>
</tr>
<tr>
<td>Progressive motor neuron, nerve and muscle pathology (but no obvious motor neuron cell body loss)</td>
<td>Misdevelopment and neurodegeneration (more similar to a juvenile syndrome)</td>
</tr>
<tr>
<td>Homozygotes exhibit more severe pathology</td>
<td>Histological characterization ongoing</td>
</tr>
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Zoe Piccus et al., unpublished
A20S carriers have slightly lower body weight
Sptlc1<sub>A20S</sub> animals produce excess sphingolipids

Sphinganine

\[
\text{Sphinganine-1-P}
\]

\[d18:0\]

\[d18:0P\]

10 to 20-week-old serum
Sptlc1\textsuperscript{A20S} animals produce excess sphingolipids

10 to 20-week-old serum
Sciatic nerve pathology in Sptlc1 A20S mutants

9-week old, myelin stain

Sptlc1 wt/wt A20S/mt A20S/A20S

25 μm
Sciatic nerve pathology in Sptlc1 A20S mutants

19-week old
Electron microscopy (EM)
Degenerative pathology in Sptlc1<sup>A20S</sup> mutant nerve

<table>
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<th>Sptlc1</th>
<th>wt/wt</th>
<th>A20S/A20S</th>
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<td>Healthy Schwann cells and axons</td>
<td>Schwann cell pathology /axonal sprouting</td>
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19-week old EM, littermates
Degenerative pathology in $\text{Sptlc1}^{\text{A20S}}$ mutant nerve

$\text{Sptlc1}$

$\text{wt/wt}$

Healthy axon

$\text{A20S/A20S}$

Axonal organelle accumulations

19-week old EM, littermates
Degenerative pathology in Sptlc1^{A20S} mutant nerve

19-week old EM, littermates
Compound Muscle Action Potential (CMAP) recordings reflect functional connectivity between nerve and muscle.

1+2: stimulating electrodes
3: recording electrode
4: reference
5: ground

Stimulation (t=0)
Latency to peak
Max amplitude

A+B: waveform in WT animals
C: diminished amplitude in SOD1 animal

Pollari, ..., van den Bosch, JoVE 2018
Nerve to muscle communication is disrupted in homozygotes
A20S homozygotes have increased NfL in serum
The Sptlc1 A20S mouse model can be used for preclinical testing

Placeholder for prevention study
Placeholder for treatment study
Closing thoughts

• Mutations in SPTLC1 associated with ALS produce neurodevelopmental and/or neurodegenerative phenotypes in gene-edited mice
  ▪ Ongoing characterization (motor neuron counts, electrophysiology, muscle histology)
  ▪ Planning prevention study and treatment study using inhibitors of SPTLC1 in Sptlc1^{A20S/A20S} mice

• Mutations in SPTLC1 have also been detected in sporadic ALS patients (Johnson et al., 2021)

• Possibility of a pathogenic role for disrupted sphingolipid metabolism in sporadic ALS

• Potential of sphingolipids as disease biomarkers for ALS
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NIMH Transgenic Core
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Vincent Schram, Chip Dye

Packard Center
ALS Association
Eunice Kennedy Shriver National Institute of Child Health and Human Development
NIH