Disorders of Cholesterol Homeostasis

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NICHD, NIH, DHHS
Disorders of Cholesterol Homeostasis

Smith-Lemli-Opitz syndrome
- Autosomal recessive
- Multiple malformations and cognitive impairment
- Inborn error of cholesterol synthesis
- Incidence: 1/40,000
- No approved therapies

Niemann-Pick disease, type C1
- Autosomal recessive
- Lethal neurodegenerative disorder
- Lysosomal storage disease: unesterified cholesterol
- Incidence: 1/100,000
- No approved therapies

Goal: Combine basic and clinical research to develop and test therapeutic interventions
Smith-Lemli-Opitz Syndrome
Smith-Lemli-Opitz Syndrome (SLOS)

Mild                   Classical                   Severe
SLOS Behavioral Phenotype

- Irritability
- Poor suck and lack of interest in feeding
- Tactile defensiveness
- Autistic and Obsessive Compulsive Traits
- Language Impairment
  - Expressive worse than receptive
- Self-injurious behavior
  - Biting and head-banging
- Aberrant sleep patterns
- Ritualistic and repetitive behavior
- Hyperactivity
- Sensory hyper-reactivity
- Temperament dysregulation
Smith-Lemli-Opitz Syndrome

7-Dehydrocholesterol → 8-Dehydrocholesterol → Cholesterol

DHCR7

NADPH → NADP⁺
ICHTHYOSIS AND SKELETAL DYSPLASIA

CDPX2

CK

CHILD

SC4MOL

4,4-dimethylcholesta-8,24-dienol

Zymosterol

Cholesta-7,24-dienol

7-dehydrodesmosterol

Desmosterol

Desmostero1osis

Lathosterolosis

“SLOS-like”

ICHTHYOSIS AND SKELETAL DYSPLASIA

(HEM Dysplasia)
Smith-Lemli-Opitz Syndrome

7-Dehydrocholesterol → Cholesterol

Steroid Hormones and Neurosteroids → Oxysterols → Bile Acids → Embryonic Development

Hedgehog Signaling → Myelin → Lipid Rafts
Smith-Lemli-Opitz Syndrome

• What is the biochemical basis of Smith-Lemli-Opitz syndrome?
  – Cholesterol deficiency
  – 7-dehydrocholesterol toxicity
    • 7-dehydrocholesterol metabolites
SLOS iPS cells exhibit defects in neural progenitor patterning and accelerated differentiation.
Aberrant SLOS iPSC neuronal differentiation is due to increased 7DHC and not decreased cholesterol.

**Lathosterolosis iPSC**

**Cholesterol Replete Culture**

**Cholesterol Deficient Culture**
7DHC Impairs Wnt/β-Signaling
Cerebral spinal fluid sterols and behavioral symptoms

Autism Diagnostic Interview-R

<table>
<thead>
<tr>
<th></th>
<th>7-DHC</th>
<th>8-DHC</th>
<th>Cholesterol</th>
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<tbody>
<tr>
<td><strong>Total</strong></td>
<td>&lt;0.0001</td>
<td>0.001</td>
<td>0.04</td>
</tr>
<tr>
<td><strong>Social</strong></td>
<td>0.0003</td>
<td>0.003</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Non-Verbal Commun.</strong></td>
<td>0.0003</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Repetitive Behavior</strong></td>
<td>0.02</td>
<td>NS</td>
<td>NS</td>
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</table>
Therapy for SLOS

To what extent are the mental and behavioral problems in SLOS due to fixed developmental abnormalities, versus to what extent are they due to functional problems secondary to the abnormal sterol composition in the CNS?
Therapy for SLOS

Glutamate-induced iontophoretic currents

Control

Mutant

Wild type (10)
Heterozygote (7)
Homozygous (13)

Homozygous + Serum (12)
Wild type + Serum (19)
Therapy for SLOS

• Dietary cholesterol supplementation
  – Multiple anecdotal reports of efficacy
  – Cholesterol does not cross the blood-brain-barrier

• Randomized, placebo-controlled, double-blind, crossover trial
  – Pasteurized egg yolk versus egg substitute
  – Two week treated versus untreated phases
Therapy for SLOS

• **Primary outcome measure**
  – Hyperactivity subscale of the Aberrant Behavior Checklist

• **Underpowered study**
  – 3 years
  – Enrolled 10/40 subjects
Therapy for SLOS

- **Simvastatin**
  - HMG-CoA reductase inhibitor ("statin drug")
  - Decrease production of 7-dehydrocholesterol
  - Increase transcription of *DHCR7* alleles with residual enzymatic function

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>SLOS</th>
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<tbody>
<tr>
<td><strong>DHCR7 Expression (Fold Increase)</strong></td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>FBS</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>FBS+Simvastatin</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>LPDS</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>LPDS+Simvastatin</td>
<td>7.5</td>
<td>10.0</td>
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<tr>
<th>Cholesterol/Total Sterols</th>
<th>0.0</th>
<th>2.5</th>
<th>5.0</th>
<th>7.5</th>
<th>10.0</th>
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<td>T93M/IVS8-1G&gt;C</td>
<td></td>
<td></td>
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</tbody>
</table>

![Graph showing cholesterol levels with Simvastatin concentration vs. DHCR7 expression]
Therapy for SLOS

- **Simvastatin**
  - Placebo-controlled, double-blind, cross-over trial
  - 23 patients enrolled, 18 completed the study
Therapy for SLOS

- **Simvastatin**

  - First controlled trial demonstrating the potential of drug therapy to modulate sterol composition and improve SLOS behavior.
Niemann-Pick Disease, type C1
Niemann-Pick Disease, type C1

Niemann-Pick disease, type C

- Autosomal recessive, progressive, lethal, neurodegenerative disorder due to mutation of either \textit{NPC1} or \textit{NPC2}
- Endolysosomal storage of unesterified cholesterol and lipids
- Incidence: \(~1/100,000\)
  - Late Onset 1/20,000-1/40,000
Niemann-Pick Disease, type C1

• Therapeutic trial issues
  – Rare Disease
  – Heterogeneous phenotype
    • Variable age of onset
    • Variable symptom complex
  – Clinical progression occurs over years
  – Goal: Stabilization or delay of neurological disease
  – Lack of defined and accepted outcome measures

Small n
Large SD
Small Δ
NPC1 Natural History Trial

• Natural History Trial

• 93 patients enrolled since August 2006
  – Age range: 3 months to 54 years (median 10 years)
  – NPC1 Neurological Severity (range 0-50, median 14)

• Goals:
  – Identify clinical or biochemical markers that can be used as outcome measures in a therapeutic trial
  – Identify a biochemical marker that can be used for diagnostic testing or screening
    • Diagnostic Delay 4-5 years
Niemann-Pick Disease, type C1

- **NPC Neurological Severity Score**
  - Likert-like scale (0-61)
    - Nine major domains (0-5)
    - Eight minor domains (0-2)
  - Retrospective and prospective
  - Inter-rater reliability
    - Cronbach’s $\alpha = 0.85$
  - Progression modeling
    - $1.9 \pm 0.2$ points/year

<table>
<thead>
<tr>
<th>Major Domains</th>
<th>Minor Domains</th>
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<tbody>
<tr>
<td>Ambulation</td>
<td>ABR</td>
</tr>
<tr>
<td>Cognition</td>
<td>Behavior</td>
</tr>
<tr>
<td>Eye Movement</td>
<td>Gelastic Cataplexy</td>
</tr>
<tr>
<td>Fine Motor</td>
<td>Hyperreflexia</td>
</tr>
<tr>
<td>Hearing</td>
<td>Incontinence</td>
</tr>
<tr>
<td>Memory</td>
<td>Narcolepsy</td>
</tr>
<tr>
<td>Seizures</td>
<td>Psychiatric</td>
</tr>
<tr>
<td>Speech</td>
<td>Respiratory</td>
</tr>
<tr>
<td>Swallowing</td>
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<table>
<thead>
<tr>
<th>Ambulation</th>
<th>Score</th>
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<tbody>
<tr>
<td>Normal</td>
<td>0</td>
</tr>
<tr>
<td>Clumsy</td>
<td>1</td>
</tr>
<tr>
<td>Ataxic unassisted gait</td>
<td>2</td>
</tr>
<tr>
<td>Assisted ambulation</td>
<td>4</td>
</tr>
<tr>
<td>Wheelchair dependent</td>
<td>5</td>
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</table>
NPC1 Diagnostics Delay

• Filipin staining of fibroblasts
  – Requires a skin biopsy
  – Specialized testing

• Molecular testing
  – Requires a high index of suspicion
  – Relatively expensive
NPC1 Diagnostics Delay

Plasma Oxysterols

3β,5α,6β-cholestane-triol
7-ketocholesterol

Porter et al. (2010) STM, 2: 56ra81
NPC1 Diagnostics Delay

Triol (24.5 ng/ml)
Sensitivity 97%
Specificity 100%

Porter et al. (2010) STM, 2: 56ra81
Jiang et al. (2011) JLR, 52:1435
Therapy for NPC

• 2-Hydroxypropyl-β-cyclodextrin
  – Cyclic oligosaccharide with a hydrophobic core
  – Pharmaceutical excipient


Allopregnanolone Therapy

NPC1 HPβCD Trial

• *Npc1* mouse model

Survival

Cerebellar pathology: 49-days

*Npc1*<sup>+/+</sup>  *Npc1*<sup>−/−</sup>  *Npc1*<sup>−/−</sup> + CD

Davidson et al. (2009) PlosOne 4: e6951

Liu et al. (2009) PNAS 106: 2377
NPC1 HPβCD Trial

- *Npc1* cat model
  - 24 week *Npc1* mutant cats (HPβCD, miglustat, untreated)

Personal Communication: Charles Vite
24(S)-Hydroxycholesterol
Pharmacodynamic Marker
Biomarkers: Pharmacodynamics

- Pharmacodynamic response
  - Serum 24(S)-hydroxycholesterol
Biomarkers: Pharmacodynamics

- Pharmacodynamic response
  - Serum 24(S)-hydroxycholesterol

![Diagram showing biomarkers and pharmacodynamics](image)

- ApoE
- Cholesterol Synthesis
- 24(S)HC
- Blood
- CSF
- Neuron
- HPβCD
- NPC1
- CYP46
- 24(S)HC
- CSF
- Blood

Graph showing change in 24(S)HC over time:

- HPBβCD
- Artificial CSF
NPC1 HPβCD Trial
NPC1 HPβCD Trial: Ommaya Reservoir

- 50 mg HPβCD
  - No drug related adverse events or reactions
- 2/3 patients had a biochemical response
  - $AUC_{8-72}$
    - Patient 1 $+ 3.1 \times sd$
    - Patient 2 $+ 0.8 \times sd$
    - Patient 3 $+ 4.1 \times sd$
- $P.\ acnes$
  - Infection/Colonization

```
<table>
<thead>
<tr>
<th>Time (hours)</th>
<th>0</th>
<th>12</th>
<th>24</th>
<th>36</th>
<th>48</th>
<th>60</th>
<th>72</th>
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<tbody>
<tr>
<td>Plasma 24-OH Cholesterol (ng/ml)</td>
<td>35</td>
<td>30</td>
<td>25</td>
<td>20</td>
<td>15</td>
<td>10</td>
<td>5</td>
</tr>
</tbody>
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- Saline
- 50 mg
NPC1 HPβCD Trial
Phase I/IIA Intrathecal HPβCD Trial

• 14 subjects enrolled
• Monthly lumbar intrathecal administration
• Open label, dose escalation study
  – Safety, biomarker evaluation, clinical efficacy
  – Dose range: 50 to 1200 mg HPβCD lumbar IT
  – Dose advancement based on safety assessments
  – Mean dose
    • 289 mg at 12 months
    • 434 mg at 18 months
Phase I/IIA Intrathecal HPβCD Trial

• Safety
  – Ototoxicity
    • Expected Adverse Event
    • Variable
    • Minimal impact on QOL
  – Hearing aids

Change in hearing by frequency after 18 months of IT HPβCD dosing

Graph showing change in hearing (dB) by frequency (Hz) for different subjects (e.g., CDA101, CDA102, CDA103, etc.).
Lumbar Intrathecal HPβCD

- Target Engagement

**Plasma 24-OHC**

**Cerebral Spinal Fluid 24-OHC**
NPC1 TRND Team

**NICHD**
Nicole Yanjanin  
Aiyi Liu (DESPR)  
Roopa Shankar

**NHGRI**
Bill Pavan

**NINDS**
Russell Lonser  
John Heiss

**TRND/NCATS**
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Liz Ottinger  
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**Albert Einstein College of Medicine**
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Bench to Bedside Awards

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National Niemann Pick Disease Foundation
SOAR-NPC
Dana’s Angels Research Trust