LBSL: Defects in energy metabolism and in-vitro response to treatment with aminolaevulinic acid

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Kennedy Krieger Institute
July 30 - August 01, 2022
No conflicts of interest related to this presentation
### Mitochondrial Disease Patients (April 2022)

**Mitochondrial Disease due to mtDNA defects**

<table>
<thead>
<tr>
<th>Causes</th>
<th>Number of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>mtDNA mutation known to be deleterious</td>
<td>18</td>
</tr>
<tr>
<td>mtDNA mutation of unknown significance</td>
<td>5</td>
</tr>
<tr>
<td>mtDNA deletion</td>
<td>7</td>
</tr>
</tbody>
</table>

**Mitochondrial Disease due to nuclear DNA defects**

<table>
<thead>
<tr>
<th>Category</th>
<th>Number of patients</th>
</tr>
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<tbody>
<tr>
<td><strong>ETC Complex / Assembly Defects:</strong></td>
<td></td>
</tr>
<tr>
<td>NDUFAF5 (3), UQRC2 (2), SURF1 (2), NUPBL (2), BCS1L, SCO2</td>
<td>11</td>
</tr>
<tr>
<td><strong>mtDNA Synthesis / Stability:</strong></td>
<td></td>
</tr>
<tr>
<td>SUCLG1, POLG1-Alpers (3), RRM2B (2), TFAM, MRPS22</td>
<td>8</td>
</tr>
<tr>
<td><strong>Protein folding, import, export and turnover. Iron cluster biogenesis</strong></td>
<td></td>
</tr>
<tr>
<td>LONP1 (2), FBXL4 (4), SPATA5 (2), SFX4, FDXR</td>
<td>10</td>
</tr>
<tr>
<td><strong>Mitochondrial Protein Synthesis</strong></td>
<td></td>
</tr>
<tr>
<td>GFM1, MTO1, NARS2 (2), EARS2 (2), KARS, VARS2, DARS2 (2), RARS2 (2), PDE12</td>
<td>13</td>
</tr>
<tr>
<td><strong>Other</strong></td>
<td></td>
</tr>
<tr>
<td>TAZ (Barth syndrome), HIBCH (2), ECHS1 (6), ETHE1 (2), PC (1), PDH (9)</td>
<td>21</td>
</tr>
</tbody>
</table>

**Total**                                     | 93                 |
### CHOC Mitochondrial Disease Patients (April 2022)

#### Mitochondrial Disease due to mtDNA defects

<table>
<thead>
<tr>
<th>Description</th>
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#### Mitochondrial Disease due to nuclear DNA defects

**ETC Complex / Assembly Defects:**

  11

**mtDNA Synthesis / Stability:**

- *SUCLG1*, *POLG1*-Alpers (3), *RRM2B* (2), *TFAM*, *MRPS22*,  
  8

**Protein folding, import, export and turnover. Iron cluster biogenesis**

- *LONP1* (2), *FBXL4* (4), *SPATA5* (2), *SFX4*, *FDXR*  
  10

**Mitochondrial Protein Synthesis**

  13

**Other**

  21

**Total**  
93
Mitochondrial tRNA Synthetases

19 mitochondrial amino-acyl tRNA synthetases (mt-aaRS)

- encoded in the nucleus
- translated in the cytosol
- transported to mitochondria

Mutated mt-aaRS and CNS-related disease

AARS2
- Ovario-LD
- Chromosome 6p21
- 10 known pathogenic variants
- Complete loss or reduced protein function in yeast homolog
- Profoundly decreased activity of RCC complex IV

EARS2
- LBTL
- Chromosome 19p13.1-p13.2
- 9 known pathogenic variants
- 25% reduction in EARS2 protein in fibroblasts
- 6% reduction in OCR
- 41% reduction in MFR
- Significantly reduced activity of RCC complex I and IV in muscle

DARS2
- LBSL
- Chromosome 14q32
- 67 known or suspected pathogenic variants
- No abnormality in mitochondrial function detected
- Variably reduced enzyme activity in fibroblasts, lymphoblasts, and muscle

Asp
- Ala
- Gln

Smith Fine et al., Journal of Neurodevelopmental Disorders 11 (2019) 29
Aspartyl tRNA synthetase (*DARS2*) deficiency

- Leukoencephalopathy with brainstem and spinal cord involvement and lactate elevations (LBSL)
- Heterogeneous clinical presentation
  - Onset in infancy, severe
  - Adult onset, mild and slowly progressive
  - Exercise Induced Paroxysmal Ataxia (rare)
- Characteristic MRI pattern
- Genotype appears to determine the phenotype

_Berge LV, et al., Brain 137 (2014) 1019–1029_
Patient 1: Initial evaluation

- Triplet pregnancy, 28-week preemie, 3 m NICU stay
- History of intermittent headaches since age 4.9 y
- Referred at age 8 y due to worsening headaches and abnormal MRI

Hyperintensities in white matter, brainstem, and spinal cord.
Increased lactate in MRS
## Targeted mutation analysis for DARS2

Sanger sequencing uncovered a novel mutation in Intron 2 and established phase

<table>
<thead>
<tr>
<th></th>
<th>Intron 2</th>
<th>Intron 5</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mother</strong></td>
<td>Normal Normal</td>
<td>c.492+2T&gt;C Normal</td>
</tr>
<tr>
<td><strong>Father</strong></td>
<td>c.228-17C&gt;G Normal</td>
<td>Normal Normal</td>
</tr>
<tr>
<td><strong>Patient 1</strong></td>
<td>c.228-17C&gt;G Normal</td>
<td>c.492+2T&gt;C Normal</td>
</tr>
</tbody>
</table>
## Family Studies

### Identification of a 2\textsuperscript{nd} affected family member

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<tr>
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<tr>
<td></td>
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<td>c.228-17C&gt;G</td>
<td>Normal</td>
</tr>
<tr>
<td></td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td><strong>Patient 1</strong></td>
<td>c.228-17C&gt;G</td>
<td>c.492+2T&gt;C</td>
</tr>
<tr>
<td></td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td><strong>Sibling 1</strong></td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td></td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td><strong>Sibling 2</strong></td>
<td>c.228-17C&gt;G</td>
<td>Normal</td>
</tr>
<tr>
<td></td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td><strong>Patient 2</strong></td>
<td>c.228-17C&gt;G</td>
<td>c.492+2T&gt;C</td>
</tr>
<tr>
<td></td>
<td>Normal</td>
<td>Normal</td>
</tr>
</tbody>
</table>
Patient 2: Initial evaluation (age 4)

- Normal pregnancy and delivery
- History of intermittent leg pain
- MRI at age 5 y: Bilateral and symmetric hyperintense T2 signal abnormalities in the supraventricular and periventricular white matter (yellow arrows). Abnormal signal intensities in the posterior limbs of the internal capsule, pyramids at the level of the medulla, and dorsal columns of the spinal cord. A right intraventricular cyst (green arrow) was also uncovered.
E-Lab
Fibroblasts studies
Skipping of exon 3 in patients

RT-PCR results suggested skipping of exon 3 in the father and the two patients harboring the intron 2 mutation.
DARS2 mRNA levels – qPCR

Carriers and patients showed lower expression of mRNA levels

qPCR Method: Taqman Assay
Primer set: Thermo Fisher Hs01016220_m1
Patients showed reduced levels of DARS2 protein
Lactic acid levels in cultured media

Patients showed elevated lactic acid levels in the culture media

Instrument: Infinite M Plex
Kit: POINTE™ Lactate Reagent Set (L7596-50)
Reactive oxygen species (ROS) levels

Patients showed elevated ROS production

- MitoSOX™ Red: Mitochondrial Superoxide
- MitoTracker™ Green: mt content (Normalization)
Oxygen consumption rate

Patients showed decreased basal respiration and spare respiratory capacity
Mitochondria morphology - Microscopy

Patients showed fragmented mitochondrial network

Fluorescent Microscope:
Live-cell staining
MitoTracker™ Green

Deconvolution: Image J plug-in
Mitochondrial Network Analysis (MiNA)

Patients showed decreased mitochondrial intensity, length, and number of branches

Footprint (Intensity)

Length

Number of branches

Quantification method
Image J, Plug-in / MiNA
Transcriptome Analysis

Method: RNAseq
Software: Kallisto and limma (R & R Studio)

![Graph showing log2 fold change vs. -log10 p-value]

-Log10 P

Log2 fold change

Total = 13589 variables
### Downregulated genes

<table>
<thead>
<tr>
<th>Gene Symbol</th>
<th>Fold change (log₂)</th>
<th>adj.P.value</th>
</tr>
</thead>
<tbody>
<tr>
<td>VARS2</td>
<td>-5.29</td>
<td>0.06</td>
</tr>
<tr>
<td>TSTD1</td>
<td>-4.70</td>
<td>0.08</td>
</tr>
<tr>
<td>PDK4</td>
<td>-2.68</td>
<td>0.24</td>
</tr>
<tr>
<td>MT-ATP6</td>
<td>-1.99</td>
<td>0.18</td>
</tr>
<tr>
<td>MT-ATP8</td>
<td>-1.91</td>
<td>0.15</td>
</tr>
<tr>
<td>MT-ND3</td>
<td>-1.78</td>
<td>0.23</td>
</tr>
<tr>
<td>COX7A1</td>
<td>-1.66</td>
<td>0.14</td>
</tr>
<tr>
<td>MT-CO2</td>
<td>-1.62</td>
<td>0.20</td>
</tr>
<tr>
<td>MT-ND4L</td>
<td>-1.45</td>
<td>0.18</td>
</tr>
<tr>
<td>OXCT2</td>
<td>-1.42</td>
<td>0.24</td>
</tr>
<tr>
<td>MT-ND6</td>
<td>-1.40</td>
<td>0.21</td>
</tr>
<tr>
<td>MT-ND4</td>
<td>-1.37</td>
<td>0.22</td>
</tr>
<tr>
<td>MT-CYB</td>
<td>-1.26</td>
<td>0.25</td>
</tr>
<tr>
<td>MT-ND1</td>
<td>-1.25</td>
<td>0.18</td>
</tr>
<tr>
<td>MT-ND5</td>
<td>-1.18</td>
<td>0.21</td>
</tr>
<tr>
<td>ACSS3</td>
<td>-1.17</td>
<td>0.18</td>
</tr>
<tr>
<td>MTHFS</td>
<td>-1.11</td>
<td>0.30</td>
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<tr>
<td>ATP23</td>
<td>-1.09</td>
<td>0.34</td>
</tr>
<tr>
<td>MT-CO1</td>
<td>-1.01</td>
<td>0.28</td>
</tr>
<tr>
<td>ACOT11</td>
<td>-1.00</td>
<td>0.19</td>
</tr>
</tbody>
</table>

### Upregulated genes

<table>
<thead>
<tr>
<th>Gene Symbol</th>
<th>Fold change (log₂)</th>
<th>adj.P.value</th>
</tr>
</thead>
<tbody>
<tr>
<td>C6orf136</td>
<td>1.76</td>
<td>0.25</td>
</tr>
<tr>
<td>PDK1</td>
<td>1.74</td>
<td>0.13</td>
</tr>
<tr>
<td>AMT</td>
<td>1.72</td>
<td>0.18</td>
</tr>
<tr>
<td>C17orf47</td>
<td>1.71</td>
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<td>HSD17B8</td>
<td>1.67</td>
<td>0.73</td>
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<tr>
<td>BNIP3</td>
<td>1.55</td>
<td>0.05</td>
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<tr>
<td>MGARP</td>
<td>1.26</td>
<td>0.32</td>
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<tr>
<td>MTFP1</td>
<td>1.20</td>
<td>0.33</td>
</tr>
<tr>
<td>MRPL23</td>
<td>1.12</td>
<td>0.16</td>
</tr>
</tbody>
</table>
DARS2 Pathway

Smith Fine et al., Journal of Neurodevelopmental Disorders 11 (2019) 29
Treatment?
DARS2 Treatment

• 5-Aminolevulinic acid (ALA)/sodium ferrous citrate (SFC) treatment in patients with different mitochondrial diseases

• Increased oxygen consumption rate, ATP levels and mitochondrial copy number after 5-ALA/SFC treatment

Shimura et al., Scientific Reports 9 (2019) Article #10549
Mitochondrial iron-containing proteins

Adapted from Maio et al, Cell Metabolism 25 (2017) 945–953
Haeme biosynthesis
Oxygen consumption rate
Seahorse

Treatment for 5 weeks on Sibling1
Oxygen consumption rate
Seahorse

Treatment for 5 weeks on Patient 1
Extracellular acidification rate (ECAR) Seahorse

ECAR
- Patient 1
- DMSO
- ALA/Fe++

Baseline ECAR
- Patient 1

Lactic Acid (mmol/g protein)
- Patient 2
- Patient 1
- Sibling 1

Lactic acid
- Treatment
- **

ECAR (mM/min/μg protein)
- DMSO
- ALA/Fe++

Basal ECAR
- **

Lactic Acid (mmol/g protein)
- DMSO
- ALA/Fe++
Respiratory chain complex enzyme activities

**Complex I activity**

Interaction: $F_{(2,12)} = 5.193; P = 0.02$
Treatment: $F_{(1,12)} = 6.770; P = 0.02$
Genotype: $F_{(2,12)} = 0.9220; P = 0.4$

**Complex IV activity**

Interaction: $F_{(2,12)} = 3.087; P = 0.08$
Treatment: $F_{(1,12)} = 81.28; P < 0.0001$
Genotype: $F_{(2,12)} = 16.57; P = 0.0004$

**Citrate synthase (CS) activity**

Interaction: $F_{(2,12)} = 6.796; P = 0.01$
Treatment: $F_{(1,12)} = 159.6; P < 0.0001$
Genotype: $F_{(2,12)} = 4.349; P = 0.03$
GSH & GSSG levels in cell homogenates

**GSH levels**

- Interaction: $F_{(2,12)} = 1.07; p = 0.374$
- Genotype: $F_{(1,12)} = 13.71; p = 0.003$
- Treatment: $F_{(2,12)} = 12.09; p = 0.001$

**GSSG levels**

- Interaction: $F_{(2,12)} = 1.86; p = 0.198$
- Genotype: $F_{(1,12)} = 3.43; p = 0.090$
- Treatment: $F_{(2,12)} = 0.88; p = 0.440$

**GSH/GSSG Ratio**

- Interaction: $F_{(2,12)} = 10.56; p = 0.002$
- Genotype: $F_{(1,12)} = 0.132; p = 0.723$
- Treatment: $F_{(2,12)} = 19.52; p < 0.001$
Haem oxygenase 1 (HO-1) content after treatment

C: Control
T: Treatment (ALA + iron)

<table>
<thead>
<tr>
<th></th>
<th>Sibling1</th>
<th>Patient1</th>
<th>Patient2</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>C</td>
<td>C</td>
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</tr>
<tr>
<td>Treatment</td>
<td>T</td>
<td>T</td>
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</tr>
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</table>

![Graph showing HO-1 expression levels after treatment comparison with control.]

- Sibling1: Untreated HO-1 expression level is high.
- Patient 1: ALA+Iron treatment shows a significant increase in HO-1 expression compared to control.
- Patient 2: ALA+Iron treatment also shows an increase in HO-1 expression compared to control.
Summary & next steps

1. Demonstrate the expression of the disease in fibroblasts
   - mRNA and protein expression
   - Oxygen consumption rate
   - Lactic Acid
   - ROS
   - Fragmented mitochondria

2. Potential treatments can rescue the phenotype
   - Treatment with ALA/Fe++
     - Oxygen consumption rate
     - Lactic Acid / ECAR
     - Antioxidant status: Improved
     - HO-1 expression

3. Generate iPSC and neuronal cells

4. Demonstrate the expression of the disease in neuronal cells

5. Potential treatments can rescue the phenotype
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L.Esqueda Admin

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