Cool Cases

A Clinician’s Perspective after 30 + Years of Mitochondrial Diagnostics

Bruce H. Cohen, MD
Professor of Pediatrics, Northeast Ohio Medical University
Director of Neurology, Akron Children’s Hospital
Elisa D

- 6 years old; Italian Heritage
- Early developmental delays
- Intellectual Disability with ASD features
- Recurrent high fevers (106°) without viral illness
- Low-grade neural crest tumor (paragangliomeuroma, abdominal)
- Persistent Lactic Acidosis
- MRI – symmetric thalamic changes
- Muscle biopsy showed early RRF changes
- Slept only a few hours a day; case discussed with Dr. Gambetti (Fatal Familial Insomnia)
- Died in her sleep during a febrile event - 1992
Elisa’s Sister “D”

- Normal growth and development
- Track and Field “star” in high school
- Repeatedly became encephalopathic and hemiparetic during races, especially on hot days
- Mild analyte abnormalities
- Normal muscle histology, ETC enzymology but ANT defect on polarography (1999)
- ANT1 sequenced in ~ 2004 and was normal
- Working now in a high power job in NYC
Katelyn D. – 1992

- 4 year old girl, developmental delays, ataxia, intellectual disability, ASD
- **Repeated events of hemiparesis** with apparent headache and encephalopathy
- Persistent **lactic acidosis**, high alanine
- MRI – corpocephaly
- Muscle biopsy – ETC Enzymology c/w complex I deficiency
- Placed on Dr. Richard Haas’ DCA study
- Event in ~ 1993; fell asleep under a tree at a church picnic on a hot day and unarousable. MRI showed **bilateral cerebellar > cortical patchy T2 changes (before FLAIR), which resolved**. Return to baseline in a month
- Genetic testing: normal karyotype and research MELAS gene test
• Katelyn D – followup
  ◦ Young adult
  ◦ Ambulatory, but ataxic with dystonia and chorea
  ◦ Few stereotyped words, dysarthria
  ◦ ASD, propensity to hug
  ◦ Further Genetic - Metabolic Evaluation
    • Normal subtelomeric FISH
    • Normal CoQ10 levels
    • Normal transferring studies including expanded glycosylated proteins but not n-glycan, o-glycan analysis
    • Normal oligo array (2009)
    • Normal mtDNA sequencing
    • Mother “deferred” further nuclear gene testing

Loose MELAS Phenotype?
Case Report 1997

1 year old boy
mild motor delays
sudden onset cardiomyopathy
carnitine deficiency
lactic acidosis
heart transplant candidate
muscle biopsy
microscopy
ETC: complex I defect
FAO: VLCAD defect
Rx: CoQ10 & l-carnitine
Outcome
Mr. SK
1994
age 61

The Man with Many “Classic” Mitochondrial Signs

- Referred to Cardiology from doctors in India for a pacemaker, got off the plane and came to his appointment
- Became anxious in the waiting room then lapsed into a deep stupor
- CT showed bilateral calcified basal ganglia
- History
  - 8 abdominal surgeries for “acute abdomen”, normal gall bladder and appendix had been removed in 2 of these surgeries
  - Progressive sarcopenia and lipodystrophy
  - Ptosis without further PEO
  - Neuropathy without DM
  - Hepatic fibrosis
  - Loss of intellectual prowess
  - Family history – mother with similar illness, son with early signs of this illness
- Labs – lactic acidosis, persistent
Evaluation

- Increased subsarcolemmal mitochondria
- Non-specific ETC Enzymatic changes, mild decrease in complex I activity
- OXPHOS showed mild decrease in substrates contributing e\textsuperscript{-} to complex I and decreased FAO

Treatment

- Very low-fat diet
- Frequent meals
- CoQ10, Carnitor, Riboflavin

Course

- Pacemaker placed for cardiac conduction defect
- Repeated episodes of coma following fasting or stress
- Died in 2000 during an illness marked by coagulopathy

What is Mr. SK’s Diagnosis?

- ?POLG?
Cold Case

- Disease not solved to the full extent
- is not the subject of a recent investigation
- which new information could emerge from re-examination of the records, retained tissue, using new technical methods developed after the case presented
- Sometimes errors of omission of testing can solve a cold case

N=200
Step-wise CNS deterioration (+Sz, + movement disorder, + ataxia)
Ptosis and PEO
Progressive optic atrophy or retinitis pigmentosa
High frequency hearing loss
Cardiac conduction defects
Myopathy or Cardiomyopathy
Hepatopathy
Neuropathy (large fiber)
Systemic Lipomatosis or lipodystrophy
Classic MRI Findings (symmetric, deep gray)
Biochemical: true LA, classic amino acid pattern, classic organic acid patterns

The Classic Mitochondrial Phenotype
2 Neat Cases
Parents identified poor hearing and low tone from birth.

- Evaluated at UCSD – Bruce Barshop
- Identified Need for Muscle Biopsy

Failure to thrive diagnosed by 2 months.

- Kinky Hair
- Seen by Dr. Menkes
- Amino aciduria
- Slow developmental progress
- No speech or language

Google Search: Taryn LA Ink
Bjornstad syndrome: pili torti and congenital hearing loss

GRACILE Syndrome: growth retardation, aminoaciduria, cholestasis, iron overload, lactic acidosis and early death

Does Genotype Drive Phenotype?
### OXPHOS Polarography

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Entry Site</th>
<th>State iii</th>
<th>State iv</th>
<th>High ADP</th>
<th>Uncoupled State</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutamate</td>
<td>Complex I</td>
<td>161 (164 ± 44)</td>
<td>17 (16 ± 7)</td>
<td>154 (175 ± 47)</td>
<td>167 (201 ± 70)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>89 – 274</td>
<td>5 – 35</td>
<td>97 – 293</td>
<td>77 – 358</td>
</tr>
<tr>
<td>2-KG + Malonate</td>
<td>Complex I</td>
<td>164 (142 ± 22)</td>
<td>28 (20 ± 6)</td>
<td>154 (151 ± 28)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>97 – 192</td>
<td>5 – 33</td>
<td>101 – 234</td>
<td>-</td>
</tr>
<tr>
<td>Succinate</td>
<td>Complex II</td>
<td>235 (295 ± 40)</td>
<td>53 (68 ± 13)</td>
<td>259 (300 ± 44)</td>
<td>270 (299 ± 47)</td>
</tr>
<tr>
<td>Duroquinol</td>
<td>Complex III</td>
<td><strong>388 (588 ± 74)</strong></td>
<td><strong>64 (182 ± 43)</strong></td>
<td><strong>264 (564 ± 75)</strong></td>
<td><strong>306 (901 ± 149)</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>433 – 766</strong></td>
<td><strong>88 – 349</strong></td>
<td><strong>406 – 779</strong></td>
<td><strong>606 – 1212</strong></td>
</tr>
<tr>
<td>TMDP + Ascorbate</td>
<td>Complex IV</td>
<td>939 (952 ± 169)</td>
<td>355 (418 ± 77)</td>
<td>809 (906 ± 169)</td>
<td>948 (1120 ± 208)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>674 – 1447</td>
<td>229 – 603</td>
<td>563 – 1376</td>
<td>757 – 1709</td>
</tr>
<tr>
<td>Palmitoyl-CoA +</td>
<td>CPTI/II</td>
<td>81 (116 ± 32)</td>
<td>11 (21 ± 6)</td>
<td>197 (152 ± 35)</td>
<td></td>
</tr>
<tr>
<td>Carnitine + Malate</td>
<td></td>
<td>61 – 196</td>
<td>5 – 38</td>
<td>87 – 293</td>
<td>-</td>
</tr>
</tbody>
</table>

Activity reported in n atoms oxygen/minute/mg protein; patient value (mean ± SD) / normal range.

### Electron Chain Enzymology

<table>
<thead>
<tr>
<th>Enzymatic Reaction</th>
<th>ETC Complexes</th>
<th>Patient</th>
<th>Control ± SD</th>
<th>Range</th>
<th>% Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>NADH-Cyt c reductase</td>
<td>I+III</td>
<td>51</td>
<td>1377 ± 554</td>
<td>307 – 2858</td>
<td>3.7</td>
</tr>
<tr>
<td>NADH-Q reductase</td>
<td>I</td>
<td>111.0</td>
<td>227.6 ± 73.1</td>
<td>111.1 – 402.6</td>
<td>49</td>
</tr>
<tr>
<td>Succinate-cyt c reductase</td>
<td>II + III</td>
<td>90</td>
<td>309 ± 154</td>
<td>82 – 771</td>
<td>29</td>
</tr>
<tr>
<td>Succinate Dehydrogenase</td>
<td>SDH</td>
<td>166</td>
<td>148 ± 75</td>
<td>21 – 347</td>
<td>112</td>
</tr>
<tr>
<td>Succinate Q reductase</td>
<td>II</td>
<td>72</td>
<td>42 ± 20</td>
<td>14 – 90</td>
<td>171</td>
</tr>
<tr>
<td>Decylubiquinol-cyt c reductase</td>
<td>III</td>
<td>671</td>
<td>4512 ± 1527</td>
<td>1417 – 8497</td>
<td>15</td>
</tr>
<tr>
<td>Cytochrome c oxidase</td>
<td>IV</td>
<td>210165</td>
<td>109695 ± 33750</td>
<td>45554 – 219110</td>
<td>192</td>
</tr>
<tr>
<td>Citrate synthase</td>
<td>CS</td>
<td>1857</td>
<td>1949 ± 704</td>
<td>1055 – 5351</td>
<td>95</td>
</tr>
</tbody>
</table>

Activity expressed in nmol/min/mg mito protein.
Case Solved

- Bjornstad syndrome: pili torti and congenital hearing loss
- GRACILE Syndrome: growth retardation, aminoaciduria, cholestasis, iron overload, lactic acidosis and early death
- Both diseases are due to a single gene \((BCS1L)\), codes for an assembly protein of complex III of the ETC, responsible for putting Fe into the Fe/S core
- Muscle biopsy showed a severe complex III deficiency
- Compound hetrozygote for G183A and G235A

Lesson Learned:

- Big deal in 2007
- 2017? Nothing special about this story – same as POLG and many other genes

3 yo girl

mother thought child had poor hearing and low tone from soon after birth

Failure to thrive with lactic acidosis and elevated liver enzymes

By June 2006 could pull to standing but lost that skill, now with further regression

Muscle biopsy normal microscopy

Liver biopsy showed hepatocellular changes “...with mild periportal fibrosis and apoptosis”
Sometimes it Takes a While

1997-2010
Great Night On-Call

- 26 yo woman, previously healthy; acute psychosis that resolved with haloperidol (1996)
- 9 months later presented with psychosis and chorea; CT showed calcified basal ganglia
- Transferred to adult neurology service in an encephalopathic state (stupor) - 1997
- CSF Lactate 7.6 mmol/L - no WBCs, normal protein
- Treated with CoQ10 200 mg bid from 1997-today
- Functionally limited, married but poor memory and insight
- One episode of encephalopathy in 2007, resolved in one month
- No deterioration, recent blood lactate 4 mmol/L
- Overall no changes in ADLs from 1996 - 2012
Diagnostic Evaluation 1997

- History: no maternal inheritance pattern
- Physical Examination: dementia - distant affect, long-term memory intact, language slow but normal, chorea, poor coordination
- Analytes: Lactic acid, concept of the lactate:pyruvate ratio, organic acids, amino acids all abnormal, elevated CSF lactate
- Pathology: normal muscle
- Biochemical: severe complex I+III, II+III
- Genetic: karyotype analysis, mtDNA screen (12 mutations + Southern blot)
<table>
<thead>
<tr>
<th>Activity (µmol/min/g wet weight)</th>
<th>ETC Complexes</th>
<th>Patient</th>
<th>Control Mean ± SD</th>
<th>Prior Controls Mean ± SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>NADH-cytochr. c reductase - rotenone:</td>
<td></td>
<td>562.0</td>
<td>805.0 ± 112.8</td>
<td>(639.0 - 915.0)</td>
<td>L</td>
</tr>
<tr>
<td>NADH-cytochr. c reductase + rotenone:</td>
<td></td>
<td>555.9</td>
<td>324.0 ± 119.0</td>
<td>(188.0 - 441.0)</td>
<td>H</td>
</tr>
<tr>
<td>NADH-cytochr. c reductase rot. sens.:</td>
<td>I, III</td>
<td>6.1</td>
<td>481.0 ± 36.5</td>
<td>(441.0 - 528.0)</td>
<td>L</td>
</tr>
<tr>
<td>NADH-ferricyanide reductase</td>
<td>I</td>
<td>2180.5</td>
<td>1620.5 ± 363.8</td>
<td>(1284.0 - 2131.0)</td>
<td>H</td>
</tr>
<tr>
<td>Succinate-cytochrome c reductase:</td>
<td>II, III</td>
<td>59.2</td>
<td>146.4 ± 41.6</td>
<td>(82.0 - 187.0)</td>
<td>L</td>
</tr>
<tr>
<td>Succinate dehydrogenase:</td>
<td>II</td>
<td>177.2</td>
<td>74.5 ± 30.4</td>
<td>(53.0 - 96.0)</td>
<td>H</td>
</tr>
<tr>
<td>Decylubiquinol-cytochrome c reductase:</td>
<td>III</td>
<td>5049.0</td>
<td>4327.5 ± 415.1</td>
<td>(4034.0 - 4621.0)</td>
<td>H</td>
</tr>
<tr>
<td>Cytochrome c oxidase:</td>
<td>IV</td>
<td>38083.0</td>
<td>73307.4 ± 22667.2</td>
<td>(38411.0 - 96774.0)</td>
<td>L</td>
</tr>
<tr>
<td>Citrate Synthase:</td>
<td></td>
<td>2408.8</td>
<td>1820.3 ± 348.2</td>
<td>(1338.0 - 2555.0)</td>
<td></td>
</tr>
</tbody>
</table>

Thanks to Charles Hoppel, MD; CIDEM
### What's Your Diagnosis?

#### 1997
- Fahr Disease
- Mitochondrial Encephalomyopathy
- Atypical MELAS
- Atypical Leigh Disease
- Complex I Disease
- CoQ10 disorder
  - low I+III, II+III, ? IV
  - responsive to CoQ10

#### 2010
- mtDNA
- homoplasmic
- G1644A
  - tRNA\_val
- Known as “adult Leigh”
Primary vs. Secondary Mitochondrial Disease

Is there a difference between primary and secondary mitochondrial disease?
Not my favorite case to talk about

- 2 year old boy with mild developmental delays and dystonia (born in 1998)
- Moderate lactic acidemia (3-4 mM) and high alanine
- MRI – moderate atrophy
- Muscle biopsy – normal microscopy, very low ETC complex I activity and low state iii rates with substrates utilizing complex I
- 100% comfortable with complex I defect
- Office visit in the summer of 2003
What has WES Uncovered in my Mito Patients?

- **SCN2a**
- **RAPSN** – Congenital Myasthenia Gravis
- **SLC52A2** - Riboflavin Transporter Defect
- Minicore Myopathy
- **SCA5**
- **CACNA1S** – hypokalemic periodic paralysis
Mitochondrial Medicine
Our Knowledge is Only the Tip of the Iceberg