Clinical Phenotype of Mitochondrial Disease

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Center for Mitochondrial Diseases
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Clinical Phenotype of Mitochondrial Disease

• What is a Jedi Knight?
• A perspective on the Dark Side of the Force.
Clinical Phenotype of Mitochondrial Disease

- Why study Mitochondria
- Mitochondrial Oxidative Phosphorylation
- Mitochondrial function in Patients
- Respirosomes ?? Supercomplexes!!
- Respirosomes in Heart Failure
- Summary and Conclusions

2008 McKinnon Conference, Duluth, MN. September 17, 2008

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Star Wars Episode I

What makes a JEDI Knight?
How do you become a JEDI Knight?

Adapted from G. Lucas, STAR WARS, Episode I (1999)
• Chip with a blood sample
  – Anakin Skywalker
• Midi-chlorian test
• Count is over twenty thousand
• No one has a count that high
  NOT even Master Yoda!

Adapted from G. Lucas, STAR WARS, Episode I (1999)

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Midi-chlorians

• Microscopic life-forms
  – reside with cells
  – Communicate with the FORCE
• We are symbiots with the midi-chlorians
  (living together with material advantage)

Adapted from G. Lucas, STAR WARS, Episode I (1999)
• Without *midi-chlorians*
  – Life cannot exist and
  – Have no knowledge of the **FORCE**.

• *Midi-chlorians*
  – Speak to us
  – Telling us the will of the **FORCE**

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**Clinical Phenotype of Mitochondrial Disease**

• Why study Mitochondria

• *Mitochondrial Oxidative Phosphorylation*

• Mitochondrial function in Patients

• Respirasomes ?? Supercomplexes!!

• Respirasomes in Heart Failure

• Summary and Conclusions

2006 McKinnon Conference, Duluth, MN, September 17, 2006
Oxidative Phosphorylation

Localize Sites of Damage to Electron Transport Chain by Use of Selective Substrates
Mitochondrial Oxidation Studies

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Translocase</th>
<th>Enzyme(s)</th>
<th>Reducing EQ.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyruvate</td>
<td>Pyruvate</td>
<td>PDC</td>
<td>NADH</td>
</tr>
<tr>
<td>Glutamate</td>
<td>Glutamate</td>
<td>Glutamate DH</td>
<td>NADH</td>
</tr>
<tr>
<td>Palmitoyl-L-carnitine</td>
<td>Translocase</td>
<td>β-oxidation</td>
<td>NADH/FADH</td>
</tr>
<tr>
<td>Succinate</td>
<td>Dicarboxylate</td>
<td>Succinate DH</td>
<td>FADH</td>
</tr>
</tbody>
</table>

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- Why study Mitochondria
- Mitochondrial Oxidative Phosphorylation
- **Mitochondrial function in Patients**
- Respirasomes ?? Supercomplexes!!
- Respirasomes in Heart Failure
- Summary and Conclusions

2008 McKinnon Conference, Duluth, MN September 17, 2008
Patient 1

- 56 yo man CC: myalgia and fatigue
- 2002 1 month after starting Lipitor (statin)
- Generalized fatigue, myalgia and minimal weakness stopped Lipitor in early 2003
- Intermittently elevated CK 506 U/L (N=0-232)
- Continued symptoms off Lipitor 5 years!!
- Exercises regularly

Patient 1

- RX:
  - Niaspan
  - Tricor
  - Metformin
  - Glucotrol
  - Lisinopril

- Total Cholesterol 188
- LDL 119
- HDL 34.4
Patient 1: Oxidative Phosphorylation
nA oxygen/min/mg mito prot

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Transporter</th>
<th>DH</th>
<th>ETC</th>
<th>Rate</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>glutamate</td>
<td>glutamate</td>
<td>GDH</td>
<td>I III IV</td>
<td>58</td>
<td>164±44</td>
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<tr>
<td>succinate</td>
<td>dicarboxy</td>
<td>SDH</td>
<td>II III IV</td>
<td>268</td>
<td>295±40</td>
</tr>
<tr>
<td>duroquinol</td>
<td></td>
<td></td>
<td>III IV</td>
<td>450</td>
<td>588±74</td>
</tr>
<tr>
<td>TMPD/Asc</td>
<td></td>
<td></td>
<td>IV</td>
<td>1461</td>
<td>952±169</td>
</tr>
<tr>
<td>Yield (mg/g)</td>
<td></td>
<td></td>
<td></td>
<td>5.1</td>
<td>5.3±1.1</td>
</tr>
</tbody>
</table>

Patient 1: Skeletal Muscle Mitochondria ETC
nmoles/min/mg mito prot

<table>
<thead>
<tr>
<th>Reaction</th>
<th>ETC</th>
<th>Rate</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rot Sens NADH-Cytochr c red</td>
<td>I III</td>
<td>303</td>
<td>1377±554</td>
</tr>
<tr>
<td>NADH Ubiquinone Reductase</td>
<td>I</td>
<td>74</td>
<td>228±73</td>
</tr>
<tr>
<td>Succ-cytochr c red</td>
<td>II III</td>
<td>127</td>
<td>309±154</td>
</tr>
<tr>
<td>Succinate Ubiquinone Reductase</td>
<td>II</td>
<td>52</td>
<td>42±20</td>
</tr>
<tr>
<td>Decylubiquinol-cytochr c reductase</td>
<td>III</td>
<td>2832</td>
<td>4512±1527</td>
</tr>
<tr>
<td>Aconitase</td>
<td></td>
<td>670</td>
<td>633±226</td>
</tr>
<tr>
<td>Citrate Synthase</td>
<td></td>
<td>1703</td>
<td>1949±704</td>
</tr>
</tbody>
</table>
Patient 1: Skeletal Muscle ETC
\( \mu \text{mol/\text{min/g wet wt}} \)

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Complex I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rot Sens NADH-Cytochr c red</td>
<td>I</td>
<td>III</td>
<td>0.6</td>
<td>2.7±2.0</td>
</tr>
<tr>
<td>NADH ferricyanide red</td>
<td>“I”</td>
<td>“I”</td>
<td>44.3</td>
<td>29.6±9.8</td>
</tr>
<tr>
<td>Succ-cytochr c red</td>
<td>II</td>
<td>III</td>
<td>2.6</td>
<td>2.4±1.0</td>
</tr>
<tr>
<td>Succinate DH</td>
<td>“II”</td>
<td>“II”</td>
<td>0.9</td>
<td>1.0±0.6</td>
</tr>
<tr>
<td>Decylubiquinol-cytochr c reductase</td>
<td>III</td>
<td></td>
<td>14.9</td>
<td>19±10.1</td>
</tr>
<tr>
<td>Citrate Synthase</td>
<td></td>
<td></td>
<td>15.7</td>
<td>17.5±4.6</td>
</tr>
</tbody>
</table>

**Patient 1 Summary**

- ETC Complex I defect
  - \( \downarrow \) NADH-linked oxidation
  - \( \downarrow \) Succinate oxidation
  - \( \downarrow \) Quinol oxidation
  - \( \downarrow \) Cyto c oxidation
  - \( \downarrow \) Complex I/III
  - \( \downarrow \) Complex I
Patient 1 Summary

• ETC Complex I defect
  Statin – Induced?
  What type of mechanism?

  Statin – uncovered a primary defect?
  In a 52 yo man!!!!

  Statin – Induced coenzyme Q deficiency?
  Complex I

Patient 2 (Zinn)

• 20 yo
  age 3 yr: bilateral hearing loss
  age 12 yr: exercise intolerance
  age 17 yr: cardiomyopathy
  plasma lactate: 4.9 mM
  L/P ratio: 21
  1995 biopsy - focal mito
  1996 biopsy - normal
  analysis negative for mDNA
Patient 2: Oxidative Phosphorylation
nA oxygen/min/mg mito prot

Substrate transporter DH ETC Rate Control

<table>
<thead>
<tr>
<th>Glutamate</th>
<th>glutamate</th>
<th>GDH</th>
<th>I III IV</th>
<th>142</th>
<th>151±44</th>
</tr>
</thead>
<tbody>
<tr>
<td>Succinate</td>
<td>dicarboxy</td>
<td>SDH</td>
<td>II III IV</td>
<td>151</td>
<td>280±47</td>
</tr>
<tr>
<td>Duroquinol</td>
<td></td>
<td></td>
<td>III IV</td>
<td>187</td>
<td>453±114</td>
</tr>
<tr>
<td>TMPD/Asc</td>
<td></td>
<td></td>
<td>IV</td>
<td>797</td>
<td>440±178</td>
</tr>
<tr>
<td>Yield (mg/g)</td>
<td></td>
<td></td>
<td></td>
<td>6.6</td>
<td>5.2±1.3</td>
</tr>
</tbody>
</table>

Patient 2: Skeletal Muscle Mitochondria ETC
nmoles/min/mg mito prot

Rot Sens NADH-Cytochr c red I III 129 481±37
NADH ferricyanide red “I” 1475 1620±364
Suce-cytochr c red II III 108 146±42
Succinate DH “II” 116 75±30
Decylubiquinol-cytochr c reductase III 343 4328±415
Cyto. Oxidase IV 98271 73307±22667
Citrate Synthase | 1910 | 1820±348 |
### Patient 2: Skeletal Muscle ETC

\[ \mu \text{mol/min/g wet wt} \]

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Complex</th>
<th>Value 1</th>
<th>Value 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rot Sens NADH-Cytochr c red</td>
<td>I III</td>
<td>0.4</td>
<td>0.7±0.3</td>
</tr>
<tr>
<td>NADH ferricyanide red</td>
<td>&quot;I&quot;</td>
<td>16.1</td>
<td>41±14</td>
</tr>
<tr>
<td>Succ-cytochr c red</td>
<td>II III</td>
<td>0.2</td>
<td>2.3±1.1</td>
</tr>
<tr>
<td>Succinate DH</td>
<td>&quot;II&quot;</td>
<td>0.4</td>
<td>0.6±0.3</td>
</tr>
<tr>
<td>Decylubiquinol-cytochr c reductase</td>
<td>III</td>
<td>3.2</td>
<td>19±6</td>
</tr>
<tr>
<td>Cyto. Oxidase</td>
<td>IV</td>
<td>81.1</td>
<td>137±47</td>
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<tr>
<td>Citrate Synthase</td>
<td></td>
<td>9.7</td>
<td>20±5</td>
</tr>
</tbody>
</table>

### Patient 2

**High ADP Respiration**

- **Pt 2**
- **Controls**

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Pt 2</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutamate</td>
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<td></td>
</tr>
<tr>
<td>Pyruvate</td>
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<td></td>
</tr>
<tr>
<td>2-KG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Palmitoyl-CoA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Succinate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Decylubiquinol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TMPD/Asc</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Patient 2 Summary

• ETC Complex III defect

| NADH-linked oxidation | \[
| Succinate oxidation | \[
| Quinol oxidation | \[
| Cyto c oxidation | \[
| Complex I/III | \[
| Complex III | \[

Patient 3

• 3 1/2 yo girl
Björnstad syndrome
Sensorineural deafness
Pili torti
Referred for muscle biopsy and isolation of skeletal muscle mitochondria
Also studied in Seidman Lab at Harvard
Patient 3: Oxidative Phosphorylation
nA oxygen/min/mg mito prot

<table>
<thead>
<tr>
<th>Substrate</th>
<th>transporter</th>
<th>DH</th>
<th>ETC</th>
<th>Rate</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>glutamate</td>
<td>glutamate</td>
<td>GDH</td>
<td>I/II</td>
<td>161</td>
<td>164±44</td>
</tr>
<tr>
<td>succinate</td>
<td>dicarboxy</td>
<td>SDH</td>
<td>II/III</td>
<td>235</td>
<td>295±40</td>
</tr>
<tr>
<td>duroquinol</td>
<td></td>
<td></td>
<td>III/IV</td>
<td>388</td>
<td>588±74</td>
</tr>
<tr>
<td>TMPD/Asc</td>
<td></td>
<td></td>
<td>IV</td>
<td>939</td>
<td>952±189</td>
</tr>
</tbody>
</table>

Yield
(mg/g)
2.1 5.3±1.1

Patient 3: Skeletal Muscle Mitochondria ETC
nmoles/min/mg mito prot

<table>
<thead>
<tr>
<th>Rot Sens NADH-Cytochr c red</th>
<th>I III</th>
<th>51</th>
<th>1377±554</th>
</tr>
</thead>
<tbody>
<tr>
<td>NADH Ubiquinone Reductase</td>
<td>I</td>
<td>111</td>
<td>228±73</td>
</tr>
<tr>
<td>Succ-cytochr c red</td>
<td>II III</td>
<td>90</td>
<td>309±154</td>
</tr>
<tr>
<td>Succinate Ubiquinone Reductase</td>
<td>II</td>
<td>72</td>
<td>42±20</td>
</tr>
<tr>
<td>Decylubiquinol-cytochr c reductase</td>
<td>III</td>
<td>671</td>
<td>4512±1527</td>
</tr>
<tr>
<td>Aconitase</td>
<td></td>
<td>543</td>
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<tr>
<td>Citrate Synthase</td>
<td></td>
<td>1857</td>
<td>1949±704</td>
</tr>
</tbody>
</table>
**Patient 3: Skeletal Muscle ETC**

\[ \mu \text{mol/min/g wet wt} \]

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Complex</th>
<th>Activity</th>
<th>Deviation</th>
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</thead>
<tbody>
<tr>
<td>Rot Sens NADH-Cytochr c red</td>
<td>I, III</td>
<td>1.6</td>
<td>2.7±2.0</td>
</tr>
<tr>
<td>NADH ferricyanide red</td>
<td>“I”</td>
<td>36.4</td>
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<td>II, III</td>
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<td>“II”</td>
<td>0.6</td>
<td>1.0±0.6</td>
</tr>
<tr>
<td>Decylubiquinol-cytochr c reductase</td>
<td>III</td>
<td>2.2</td>
<td>19±10.1</td>
</tr>
<tr>
<td>Citrate Synthase</td>
<td></td>
<td>16</td>
<td>17.5±4.6</td>
</tr>
</tbody>
</table>

**Patient 3 Summary**

- ETC Complex III defect

- NADH-linked oxidation
- Succinate oxidation
- Quinol oxidation
- Cyto c oxidation
- Complex I/III and II/III
- Complex III
Conclusion

- BCS1L mutations cause disease phenotypes ranging from highly restricted pili torti and sensorineural hearing loss (the Björnstad syndrome) to profound multisystem organ failure (complex III deficiency and the GRACILE syndrome).
- All BCS1L mutations disrupted the assembly of mitochondrial respirasomes (the basic unit for respiration in human mitochondria), but the clinical expression of the mutations was correlated with the production of reactive oxygen species.
- Mutations that cause the Björnstad syndrome illustrate the exquisite sensitivity of ear and hair tissues to mitochondrial function, particularly to the production of reactive oxygen species.
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What is Blue Native Electrophoresis?

Native PAGE (no SDS) - acrylamide gradient from 3-4% down to 12-20%
Nonionic detergents - stabilize hydrophobic membrane protein complexes
Add Coomassie G-250 - makes all such complexes negatively charged
Complexes "stick" at gel pore size ~ complex diameter

Individual Complexes
Triton X-100, 3 mg / mg protein
Coomassie
G-250
Restain
What is Blue Native Electrophoresis?

Native PAGE (no SDS) - acrylamide gradient from 3.4% down to 12-20%
Nonionic detergents - stabilize hydrophobic membrane protein complexes
Add Coomassie G-250 - makes all such complexes negatively charged
Complexes “stick” at gel pore size ~ complex diameter

Individual Complexes
Triton X-100, 3 mg / mg protein
Coomassie
Restain
Complex I
NDUF8
Rieske
COX4

Supercomplexes
Digitonin, 6 mg / mg protein
G-250
Restain

Complex I
(Complex III)_2
Complex IV
What is Blue Native Electrophoresis?

Native PAGE (no SDS) - acrylamide gradient from 3-4% down to 12-20%
Nonionic detergents - stabilize hydrophobic membrane protein complexes
Add Coomassie G-250 - makes all such complexes negatively charged
Complexes “stick” at gel pore size ~ complex diameter

### Individual Complexes

<table>
<thead>
<tr>
<th>Triton X-100, 3 mg / mg protein</th>
<th>Digitonin, 6 mg / mg protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complex I</td>
<td>Complex I</td>
</tr>
<tr>
<td>Complex II</td>
<td>Complex III</td>
</tr>
<tr>
<td>Complex IV</td>
<td></td>
</tr>
</tbody>
</table>

### Supercomplexes

<table>
<thead>
<tr>
<th>Coomassie G-250 Restain</th>
<th>NDUF88 Rieske COX4</th>
</tr>
</thead>
</table>

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What is Blue Native Electrophoresis?

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Nonionic detergents - stabilize hydrophobic membrane protein complexes
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### Individual Complexes

<table>
<thead>
<tr>
<th>Triton X-100, 3 mg / mg protein</th>
<th>Digitonin, 6 mg / mg protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complex I</td>
<td>Complex I</td>
</tr>
<tr>
<td>Complex II</td>
<td>Complex III</td>
</tr>
<tr>
<td>Complex IV</td>
<td></td>
</tr>
</tbody>
</table>

### Supercomplexes

<table>
<thead>
<tr>
<th>Coomassie G-250 Restain</th>
<th>NDUF88 Rieske COX4</th>
</tr>
</thead>
</table>

---

Complex IV (Complex III)
What are supercomplexes?

(Complex I monomer) + (Complex III dimer) → core S₀ supercomplex
S₀ + 1 to 4 (Complex IV monomer) → "respirasome" supercomplexes S₁ through S₄

S₁ architecture at ~ 4 Angstrom resolution

White = Complex I
Red = (Complex III)₂
Green = Complex IV

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- Summary and Conclusions
Localize Sites of Damage to Electron Transport Chain by Use of Selective Substrates

State 3 respiratory rates
Uncoupled (200 µM dinitrophenol) respiration
ETC complexes in isolated heart mitochondria

ETC supercomplexes in heart IFM
CONCLUSION

In HF the mitochondrial defect lies in the supermolecular assembly of the ETC in functional respirasomes.

We propose that phosphorylation of specific complex IV subunits is involved in disruption of supercomplex assembly of the mitochondrial ETC in HF.
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  Baltimore, MD
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