**Mitochondrial Cytopathies in Children: Pediatric Grand Rounds, Buffalo, 2012**

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- GSK - speaker honoraria 2011.
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**Mitochondrial Disorders – Review.**

- 1.5 billion y ago – purple photosynthetic bacteria.
- Has its own mtDNA (37 genes).
- Most proteins encoded by nDNA - ~ 1,500 total.
- Intermediary oxidative metabolism (ETC).
- Apoptosis.
- ROS production.
- Inflammasome activation.
- Linked to telomere length.

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**Electron Transport Chain**

- Formation ETC under dual genomic control.
- Series circuit I-III-IV and V conserved in vertebrates (13 sub-units).

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**Human mtDNA**

- 16,569 base pairs.
- Encodes for: 22 tRNA, 2 rRNA, 13 polypeptides.
- Maternal inheritance.
- Higher mutation rate (lack of histones).
- 2 – 10 copies/mito.
- Up to 1,000s/cell.

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**Mitochondrial Cyopathies**

- Molecular etiology
  - nuclear defects
  - mtDNA defects
    - Deletions, point mutations
    - Maternal vs sporadic
- Heteroplasmia
- Threshold effect

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**From Zeviani and Di Donato, Brain 127:2153, 2004**
Mitochondrial Dysfunction

Cytochrome b mutation

ATP
Alt. E. Source
ROS (free radicals)
Lactate
Mito proliferation
Apoptosis
Anti-oxidant enzyme

Mitochondrial Disorders

- Usually refers to disorders affecting the ETC.
- First mutations were discovered in the mtDNA as deletions (KSS) and LHON (1778) and MELAS (3243) in 1988.
- There are now > 100 known mtDNA mutations and an increasing number of nDNA mutations.

Mitochondrial Diagnosis

- Multi-system history.
- Family history (maternal – rule IN).
- Lactate (SEN = 0.65; SPEC > 0.90).
- Urine – organic acids (ethylmalonic, 3-methylglutaconic, lactate, TCAi).
- Plasma amino acids (alanine).
- Exercise testing.
- MRI/MRS.
- Muscle histology/EM.
- Muscle enzymology.
- Specific point mutations.

Clinical Phenotypes in Adults

- Mitochondrial Encephalomyopathy
- Lactic Acidosis and Stroke-Like Episodes.
- Chronic Progressive External Ophthalmopathy.
- Kear-Sayre Syndrome.
- Leber’s Hereditary Optic Neuropathy.
Clinical features of mito. in children

- Seizures
- Developmental delay or regression
- ADHD
- FTT
- Recurrent encephalopathy
- Cardiomyopathy
- Hypotonia

DIFFERENTIAL DIAGNOSIS IS HUGE

My general approach in pediatrics.

- History suggestive of mitochondrial disease but no characteristic pattern:
  - Blood (lactate, ammonia, amino acids, LFTs).
  - Urine (organic acids, creatine/creatinine ratio).
- +ve screen or high clinical index:
  - Muscle biopsy.
  - + CNS = L/P (L/P ratio, neurotransmitters and folate) and MRI with MRS.

My general approach.

- Muscle biopsy (+ fibros +/- punch):
  - LM – MGT, COX, SHD, ORO + routine.
  - EM – lipid, pleomorphic, electron densities, PCls.
  - Enzymes (fresh vs frozen – know the norms).
  - Deletions and depletion.
  - Keep a piece for mtDNA.

Muscle Biopsy

Tarnopolsky et. al, Muscle and Nerve, 2011.

Measuring mitochondrial function.


   - Functional assays on isolated mitochondrial fractions (Clark electrode, Oroboros, Seahorse).

3. VO_{2max} (maximal oxygen consumption) ~ human state III respiration.
Mitochondrial Diagnosis – Genetic.

- mtDNA point mutation analysis (MELAS 3243, LHON 11778, MERRF 8344, etc.).
- Deletions (LR-PCR, qPCR, Southern):
  - Single – KSS, CPEO.
  - Multiple – mtDNA maintenance (POLG1, OPA1, twinkle, etc.).
- nDNA mutations (SURF1, SCO2, NDUFV1, POLG1, twinkle, etc.).
- mtDNA (maternal inheritance – non-targetted approach):
  - Sequencing, denaturing HPLC ?

Mitochondrial disorders in children

- N = 400 children referred for mitochondrial disease – N = 113 children with disease according to the Walker criteria (mean age at presentation = 40 mo).
- 32% complex I; 26% combined; 19% COX.
- mtDNA mutations = 11.5%.
- 40% cardiomyopathy (58% hyper, 29% dil and 13% non-compaction).
- 60% primarily neuromuscular.
- Survival =
  - + cardiomyopathy = 18% @ 16 y.
  - - cardiomyopathy = 95% @ 16 y.


Mitochondrial disorders in children

- mtDNA depletion (polg, DGUOK, TK)
- Leigh’s disease I (NDUF), II, IV (SURF-1), PDH, mt8993
- Congenital lactic acidosis
- Cardiomyopathy
- MNGIE syndrome (thymidine phosphorylase, TYMP)
- Alper’s Syndrome (polymerase gamma mutations)
- CoQ10 deficiency (ataxia and myopathy)
- Isolated ataxia (IOSCA (twinkle), MIRAS, CoQ10)
- Isolated complex I deficiency (most common)

Rule In/Rule Out Mimics – Children.

- Complex I deficiency:
  - Dystrophinopathy with skewed X-inactivation.
  - Novel Chr. gain/del. detected by Chr. microarray.
  - Rett syndrome (MECP2 mutation).
  - Nemaline rod myopathy (ACTA1 mutation).
  - P.W.S. (abnormal methylation pattern).
  - Zellweger (4X increase in VLCFFAs – PEX1-P).
  - SCN1A mutation (Dravet’s syndrome).
  - CDG.

Encephalo-hepatopathy

- Often triggered by concurrent viral infection.
- Valproate is a common medication trigger.
- Transaminitis, α-feto-protein.
- Specific causes:
  - DGUOK, POLG1, MPV17, SCO1, BCS1L
  - RRM2B, SUCLG1, C10ORF (twinkle).

Human disorders due to polymerase gamma mutations

- ~ 200 mutations known in catalytic, linker and proof reading regions.
- Alper’s syndrome (AR) – psycho-motor regression, hepatopathy, encephalopathy.
- AR spino-cerebellar ataxia and neuropathy – onset in middle age.
- AD CPEO – later onset CPEO +/- depression.
- AR SANDO – sensory neuropathy, ataxia, PEO, dementia.
mtDNA depletion/+/- deletions

- POLG1 (MIRAS, Alper’s)
- DGUOK (hepatic)
- TK2 (encephalo-myo-pathic)
- C10ORF (twinkle) (encephalopathic, IOSCA)
- SLC25A4 (encephalomyopathy)
- MPV17 (hepatic involvement).
- SULCA2, SULCG1 (encephalo-hepato-myopathy)
- RRM2B (encephalomyopathy/myopathy)

Coenzyme Q10 Deficiency

- Suspect if I + III and II + III are low.
- Defect biosynthesis – COQ2, PDSS1, aprataxin.
- Myopathic-plus form: myoglobinuria, encephalopathy, RRR and lipid storage myopathy
- Myopathic only form: RRF + lipid myopathy.
- Cerebellar forms: pyramidal, neuropathy, +/- Sz.
- Rx: high doses of CoQ10 (25 mg/kg/d).

Case 1 - ? SCA

- 42 y old female.
- Dx: AD-SCA?.
- Phenotype: progressive ataxia, dysarthria, sensory neuropathy + mild pes cavus, mild cerebellar atrophy on MRI.
- SCA 1,2,3,6,7,8,17, FA – ve - lactate normal.
- Asked to see her daughter and grandson (age 2) – both with severe ataxia and global developmental delays.

mtDNA Sequencing

- C9035T (Leu → Pro, ATPase 6): L is conserved in all mammals, birds, snake, yeast.
- Not found in over 100 controls.
- Screened > 50 sporadic ataxias.
- Cybrids: ATP (50 %); ROS is 7 fold higher; rescued by COQ10 and vitamin E.


Case 2 - ? Fatty acid oxidation defect

- 6 y old male – admitted to hospital with 2 day history of “groggy and sleepy” – vomiting.
- CK = 1,500 iU (N < 220).
- Rx – rehydration with glucose and saline.
- Better in 3 days and CK was 500 iU.
- Development = walk = 12 months; BUT poor endurance (sore stomach, SOB), not jump.
? Fatty acid oxidation defect

- Fhx: mom and dad – N; large family no NMD but a remote great aunt on dad’s side = DM1; younger brother doing “much better” and almost same weight @ 2 y of age.
- Pmhx: strabismus surgery @ 3 y of age.
- O/E: MS = N, irritable, CN = N (epicanthal folds), M = mild hypotonia, generalized proximal weakness, Sensory/MSR = N; left toe upgoing, skin = N.

? Mitochondrial disease

- Labs: CK – returned to normal, lactate normal X 3, organic acids= N (ketones), acyl-carnitine = N, total carnitine = low (free), ammonia, LFTs, renal all N.
- Muscle Biopsy: borderline RRF, EM = pleomorphic, complex I + III (< 30 % of LLN).
- Diagnosis: Possible/probable mitochondrial disease.
- F/U: MRI normal age 9 y, ADHD Dx, WPW age 13 y, lactate = 7.8 mmol/L.

? Mitochondrial disease

- Repeat Biopsy (age 13 y): 20 % RRF (COX +ve), many paracrystalline inclusions, complex I + III ~ 40 % of LLN; deletion analysis = N.
- mtDNA analysis (dHPLC + SEQ.):
  - blood = few polymorphisms; muscle = heteroplasmic 15161 T>C in cytochrome b (highly conserved); mom = negative by Seq. and normal histology and ETC.

Mitochondrial Dysfunction

- Cytochrome b mutation
- ATP
- Alt. E. Source
- ROS (free radicals)
- Lactate
- Mito proliferation
- Apoptosis
- Anti-oxidant enzyme

Mitochondrial Disease Rx Strategies

**General Issues**
- Avoid stressors (heat, dehydration, prolonged fasting, excessive exercise, VPA, statins, etc.).
- Check sleep (abnormal delta waves, apnea, no stage 3 or 4, nocturnal myoclonus, Sz, RLS) – melatonin (0.1 mg/kg/d).
- Check for contractures, treat spasticity.
- Optimize nutrition for growth – G-tube is often very beneficial if sub-optimal intake is present (also allows for optimal delivery of supplements and medications).

**Specific**
- High cholesterol = Fibric acid (PPARα agonist) – not statins (deplete CoQ10).
- Cochlear implants – hearing loss.
- MNGIE (allogenic BMT (reduce thymidine)).
- CPEO – blepharoplasty.
- L-arginine – hypocitrullinaemia and hypoargininemia in MELAS (30 g in early phase, Neurology, 2005).
## Mitochondrial Disease Rx Strategies

<table>
<thead>
<tr>
<th>Strategy</th>
<th>Formula/Compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bypass Defect</td>
<td>CoQ10, succinate, riboflavin.</td>
</tr>
<tr>
<td>Reduce Lactate</td>
<td>Dichloroacetate, thiamine.</td>
</tr>
<tr>
<td>Anti-Oxidants</td>
<td>Vit E, lipoic acid</td>
</tr>
<tr>
<td>Alternative Energy</td>
<td>Creatine monohydrate</td>
</tr>
<tr>
<td>Exercise training</td>
<td>Aerobic vs strength</td>
</tr>
<tr>
<td>Vasodilatation</td>
<td>L-arginine</td>
</tr>
<tr>
<td>Folate deficiency</td>
<td>Folate, folic acid</td>
</tr>
<tr>
<td>Nucleotide precursors</td>
<td>Triacetyluridine</td>
</tr>
</tbody>
</table>

## Creatine

- **glycine**
- **arginine**
- **methionine**

\[ C \quad H_2N \quad C \quad NH_2 \]
\[ H_3C \quad N \quad CH_2 \quad COO^- \]

**Creatine in the body:**

- **Exogenous consumption:** (~ 1 g/day)
- **Skeletal Muscle (~ 90% of Creatine)**
- **Creatinine**

**Creatine in Mitochondrial Disorders.**

<table>
<thead>
<tr>
<th>Benefit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat-free mass</td>
</tr>
<tr>
<td>Strength/Power</td>
</tr>
<tr>
<td>Neuro-toxicity (ALS, HD, PD)</td>
</tr>
<tr>
<td>Anti-oxidant (direct and indirect)</td>
</tr>
<tr>
<td>Anoxia protection</td>
</tr>
<tr>
<td>Mitochondrial function (mdx, ? humans)</td>
</tr>
<tr>
<td>Apoptosis/ΔΨm (traumatic brain injury)</td>
</tr>
</tbody>
</table>

**Creatine in Mitochondrial Disorders.**

- N = 7, RCT, cross-over,
- CrM 10g/d X 2 week and 4 d/d X 1 week:
- Handgrip and dorsi-flexion power.
- VO2max.


**Not performing well?**

- 26 y male triathlete.
- Study volunteer.
- EM for lipids.
- Surprised to find paracrystalline inclusions in muscle.
- Discovered a novel cytb “mutation” (G15497A).

**Tarnopolsky MA, et al, Muscle Nerve, 2004.**
Is the sequence variant pathogenic?

Cybrid generation:
- Expose to stressors:
  - Oxygen and glucose (OGD).
  - SIN1 – peroxynitrite donor.
- ? Protection from Rx?

1. Deplete mito. in immortalized cell (EB).
2. Enucleate the patient’s + con. cells (centrifuge).
3. Fuse cells with PEG.

Mitochondrial Cocktail


- 2 month RCT, 2 month W/O, cross-over: CoQ10 120 mg bid + 150 mg Vit E + creatine 0.1 g/kg/d + LA 300 mg bid in 16 patients with definite mitochondrial disease.

CoQ10 (mg/L), P < 0.001
8-OH-2dG (ng/g creatinine), P = 0.065

Mitochondrial Cocktail


Lactate (mmol/L), P < 0.05
8-isoprostanes (umol/g creatinine), P < 0.05

Putting an END to AGING...

3 Groups of POLG (3 months > 8 months):

Safdar et al., PNAS, 2011.
Exercise attenuates many aging features. Safdar et al., PNAS, 2011.

Endurance Exercise Promotes Systemic Mitochondrial COX Activity

P < 0.05

Safdar et al., PNAS, 2011.

Endurance Exercise Restores Skin Ultra-Structure

Safdar et al., PNAS, 2011.

Endurance Exercise Lowers Inflammation and Restores Growth Factors

P < 0.05

Abadi and Safdar et al., unpublished

Thanks

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