Mitochondrial Disorders
The New Frontier

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What Are Mitochondria?
- Subcellular organelles
- 1 micron in length
  - Cigar shaped (in vitro)
  - Complex structure in vivo
- Comprised of outer membrane and a heavily infolded inner membrane
- Made from ~1500 structural and enzymatic proteins
- Most of these proteins are encoded by nDNA
- 13 proteins of the ETC are encoded by mtDNA, resides in the mitochondria
  - ~16.5kB
  - 37 genes
    - 2 rRNA
    - 22 tRNA
    - 13 structural proteins of the ETC

What do mitochondria do?
1. Generate ATP
2. Critical component of apoptosis
3. Generate free radicals
4. Serves as a genetic barometer that allows immediate intracellular changes (seconds, minutes, days) and quick evolutionary changes (~1000s of years)
5. Roles in most neurodegenerative diseases and some cancers

Energy is stored in covalent bonds

Goals
- Mitochondria convert food into cellular energy
- Mitochondria are comprised of gene products from two different DNAs
- Mutations in either mtDNA or nDNA can cause disease
- Pearls to Disease Identification
- Tips to Diagnosis
- Treatment Options

H₂ + CH₃CH₂CH₂CH₂CH₃ → CH₃CH₂CH₂CH₂CH₂CH₂CH₃

Energy is stored in covalent bonds
**Vitamins and Cofactors**
- CoQ10
- Lipoic Acid
- B1, B2, B3, B5, Folate
- Fe/S Core, Cu Core

**Step 1 - Glycolysis**
- 6 carbon sugar
- 9 metabolic steps, high kinetic flux
- generates two 3-carbon molecules (pyruvate)
- 2 net molecules of ATP
- no oxygen required

**Step 2 - Citric Acid Cycle**
- 1 carbon removed by PDH (CO2)
- the 2 carbons left are "activated" to acetyl-CoA & condensed onto a 4 carbon molecule (oxaloacetate) to make citrate
- 2 carbons removed (CO2)
- 3 NADH’s generated and an FADH2 is generated
- last product is oxaloacetate

**Step 3 - Electron Transport Chain**
1. Embedded in the IMM
2. 5 enzyme complexes
   - Complex I accepts NADH
   - Complex II accepts FADH2
3. Electrons flow to complex III via CoQ10 and to complex IV through cytochrome c
4. complex IV reduces oxygen to water
5. As electrons flow, H+ are pumped into the inner membrane space
6. Protons flow thru channel in complex V and ATP is formed

Under normal conditions, 2% of the electrons leak from the ETC to reduce oxygen to the superoxide that triggers a cascade of free radical formation that indiscriminately damages macromolecules: lipids, mtDNA

**Mitochondrial Genetics 101**
- The mitochondria contain its own DNA
  - Small ~ 16,569 base pairs
  - Compact, no introns
  - 2-10 copies per mitochondria
  - Not all base pair substitutions are pathogenic
  - maternally inherited
  - mutations, when they occur, generally appear in < 100% of all mtDNA (heteroplasmy); health of mitochondria is based on percent of wild type mtDNA (population genetics with each mitochondria)
  - Critical regions of mtDNA are Evolutionary conserved
  - mtDNA contains the coding sequence for its own rRNA, tRNA and a fraction (25%) of the genes that encode the protein of the ET
- Most of the mitochondria structure is encoded by the nDNA; only 13 of ~ 1500 mitochondrial proteins are encoded by mtDNA
  - All the chaperone proteins and assembly proteins are encoded by nDNA
  - The polymerase required for mtDNA replication is encoded by nDNA (15q25)
Mitochondrial Genetics

What does the nDNA contribute?

- The other ~1500 genes are scattered across the autosomes
- Unlike mtDNA, nDNA replicates along with cell replication
- Encode for the polymerase apparatus of the mtDNA
- Encode for all the chaperone proteins of the mtDNA
- Encode for all the assembly proteins of the mtDNA
- Encodes for most of the ETC proteins
- Encodes for the matrix enzymes and the rest of the mitochondrial structure

Mitochondrial Replication

- In both cells replicating and non-replicating, the mitochondrial (and the mtDNA) are undergoing constant replication
- The nDNA only replicates when a cell replicates

... therefore cells that are post-mitotic at birth are at risk for those disorders that require mtDNA replication

Summary

- Mitochondria generate energy in the form of ATP from oxidation of food
- The mitochondria contains about ~1500 structural and enzymatic proteins
- The mitochondria contains a small piece of DNA (mtDNA) that encodes for 13 proteins that make up parts of electron transport chain complexes I, III, IV and V
- Nuclear genes scattered across the autosomes make up
  - the structural proteins of the mitochondria
  - the genes for the mtDNA replication apparatus
  - the chaperone proteins that get the nuclear gene products into the mitochondria
  - the majority of the enzymes of complexes I, II, IV and V and all of complex II
  - all the enzymes of beta oxidation, urea acid cycle and the tricarboxylic acid cycle
- mtDNA is derived solely from the ovum (mother)
- For diseases caused by mutant mtDNA, the severity is a reflection of the specific pathogenicity of the mutation, the % mutant mtDNA and organ distribution of this % mutant mtDNA
- An elegant system exists to assemble the mitochondrial components
- The majority of the mitochondria, and genetic diseases that affect mitochondrial function, are a result of mutations in nuclear genes

What are Mitochondrial Diseases?

- Clinical syndromes and diseases caused by a reduction of ATP production within the mitochondria, as a result from
  - A genetic mutation
  - Toxin or other environmental event that damages the non-genetic structure or function
  - An acquired mutation
- Aging is a mitochondrial syndrome with a 100% mortality rate

Brain: the most common organ affected in children

- Global developmental delays
- ‘cerebral palsy’
- Autism and ASD
- Seizures
- Migraine
- Dementia
- Movement Disorders
- Stroke and stroke-like episodes
- Neuropsychiatric symptoms

4 yr old with ataxic gait, viral infection showing deep gray matter involvement
Leigh Syndrome
Muscle: Skeletal, Cardiac and Smooth

- Hypotonia
- Weakness*
- Cramping (?)
- Pain*
- Ptosis
- Ophthalmoplegia*
- Intestinal dysmotility
- Cardiomyopathy*
  - also adult onset

Nerve: It takes a lot to be an axon, dendrite, Schwann Cell and Oligodendrocyte

- Cardiac Conduction Defects
- Neuropathic Pain and weakness
- AIDP/CIDP
- Gastrointestinal dysmotility
  - Reflux, pseudo-obstruction
- Fainting
- Abnormal sweating & temperature regulation

Systemic

- Failure to gain weight & "anorexia nervosa"
- Short stature
- Fatigue
- Intermittent air hunger

Other Organ Involvement

- Kidney: RTA
- Eyes: retinitis pigmentosa, optic atrophy
- Ears: sensorineural hearing loss, aminoglycoside sensitivity
- Pancreas: DM and exocrine pancreatic failure
- Liver: hypoglycemia, nonalcoholic steatohepatitis (NASH)

Leigh Syndrome

- Healthy child until age 3
- Non-specific viral infection
- Lost the ability to ambulate due to ataxia, hemiparesis then bilateral hemiparesis
- Lactic acidosis
- Eye movement and bulbar dysfunction
- Dystonia
- Neuropathy
- Some recovery over 6 months followed by deterioration
  - occurs in infancy
  - affects multiple systems
  - muscle weakness
  - ataxia
  - optic atrophy
  - Retinitis pigmentosa
  - Sensorineural hearing loss
  - Sensorineural hearing loss
  - Cardiac conduction defects
  - Sleep apnea
  - Other


Liver Mitochondria of late adolescent with MS picture and dementia

17 yo young man with acute encephalopathy and profound lactic acidosis showing axonal sprouting
Kearns Sayre Syndrome

- Triad
  - high frequency hearing loss
  - cardiac conduction defect
  - PEO
- Other features
  - myopathy
  - diabetes
  - retinopathy
  - dementia and seizures
  - cardiomyopathy
  - dysphagia and weight loss
- 5 kb deletion in all (>97%) of the mtDNA from middle of N5 to ATP8: (4977 base pairs from 8488 to 13460; 13 base pair repeat at mutation break point)

Kearns T, Sayre G (1958). "Retinitis pigmentosa, external ophthalmoplegia, and complete heart block: unusual syndrome with histologic study in one of two cases". A.M.A. Archives of Ophthalmology 60(2): 280-9

Pearson Syndrome

- My index patient
- DOB 6/20/82
- Anemia (Hb 8) and FFT noted at 8 months
- Presented with severe lactic acidosis (8 mmol/l)
- Sideroblastic anemia
- Digestive problems
- Myopathy
- Neuropathy
- Photos and slow PEO
- Progressive hearing loss
- Diabetic retinopathy
- Heart block
- Cardiomyopathy
- Died in 1995
- Definitive diagnosis showing common KSS deletion 1993

Case 3: MELAS

Family History Gives Clues

- 3 year old girl presents in 2003 with WPW, treated with ablation
  - Myoclonic seizures at age 5
  - Loss of cognitive skills
  - Hearing loss
  - Short stature
  - Family Hx: Mother short stature, diabetes and hearing loss and bipolar disorder
- Labs in 2006
  - Lactic acid 4.5 mEq
  - MELAS 3243 mutation
- Course
  - Progressive hearing loss, epalipot and dementia
  - Death in early 2008
MELAS
- Variable Age of Onset (6 months through adult)
  - FTT and ID in infancy, ADD common
  - awareness and forgetfulness
  - Short Stature
  - Strokes and stroke-like episodes
  - Progressive Dementia
  - Hearing loss and DM
  - Anorexia from autonomic gut neuropathy
  - Migraine
  - Myoclonic or tonic-clonic seizures
  - WPW, hypertrophic > dilated cardiomyopathy
  - Ophthalmoplegia
  - RTA
- A3243G tRNALeu (UUR) gene; 1:6135 (Finnish) (80%)
- C3271G tRNALeu (UUR) gene


Hypotonic, ventilator-dependent, g-tube fed; began to improve from 6-30 months of life

Reversible COX Deficiency
Explanation for this tissue specific and developmentally timed process:
1. mtDNA copy number increases in the first year of life (not confirmed in this study)
2. Isoform switching COX VIa and COX VIIa (Tritschler, 1991)

Most profound infantile disorders have a fatal outcome and this disorder may be an exception

homoplasmic m.14674T>C mt-tRNAGlu

Case Report
- 15 year old young woman evaluated for jerky limb movements -- diagnosis was “mannersisms” (1993)
- At age 19, patient has her first seizure (1997)
  - EEG multifocal spikes
  - MRI normal
  - started on valproate
- Three months later
  - florid liver failure
  - undergoes a liver transplant
- During recovery doctors suggest she may have a mitochondrial disorder, preliminary evaluation not revealing

Case Report
- After liver transplant, traveled to Emory for a muscle biopsy and mitochondrial evaluation
  - Biochemical defects identified (complex I)
  - normal muscle histology
  - common mutations not revealing (MERRF, MELAS)
  - complex I mtDNA genes normal
  - started on supplements
- 1998: First CCF evaluation
  - seizures, poorly controlled
  - jerky movements, appeared both like myoclonus or chorea
  - bright, energetic
  - returned to college, working in the radio business
- 2000: No Change
- 2002: No Change
Case Report

- November 2005
  - Symptoms
    - seizures better controlled
    - PEO - scheduled for surgery
    - slurred speech
    - poor night vision
    - hearing loss
    - memory difficulties
    - inability to maintain employment
  - Signs
    - PEO
    - RP
    - Ataxia
    - Hearing Loss
    - Dysarthria
    - Loss of DTRs

Step back 75 years

Alpers Syndrome

- Infantile fatal cerebral disorder that was first described by Bernard Alpers in 1931: “poliodystrophy” — refractory seizures, developmental regression, cortical blindness, age of onset 3-7 years, some with identified developmental disabilities and others normal
- 1975 - study showing mitochondrial ultrastructural changes in Alpers poliodystrophy
- 1976 Huttenlocher described the hepatic features (micronodular necrosis) and familial nature of the disorder; “Huttenlocher variant” or “Alpers-Huttenlocher syndrome”
  - Based on pattern of illness - autosomal recessive
  - many children present with epilepsia partialis continua or status epilepticus
  - progressive neurosensory and spasticity
  - Death 1-20 years into the course, often from liver failure

Alpers Poliodystrophy

- 1992 Bicknese et al describe fatal valproate toxicity in a boy with “Huttenlocher variant of Alpers’ syndrome”
- 1996 Copeland characterizes and clones human the polymerase gamma gene
- 1996 Naviaux reported in abstract form biochemical evidence of mitochondrial dysfunction
- 2001 First mutations described in this gene causing progressive ophthalmoplegia
- 2004 Naviaux et al find several families with Alpers harboring 7 distinct mutations in polymerase gamma (POLG) 4999

and one year later……

Mutations in DNA polymerase γ, POLG

November 2005
n=49
When to Consider POLG?

Child

- Developmental Regression
- Epilepsia Paroxysmal-Status Epilepticus-Refactory Seizures
- Generalized-Child MIB Progression
- Valproate-induced liver toxicity
- Cortical Blindness
- Myoclonus
- Ataxia
- Neuropathy
- Liver Failure

Adult

- PEO
- Myopathy
- Psychiatric illness
- Parkinsonism or EP movement
- Ataxia
- Dysarthria
- Seizures
- DMD
- Ataxia - dystonia
- Neuropathy
- Myoclonus
- Dementia

How Common are these disorders?

Remember gene frequency may vary in different populations...

- Neurofibromatosis type I: 1:3000
- Duchenne Muscular Dystrophy: 1:3000 boys
- Cystic Fibrosis: 1:2700
- Marfan Syndrome: 1:5000
- PKU: 1:10,000

- Mitochondrial
- MELAS A3243G: 1:1000 (gene frequency)
- 1:5000 (disease frequency)
- POLG mutations: 1:10,000
- Total estimated disease frequency of only the two most common genes: mtDNA + POLG = 1:4400

> denovo mutation rates for the 10 most common mtDNA mutations is > 1:1000
> > 1:200 people carry 1 of these 10 pathogenic mtDNA mutation

Pathogenic Mitochondrial DNA Mutations Are Common in the General Population

H. R. Elliott, D. C. Samuels, J. A. Eden, C. L. Relton, and P. F. Chinnery
My Typical Lab Evaluation

**Blood**
- Lactic Acid
- Amino Acids
- Total and Free carnitine with acylcarnitine profile
- B12 level
- Methylmalonic acid
- Ammonia
- CK
- CMP + ? HBA1C
- CBC
- CoQ10 (WBC)
- Free T4 + TSH

**Urine**
- Routine Urine Analysis
- Organic Acids
- Amino Acids
- Carnitines + Acylcarnitine Profile
- Acylglycine
- Guanidoacetate + Creatine
- Purine and Pyrimidines
- Neurotransmitters

Dysmorphic

Cognitive Problems?

Specific mtDNA disorder?

Specific nDNA gene sequence

Initial Evaluation:
May Also Include
- EKG and Echocardiogram
- Retinal Exam
- Audiogram
- MRI and MRTS
- Skin biopsy for EK and Fibrotast Culture: acylcarnitine probe
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Muscle Biopsy
When is it Indicated?
- Myopathy
  - after formal evaluation
  - EMG changes
  - diagnosis not available by genetic testing
    - myoadenylate deaminase deficiency
    - myophosphorylase deficiency
    - CPT2 deficiency
- Elevated CK
- The need for a non-replicative tissue
- After careful consideration for mitochondrial disease

Muscle Biopsy: Informed Consent
1. Microscopy is key
2. For enzymology to be accurate flash freezing is essential
3. Most muscle contains 5 mg of mitochondria per gram of muscle so the enzymatic results are based on a very dilute amount of mitochondria
4. Because of technical limitations, even under the best of circumstances, true rotenone-sensitive complex I activity has a large error bar
5. Many labs have not changed their protocol or refined their controls in decades
6. Immobility from any reason will result in a reduction of mitochondrial function
7. The muscle biopsy is a test, it is not a gold standard

Muscle Biopsy
- Light Microscopy
- Electron Microscopy
- Mitochondrial Studies
  - OXPHOS Polarography
  - Electron Transport Spectrophotometry

Treatment
- Symptomatic treatment of symptoms
  - Pain: Neurontin/Lyrica, Gabitril (spasms), Lamictal and Topamax
  - Seizures: Lamictal, Topamax
  - Depression: ACDs, Psychostimulants, SSRI, Effexor
  - Constipation: Miralax
  - Treat all other medical problems vigorously
- Diet Changes may include
  - Frequent smaller meals (continuous feeding)
  - Lower fat diet
  - Overhydration
  - Calorie neutral diet or even calorie restriction
Rationale for Vitamin and Cofactor Therapy

- Stimulate poorly functioning enzymes in the energy pathways
- Act as antioxidants
- Alternative energy sources
- Improve muscle bulk
- Scavenge free-fatty acids and poisonous organic acids
- Bypass blocked components of the electron transport chain

Goals of Therapy

- **Brain**
  - Reduce seizures
  - Improve attention and concentration
  - Improve intellectual functioning
  - Prevent strokes
  - Improve motor control

- **Muscle**
  - Improve strength
  - Lessen pain
  - Lessen fatigue
  - Reverse cardiomyopathy

- **Liver**
  - Improve synthetic function

- **Nerve**
  - Improve autonomic function
  - Lessen pain
  - Improve nerve conduction (all)
  - GI
  - Improve gastric motility
  - Improve intestinal motility
  - Eyes
  - Prevent further retinopathy or optic atrophy
  - Ears
  - Prevent further hearing loss
  - Systemic
  - Growth: prevention of failure to thrive

Vitamins and Supplements

- **Coenzyme Q10**  $3 for 540 mg
- **Idebenone** $2 for 200 mg
- **L-Arginine** $3 for 990 mg
- **Vitamin B1**
- **Vitamin B2** (pennies)
- **Vitamin B3**
- **Vitamin B5**
- **Vitamin B6**
- **Vitamin B12**
- **Folic Acid**
- **Biotin**
- **Vitamin C**
- **Vitamin K3**
- **Betacarotene**
- **Alpha Lipoic Acid** $1 for 600 mg
- **Succinate**
- **Urinine**
- **L-Arginine** pennies
- **NADH**
- **Ribose**
- **Magnesium**
- **Zinc**
- **Dichloroacetate**
- **Orotate** $1 for 1000 mg
- **Allopurinol**
- **Creatine monohydrate** pennies
- **Folinic Acid (Leucovorin)** 25 mg bid ~ $1000/month

**My Typical Treatment**

CoEnzyme Q10  5 - 20 mg/kg/day ÷ tid
L-Carnitine 30-100 mg/kg/day ÷ tid; 990 mg tid max
Riboflavin 100-600 mg/day qHS
Lipoic Acid  10 mg/kg/day + bid
Creatine Monohydrate 100 mg/kg/day; 5 gms max

**What Makes Sense (to me)**

L-Arginine 300 mg per kg per day
Folinic Acid 5-50 mg a day
Metabolic Medicine
Our Knowledge is Only the Tip of the Iceberg