What are Mitochondria?

- Subcellular, cytoplasmic organelles
- Arose from ancient symbiont ancestor: purple sulfur bacteria that could handle oxygen
- Regulate many cellular functions
  1. Energy production
  2. Calcium homeostasis
  3. Apoptosis
  4. Radical species generation
  5. Radical species scavenging
  6. Steroid biosynthesis
  7. Orchestrate metabolism

\[
\text{TCA CYCLE: } \quad \text{H}^+ + \text{NADH} + \text{H}^+ + \text{FAD} \rightarrow \text{H}^+ + 2 \text{FADH}_2 + 2 \text{NAD}^+ + 2 \text{H}^+ \quad + \quad \text{H}^+ + \frac{1}{2} \text{O}_2 \rightarrow \text{H}_2\text{O}
\]

\[
\text{IMM} \quad \text{Matrix} \quad \text{ADP} + \text{P} \rightarrow \text{ATP}
\]
Mitochondrial Disease: Clinical Features

*Gorman G et al, Nat Rev Dis Primers, 2016*
Mitochondrial Disease:
Rapidly changing molecular understanding


- No common biomarker for mitochondrial disease
- Many genetic causes across 2 genomes
  - Mitochondrial DNA: 37 genes
  - Nuclear DNA: >250 genes
- Collectively affect > 1 in 4,300 people

*McCormick et al, Neurotherapeutics, 2013*
Mitochondrial Disease: Molecular pathways effected by genetic disorders

*Gorman G et al, Nat Rev Dis Primers, 2016*
Mitochondrial disease has >300 causes

- Mutations across 2 genomes cause mitochondrial disease
Next generation sequencing has revolutionized causative mitochondrial disease gene discovery and diagnosis.

Mutation #1:
G>T transversion (p.P308Q)
29 of 63 total reads
(paternally inherited)

Mutation #2:
G>C transversion (p.N251K)
112 of 231 reads
(maternally inherited)
Whole exome sequencing:
Disease diagnosis relies on computer algorithms.

Young girl with Leigh syndrome, chronic lactic acidosis, OXPHOS deficiency. Normal prior testing for 18 individual genes. Only child in otherwise healthy family.

Sequence Variants

Total (SNPs/Indels) 149,953 (130,948/19,005)
Coding 20,828 (20,468/360)
Non-Synonymous 11,179 (10,819/360)
Synonymous 10,936

Gene Candidates

212 genes
8 genes
2 genes
1 gene

Biparental compound heterozygous
MitoCarta
Novel
Predicted pathogenic

Gai X et al, Amer J of Hum Genet, 2013
Using GENESIS to identify a novel mitochondrial disease gene
Mitochondrial Disease Sequence Data Resource: https://mseqdr.org

MSeqDR is a Global Genomics Resource for Mitochondrial Disease

- Improve ability to diagnose “primary” mitochondrial diseases
  - Use knowledge of disease-causing variants to prioritize variants in specific cases
  - Identify additional “rare” cases whose genetic diagnosis may not have been known
  - Useful to both clinical and research efforts in molecular diagnosis of mito disease

- Identify genetic links to “secondary” mitochondrial conditions

- Facilitate research to investigate mechanisms underlying specific genetic causes or biochemical categories of mitochondrial disorders
  - Investigate modifier genes for known mitochondrial disorders or phenotypes
  - Link anonymous sequence data to meaningful clinical and laboratory information

- Increase potential for new treatments targeted to precise disorders
  - Clarify specific nature of individual patients’ diseases
  - Group patients into “similar” classes of rare mitochondrial disorders to facilitate focused clinical trial evaluations
  - Characterize genetic factors that may influence therapeutic response
No proven effective therapies or cures exist for human mitochondrial disease

- Why are there so few proven effective therapies?
  - Individually rare disorders
    - Highly heterogeneous genetic causes & clinical features
  - Exercise has therapeutic value in mitochondrial disease
  - Lack clarity on optimal diet in mitochondrial disease
  - One-size-fits-all empiric "supplement cocktails" theoretically target mitochondrial enzymes and stress with variable use*
    - Increase free CoQ pool (carnitine, pantothenate)
    - Enzyme co-factors (vitamin B1 or B2)
    - Metabolite therapies (arginine, folinic acid, creatine)
    - Enzyme activators (dichloroacetate, >5 years in clinical trial planning)
    - Antioxidants (vitamin C or E, lipoic acid, coenzyme Q)

*Parikh S et al, Curr Treat Options in Neurol, 2009
Mitochondrial Disease Community Partners

**NAMDC / RDCRN**
- Physician-entered registry
- Natural history Studies
- Biorepository

**Medical Centers**
- Clinicians
- Researchers
- Clinical Trials

**Advocacy Groups**
- UMDF
  - Education
  - Support
  - Research Funding
  - Patient registry
- MitoAction

**Government**
- NIH
- FDA
- DOD
- Legislators
- Lobbyists

**Patients/Families**
- Participation
- Philanthropy

**Pharma**
- Clinical Trials
- Drug Development
Few clinical trials have been performed for human mitochondrial disease

• No universal clinical trial design, outcome measure, or biomarker

Critical Path Innovation Meeting, FDA, October 19, 2015:
‘Planning Clinical Treatment Trials in Mitochondrial Disease’

• Current trials now emerging involve common clinical outcomes in genetically-confirmed mitochondrial disease cohorts

ANTI-OXIDANTS:
- Coenzyme Q10 – trial never filled/completed
- Idebenone – approved in Europe in 2015 for LHON
- EPI-743 (Edison) – failed primary outcome in Leigh disease
- RP-103 (Cysteamine bitartrate, Raptor)-phase II, Leigh disease, discontinued

OTHER MECHANISMS:
- Elamipratide (Stealth BioTherapeutics) – phase II, myopathy/EL/LHON
- RT-408 (nrf-2 agonist, Reata) – phase II/III, myopathy/El
- Dichloroacetate (FDA) – Phase III, PDH deficiency observer-reported outcome
Mitochondrial Disease Patient Symptom Frequency

Survey of 290 Mitochondrial Disease Patients*

Top 5 symptoms experienced by mitochondrial disease patients

- Muscle Weakness
- Chronic Fatigue
- Exercise Intolerance
- Gastrointestinal Problems
- Balance Problems

% of 290 patients who do or do not experience each symptom:

*In Preparation, Zolkipli-Cunningham Z et al
Clinical Trial Participation Willingness Among Mitochondrial Disease Patients Stratified by Symptoms

Survey of 290 Mitochondrial Disease Patients*

Symptomatic patients’ willingness to participate in trial by symptom:

- Muscle Weakness
- Exercise Intolerance
- Chronic Fatigue
- Gastrointestinal Problems
- Balance Problems
- Peripheral Neuropathy
- Difficulty Falling or Staying Asleep
- Eye Muscle Problems
- Headache
- Decreased Vision
- Dehydration
- Dysautonomia
- Ptosis
- Speech Problems
- Delayed Milestones
- Mood Disorder
- Learning Disability
- Optic Nerve Problems
- Intellectual Disability
- Sleep Apnea
- Heart Rhythm Problems
- Retinal Problems
- Hearing Loss
- Difficulty Losing Weight
- Heart Muscle Problems
- Hyperlipidemia
- Tinnitus
- Behavioral Problems
- Stroke
- Difficulty Gaining Weight
- Seizures
- Diabetes
- Liver Disease
- Kidney Disease
- Autism Spectrum Behaviors

*In Preparation, Zolkipli-Cunningham Z et al
New model for getting to effective therapies for mitochondrial diseases

**Disease Definition**
- Phenotype + Function
- Biochemical
- Organelle
- Genetic etiology
- Molecular Pathway

**Outcome Prioritization**
- Organ system(s)
- Pathophysiology
- Function
- Biomarker

**Treatment Options**
- Off-purpose FDA drugs
- Medical Foods
- Dietary Supplements
- Vitamins
- New drugs from Pharma

**In Vitro Laboratory**
Drug testing in Mito Disease
- Patients’ cells (Fibroblasts vs Tissue-specific)
- Genetic models of RC disease
- Integrated physiologic endpoints
- Toxicity studies

**Clinical Trials**
Standard of Care
Mitochondrion

TCA CYCLE

ADP + P → ATP

IMM

Matrix

Ribosome

AMPK

mTORC1

S6

Proteotoxic Stress

Lysosome

Ribosome

Proteotoxic Stress
Probucol rescues Coenzyme Q deficient \textit{Pdss2} mice

Control, 7dpf

100 nM Rotenone, 7 dpf

Byrnes et al, *Neurochem Intl*, In press
Emerging therapeutic arsenal for mitochondrial disease

Therapeutically targeting central alterations in the nutrient-sensing signaling network & basic cellular processes regulating proteotoxic stress may offer a personalized way to modify sequelae of OXPHOS dysfunction and improve health outcomes in primary mitochondrial disease.

**SIRT Agonists**
- Nicotinic Acid
- Resveratrol

**mTORC1 Inhibitors**
- Rapamycin
- Probucol

**PPAR Agonists**
- Probucol
- Rosiglitazone
- Fenofibrate

**AMPK Agonists**
- AICAR

**Translation Inhibitors**
- Cycloheximide
- Actinomycin
- Anisomycin

**Autophagy Inhibitors**
- Lithium chloride
- 3-methyladenine

**Nutrients**
- Glucose

**Antioxidants**
- Vitamin E
- N-acetylcysteine
New Paradigm for Rare Disease:

N of 1 Individualized Treatment Trials

Perform multiple individual “N of 1” clinical research trials using experimentally-validated therapies found most effective in each mitochondrial disease patient cell line or animal model study.

Multiple, blinded, cross-over studies conducted in single individuals is a relevant clinical trial approach to consider in mitochondrial disease because:

1. Patients differ from one another
2. Patients’ conditions vary over time
3. Optimal treatment may differ between patients
4. There are too few similar patients to pool for study
EVERY PATIENT IS A TRANSLATIONAL RESEARCH PROJECT

- Most rare diseases have no effective treatment or cure
- Clarify the causes and consequences of peoples’ individual diseases
  - Research mechanisms of 100s of individually distinct genetic diseases within a group
  - Understand overlap with common chronic diseases that may spread research interest
    - Alzheimer's, Parkinson's, ALS, Diabetes, Cancer, etc...
  - Characterize treatments effects in cell and animal models of genetic diseases
- Improve health by precision targeting of rare disease manifestations
  - Train knowledgeable clinicians to diagnose, care for and effectively manage complex patients
  - Validate common scales and biomarkers to diagnose and monitor disease in subgroups
  - Perform natural history trials to understand the spectrum of disease
  - Unite experts to maximize meaningful studies of existing and emerging therapies
  - Partner with patient advocacy groups to develop treatments for prioritized problems
  - Integrate pharma, academia, and government resources to lower barriers and channel limited resources into meaningful studies that broach accurate disease mechanism and develop efficacious treatments for distinct disease sub-groups
  - Consider innovative ‘N of 1’ precision trials tailored to each rare disease patient