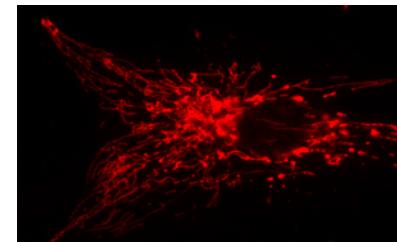
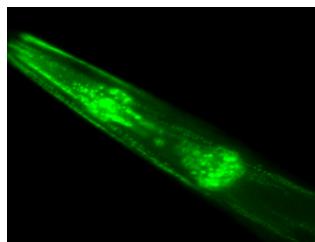
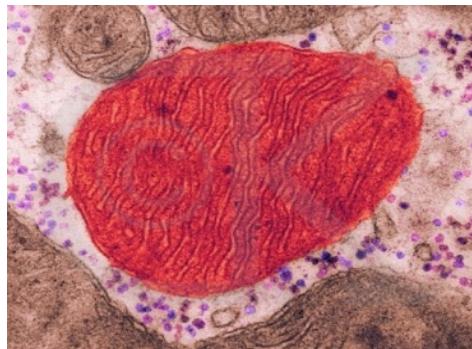




The Children's Hospital *of* Philadelphia®  
RESEARCH INSTITUTE

# Using Genetics in Clinical Trials for Mitochondrial Disease

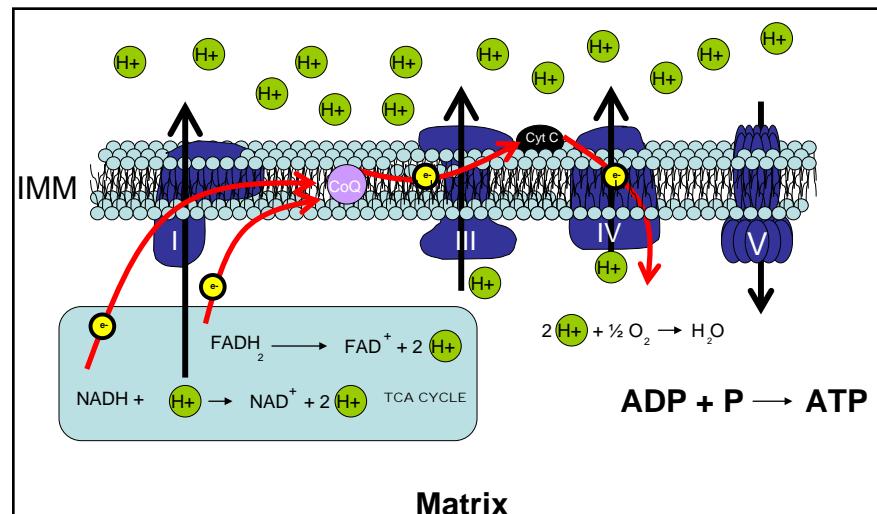


4

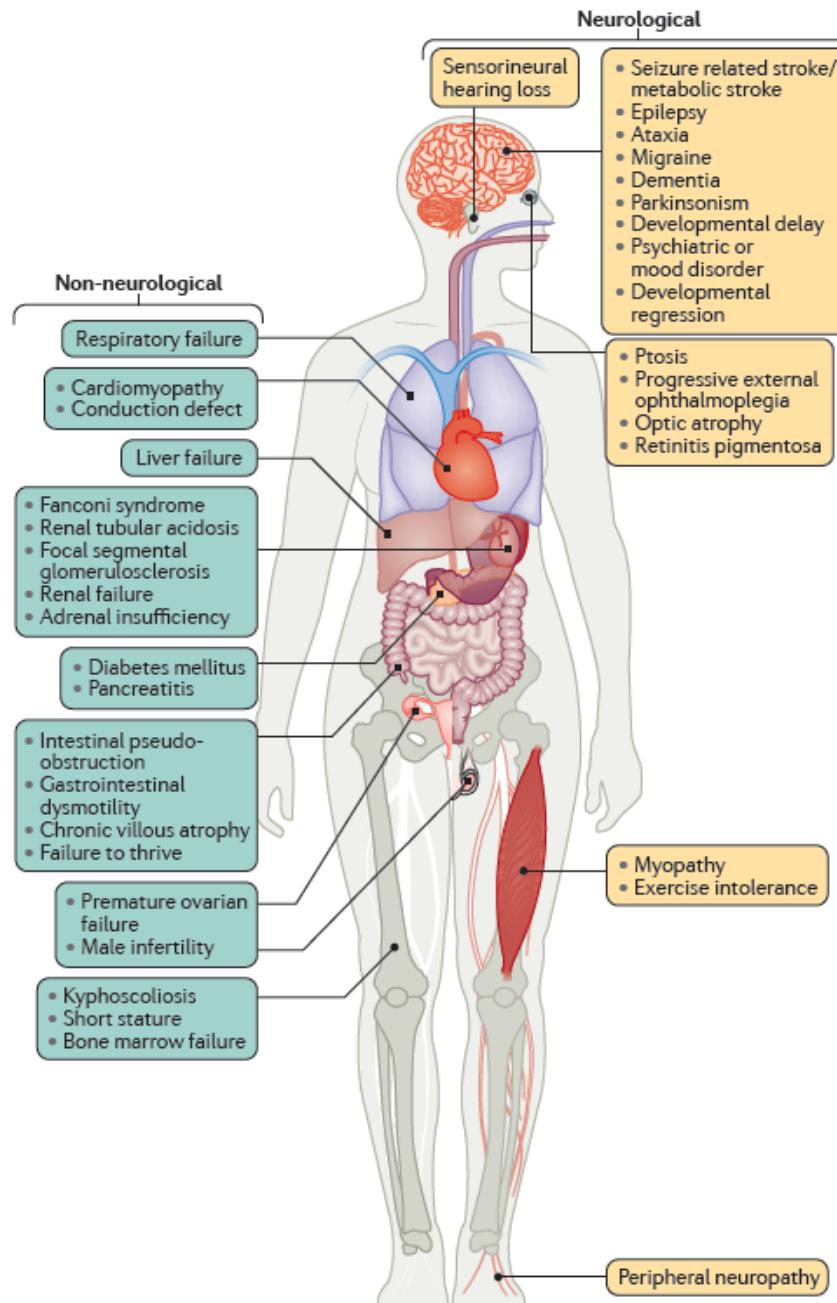
**Marni J. Falk, M.D., FACMG**  
Associate Professor of Pediatrics  
Executive Director, Mitochondrial Medicine Center  
Division of Human Genetics  
The Children's Hospital of Philadelphia  
University of Pennsylvania Perelman School of Medicine  
Philadelphia, Pennsylvania

# 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



# Mitochondrial Disease: Clinical Features



**\*Gorman G et al,  
Nat Rev Dis Primers,  
2016**

# Mitochondrial Disease:

## Rapidly changing molecular understanding

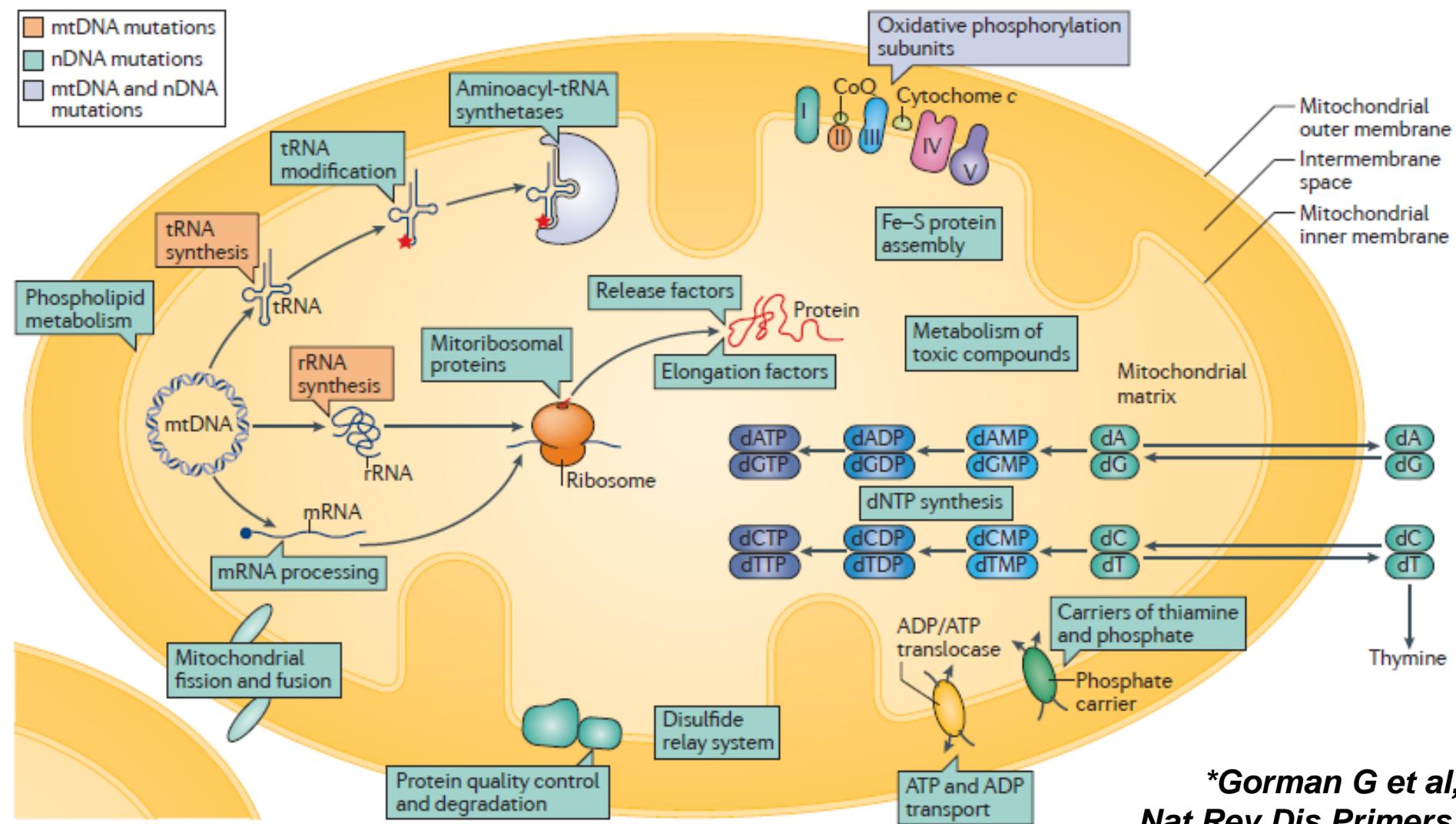
**“Any symptom, any organ, any age, any mode of inheritance”** - - Munnich & Rustin (*Am J Med Genet* 2001,106:4-17)

- 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



# Mitochondrial Disease:

## Molecular pathways effected by genetic disorders

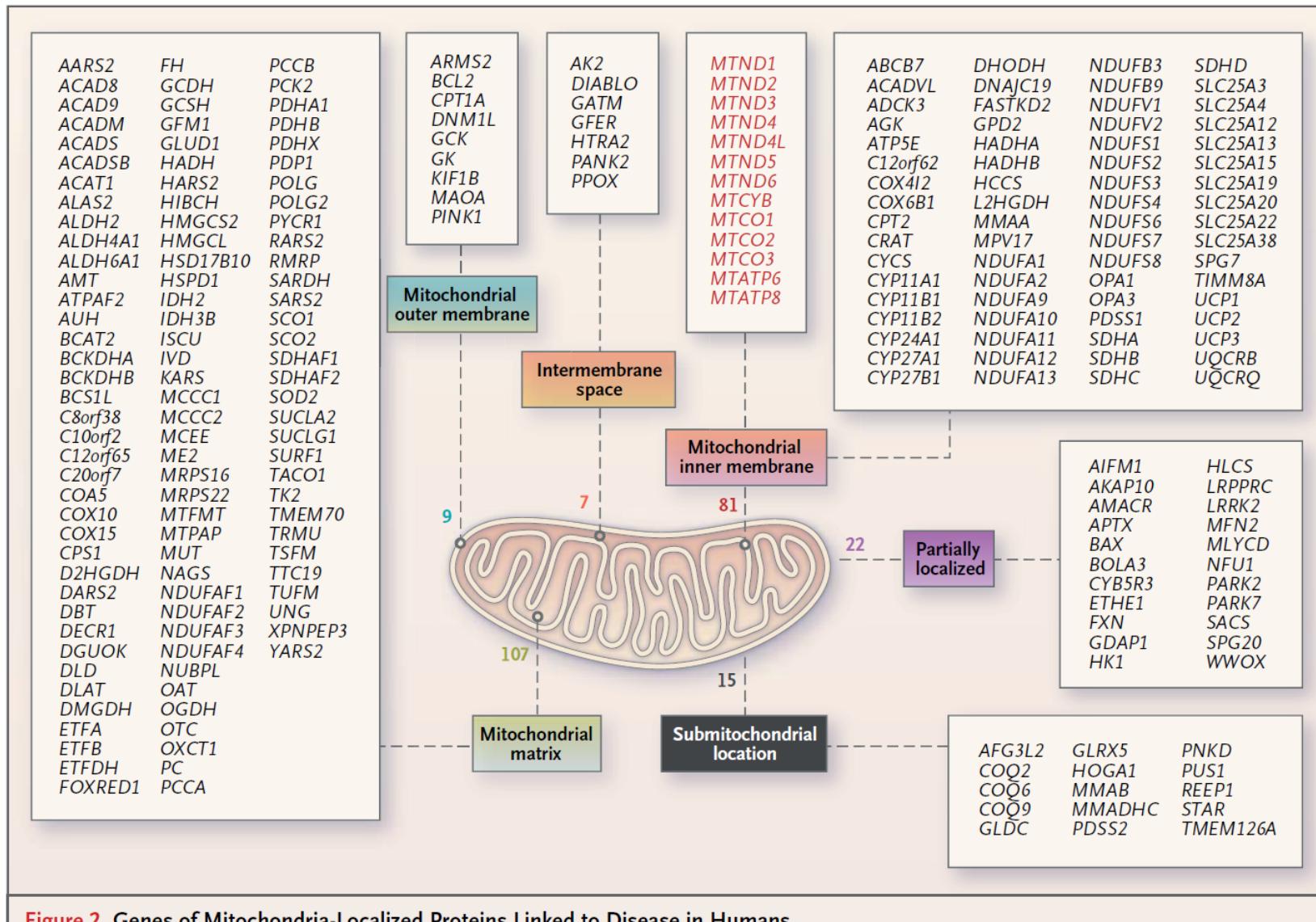


\*Gorman G et al,  
Nat Rev Dis Primers,  
2016



# 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)

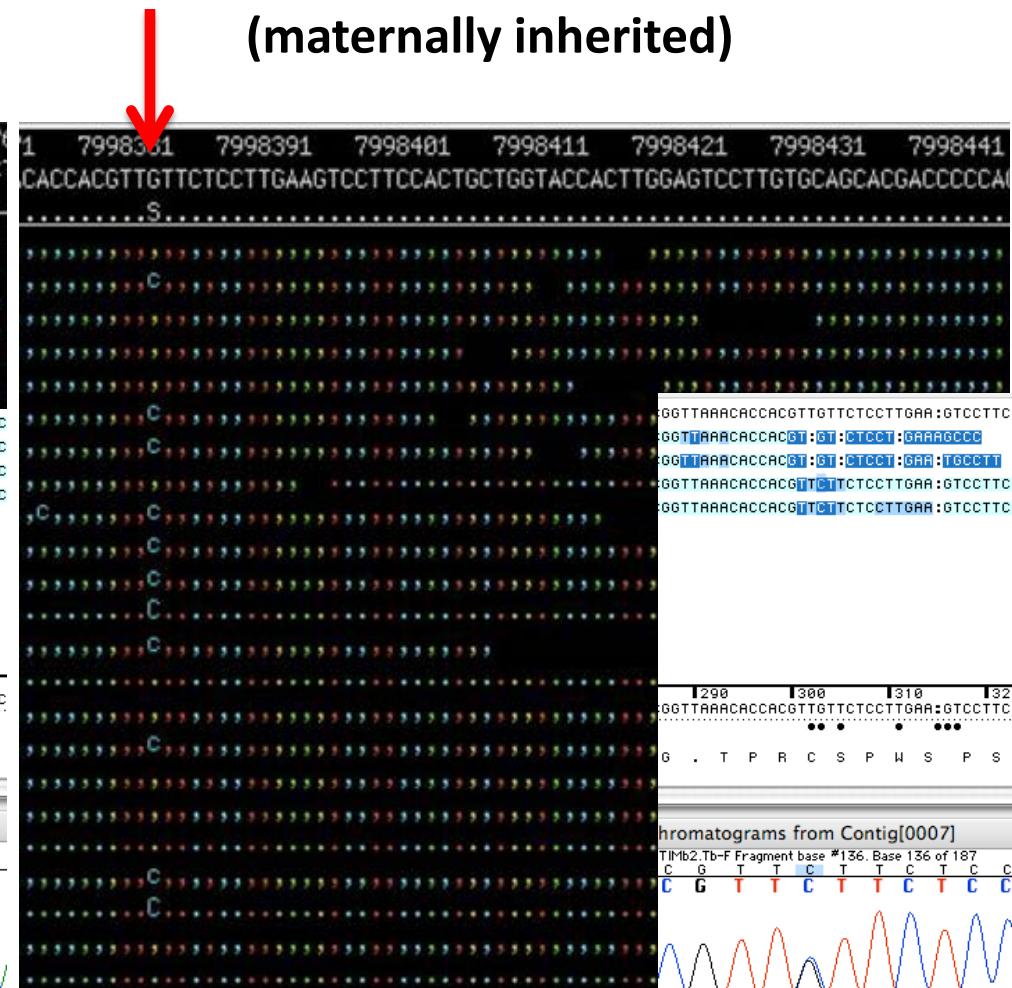


## Mutation #2:

G>C transversion (p.N251K)

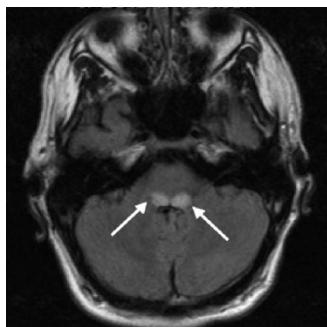
112 of 231 reads

(maternally inherited)

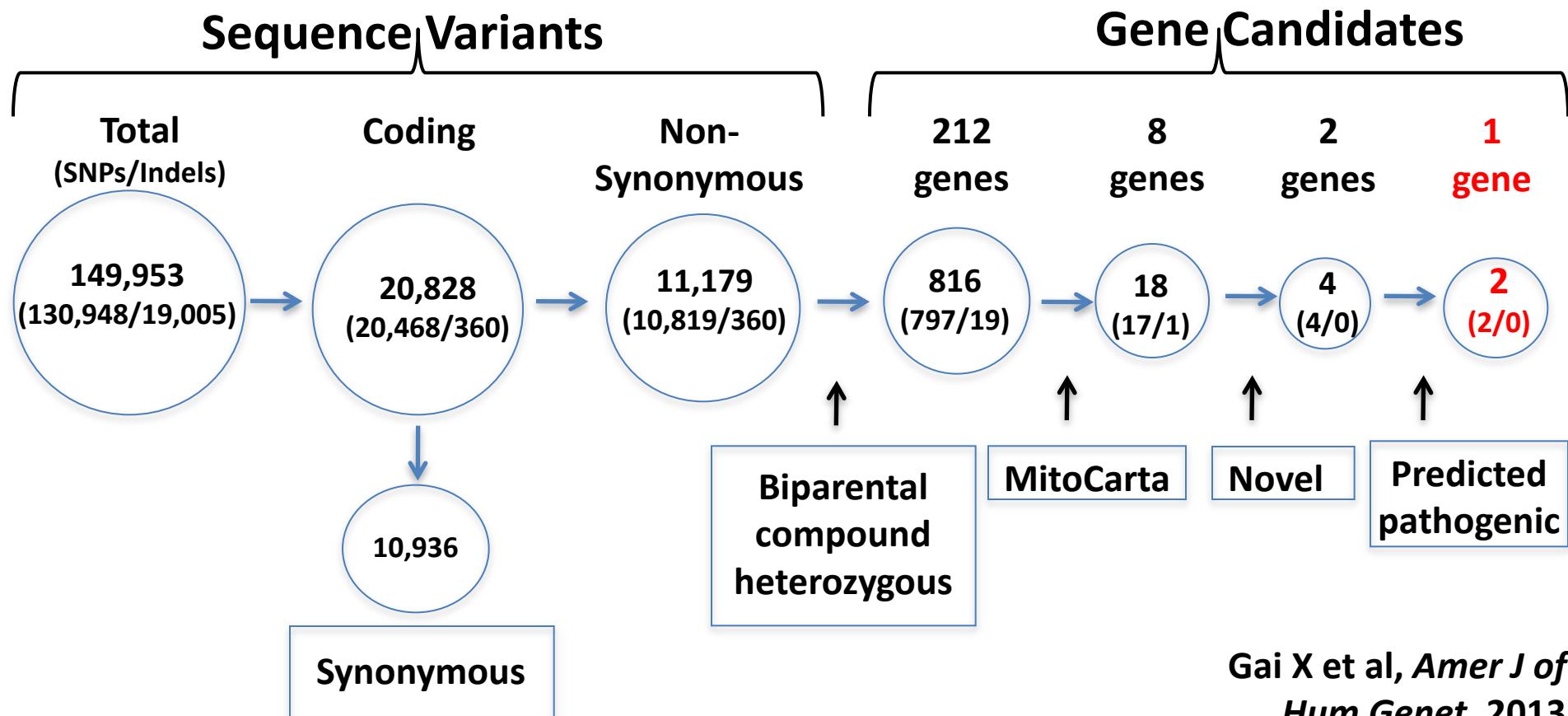


# Whole exome sequencing:

## 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.





# Using GENESIS to identify a novel mitochondrial disease gene

GEM.app - Genomes Management Application  
https://genomics.med.miami.edu/gem-app/main/gem-main.php#tab1

Reset **Query** Help Logout

**Inheritance Model...**  
Select a Mendelian inheritance model. If none is selected all models will be displayed. If analyzing multiple families, you could limit analysis on families with a particular inheritance pattern.  
Inheritance Model: X-Linked Dominant  
Limit Analysis to: All Family Types

**Predefined Filters...**  
Select an Inheritance Model to use these predefined filters.  
 User Defined  
 Relaxed  
 Moderate  
 Strict

**Family Selection...**  
Select single or multiple families for analysis. If none is selected all your families will be displayed. Enter Family ID(s) separated by commas (i.e 25011,76542)  
Family ID: 93117

**Reduced Phenotypic Penetrance...**

**Genomic Position...**

**Gene Selection ...**

**Variant Function Class ...**

Coding-Synonymous  
 Missense  
 Nonsense  
 In-Frame Indels  
 Frameshift Indels  
 Splice Site  
 UTR

Select Synonymous Variant Only

Back **Submit**

GEM.app - Genomes Management Application  
https://genomics.med.miami.edu/gem-app/main/gem-main.php#tab1

Reset **Query** Help Logout

**H.sapiens**

Variants Wit... Query 2 Query 3

Modify Filters Show / Hide Columns Max Column Width Download

GVD-HD	VARIAN...	CHR	POS	DBSNP137 RSID	REF ALL...	LIST GENO...	VARIANT ...	GENE	OMIM GE...	PUBMED LINK	PHENOT...	FAMILY ...	SOLVED ...	SAMPLE...	TOTAL # OF SNV...	TOTAL # OF INDE...	# SAMPLES WITH GENOTYP...
GVD <sup>10</sup>	SNV	X	129149783		T	Y;T;C	missense	N S	BCORL1	300688	mito	93117	522;523;524	194	4	1	
GVD <sup>10</sup>	SNV	X	129149783		T	Y;T;C	missense	N S	BCORL1	300688	mito	93117	522;523;524	194	4	2	



# Mitochondrial Disease Sequence Data Resource:

## <https://mseqdr.org>

About GBrowse MSeqDR-LSDB Tools Phenome GEM.app Submission

Hello! [LSHEN](#) [Log Out](#)



Genomic Search

Enter search term here. Mouse-over for examples.



### MSeqDR: the Mitochondrial Disease Sequence Data Resource Consortium

A global effort, 100+ mitochondrial disease experts.

#### Statistics

Diseases: 177 Genes: 1,363 Variants: 3,658 mtDNA Tracks: 22

Securely [collects](#) and [shares](#) data for [rare diseases](#), patients and causative [mutations](#).

[Tools](#) designed for mitochondrial diseases and mtDNA mutations.

[Read Feedbacks](#) to our [SIMD'15 Tutorial Workshop](#)

Proven rare disease gene discovery platform at [GEM.app](#) with family level data mining.



[Learn more...](#)



MSeqDR is developed and hosted by the MEEI Bioinformatics Center  
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All rights reserved

For [feedbacks](#) about the webpages, contact [MSeqDR webmaster](#)

Falk MJ et al, *Mol Gen Metab*, 2015; Shen L et al, *Hum Mut*, 2016



# 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)



## NAMDC / RDCRN

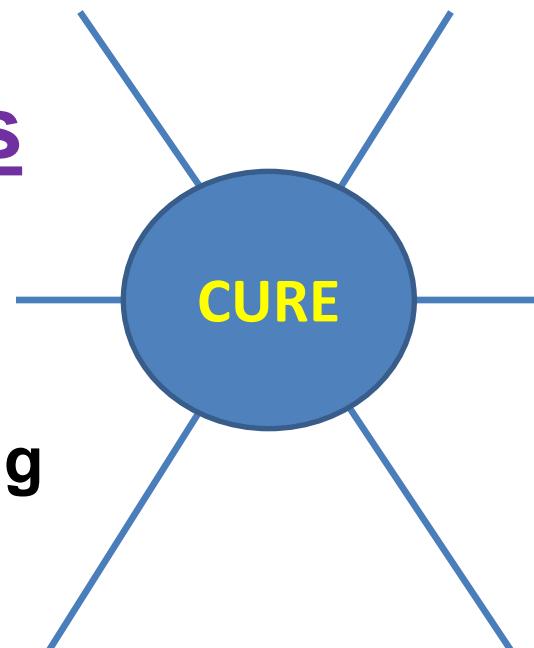
- Physician-entered registry
- Natural history Studies
- Biorepository

## 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

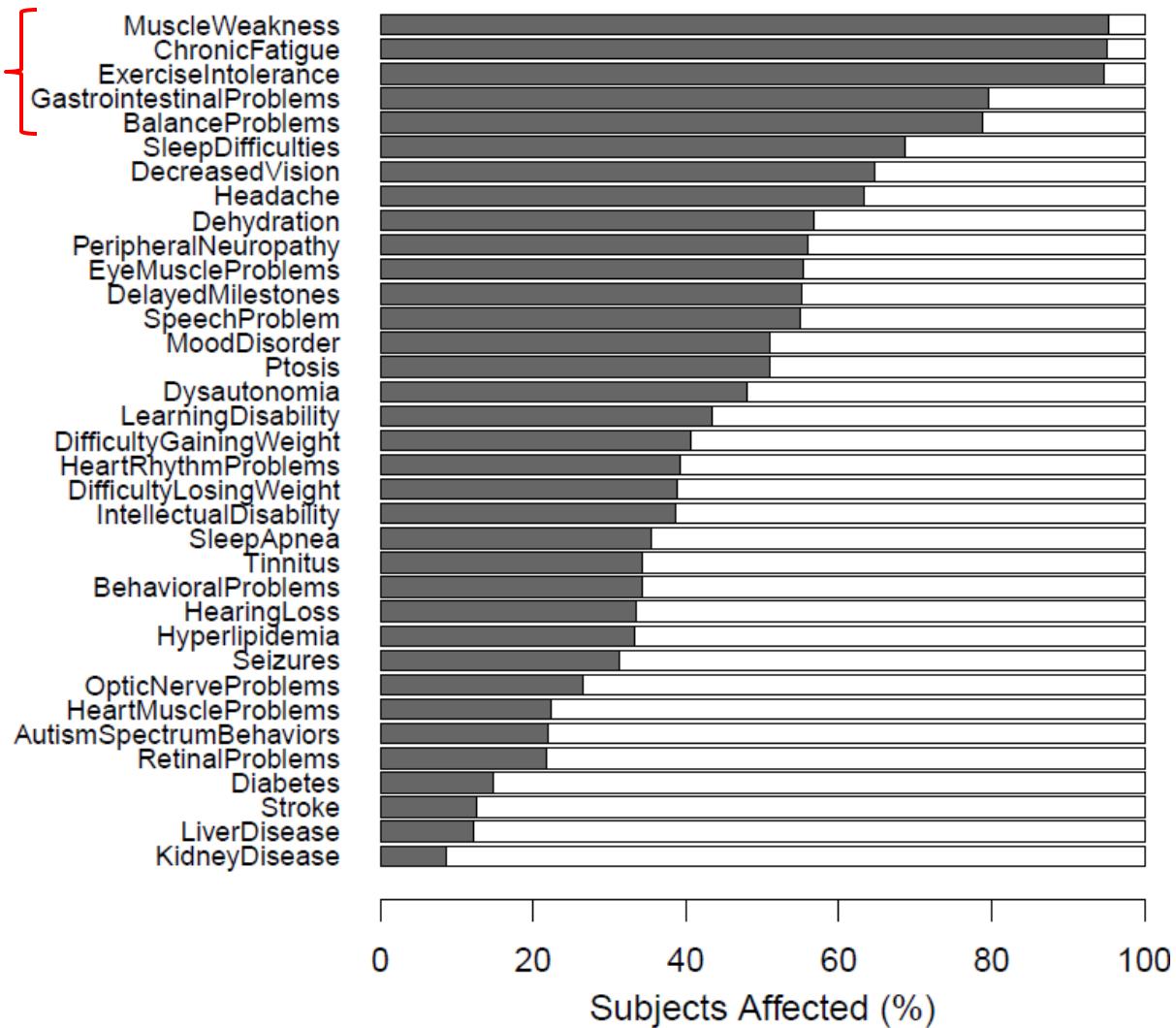
## OTHER MECHANISMS:

- Elamiprotide (Stealth BioTherapeutics) – phase II, myopathy/EI/LHON
- RT-408 (nrf-2 agonist, Reata) – phase II/III, myopathy/EI
- Dichloroacetate (FDA) – Phase III, PDH deficiency observer-reported outcome



# Mitochondrial Disease Patient Symptom Frequency

Top 5 symptoms experienced by mitochondrial disease patients



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

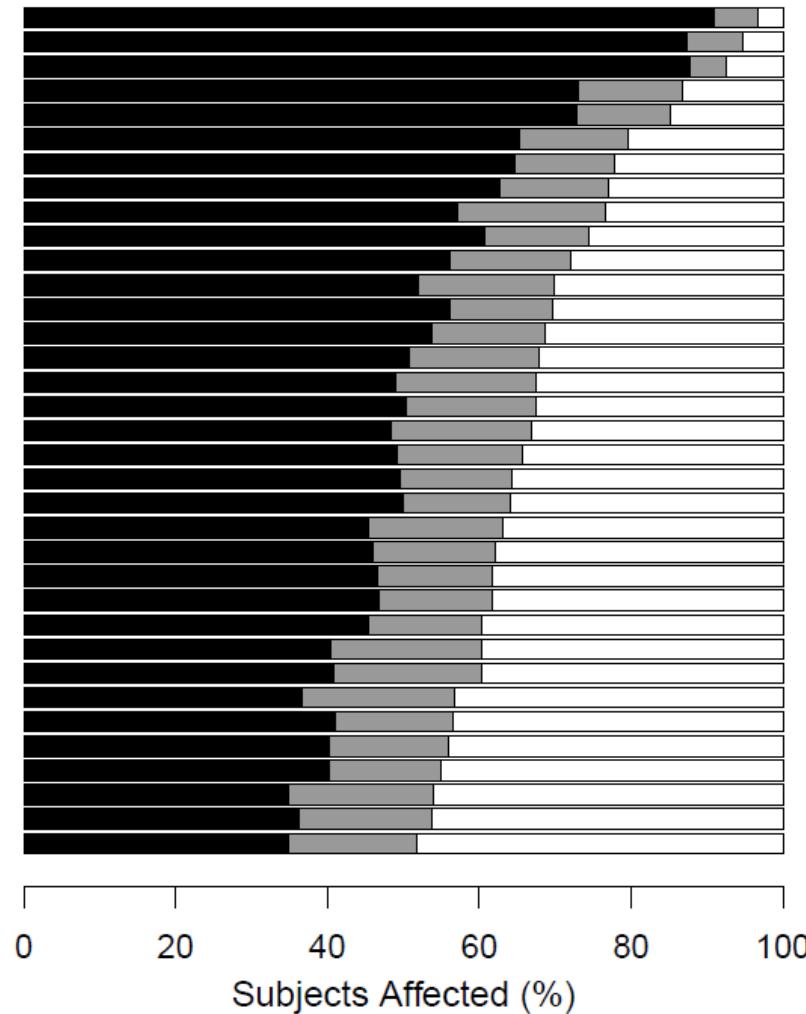
■ Yes □ No

Survey of  
290  
Mitochondrial  
Disease  
Patients\*

# Clinical Trial Participation Willingness Among Mitochondrial Disease Patients Stratified by Symptoms

Top 5 symptoms for which patients would participate in clinical treatment trials

MuscleWeakness  
ExerciseIntolerance  
ChronicFatigue  
GastrointestinalProblems  
BalanceProblems  
PeripheralNeuropathy  
DifficultyFallingOrStayingAsleep  
EyeMuscleProblems  
Headache  
DecreasedVision  
Dehydration  
Dysautonomia  
Ptosis  
SpeechProblem  
DelayedMilestones  
MoodDisorder  
LearningDisability  
OpticNerveProblems  
IntellectualDisability  
SleepApnea  
HeartRhythmProblems  
RetinalProblems  
HearingLoss  
DifficultyLosingWeight  
HeartMuscleProblems  
Hyperlipidemia  
Tinnitus  
BehavioralProblems  
Stroke  
DifficultyGainingWeight  
Seizures  
Diabetes  
LiverDisease  
KidneyDisease  
AutismSpectrumBehaviors



Symptomatic patients' willingness to participate in trial by symptom:

■ Yes   ■ Neutral   □ No

# New model for getting to effective therapies for mitochondrial diseases

## Disease Definition

- Phenotype + Function
- Biochemical
- Organelle
- Genetic etiology
- Molecular Pathway

## Outcome Prioritization

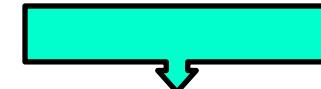
- Organ system(s)
- Pathophysiology
- Function
- Biomarker

## Treatment Options

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



## Clinical Trials



## Standard of Care

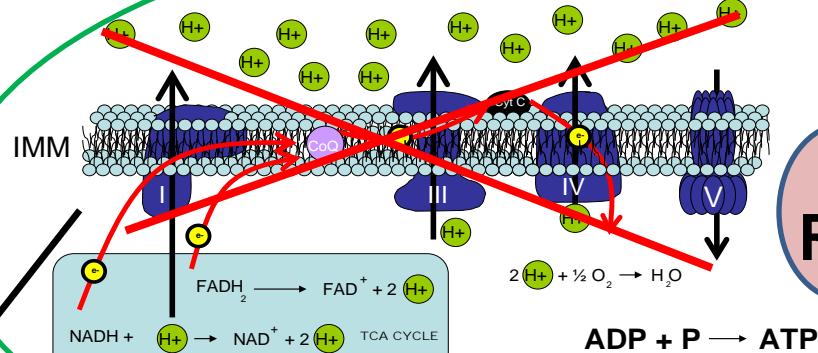
## *In Vitro Laboratory*

## Drug testing in Mito Disease

- Patients' cells (Fibroblasts vs Tissue-specific)
- Genetic models of RC disease
- Integrated physiologic endpoints
- Toxicity studies



# Mitochondrion



AMPK

mTORC1

S6

Ribosome

Proteotoxic  
Stress

Lysosome

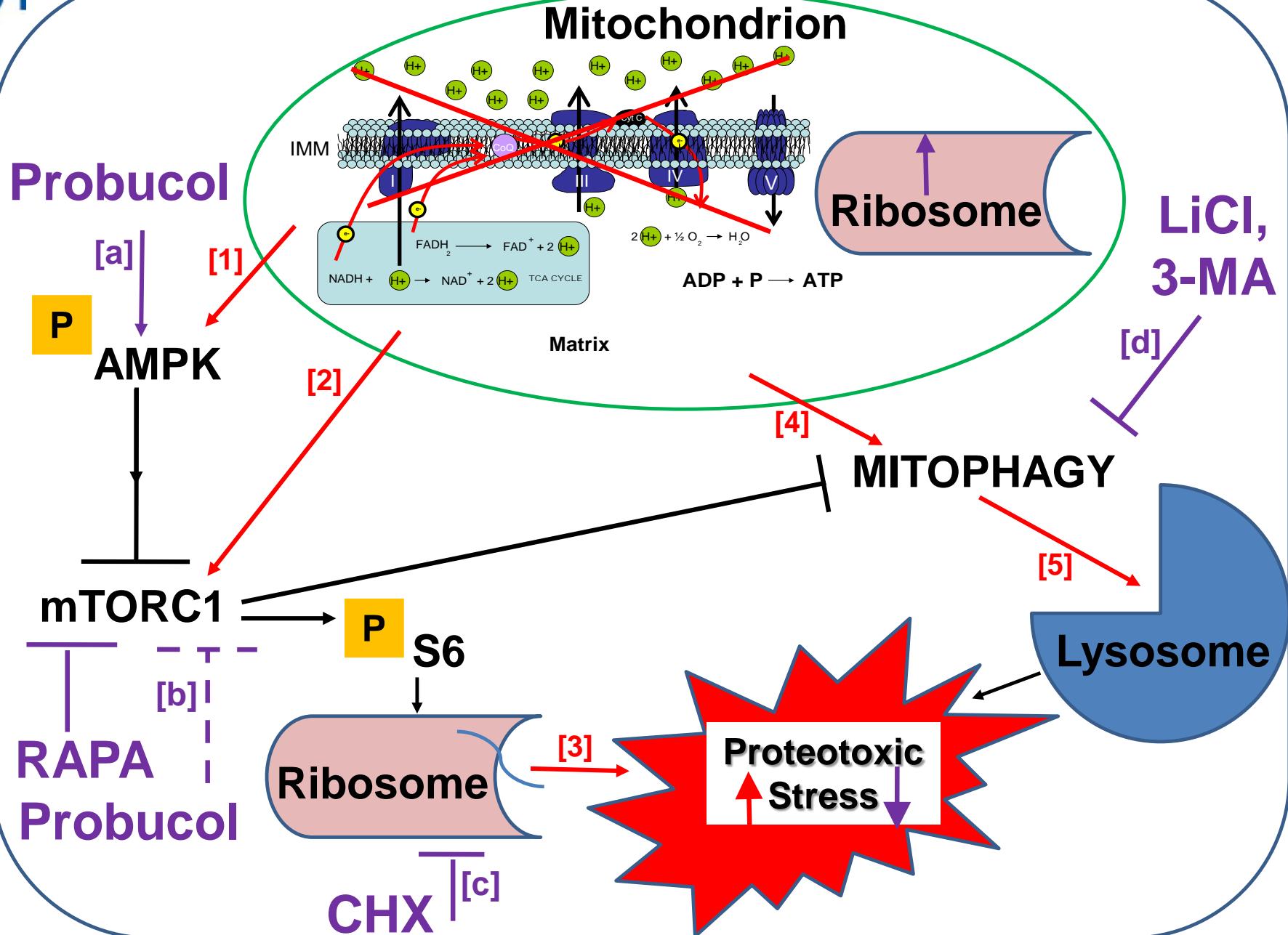
MITOPHAGY

↑ P

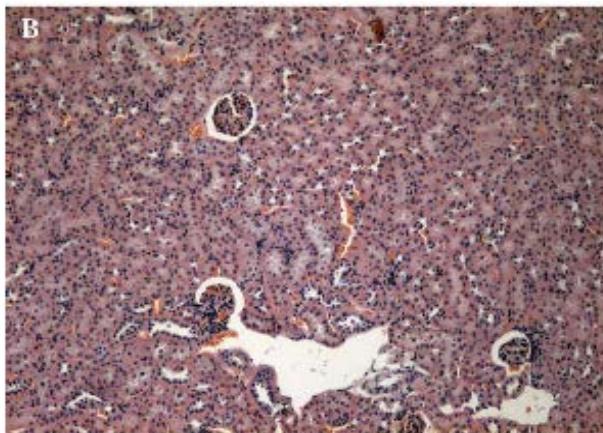
↑ P

↓

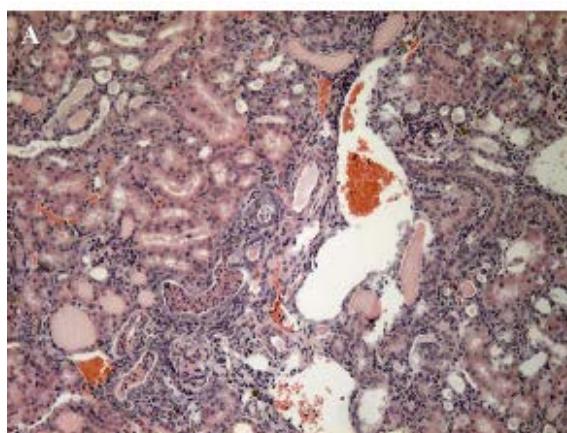
→



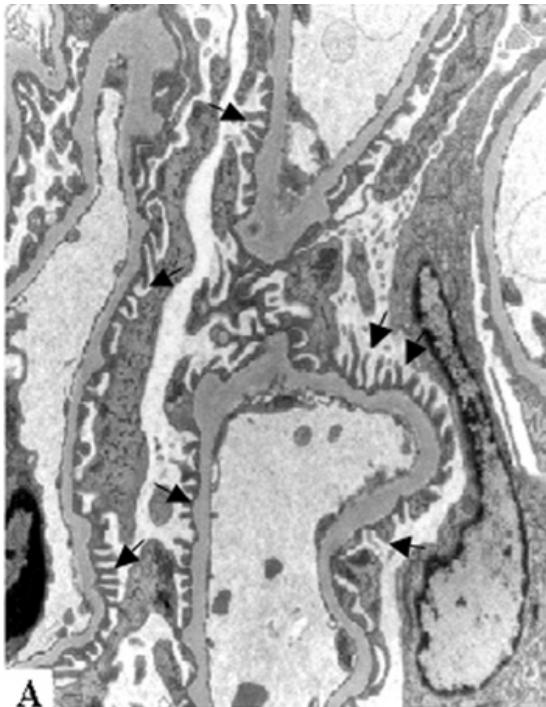
# Probucol rescues Coenzyme Q deficient *Pdss2* mice



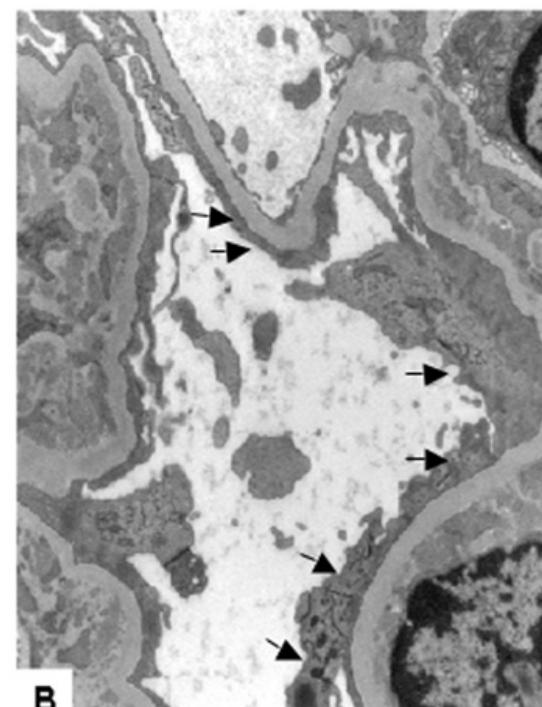
B6 wild-type mice



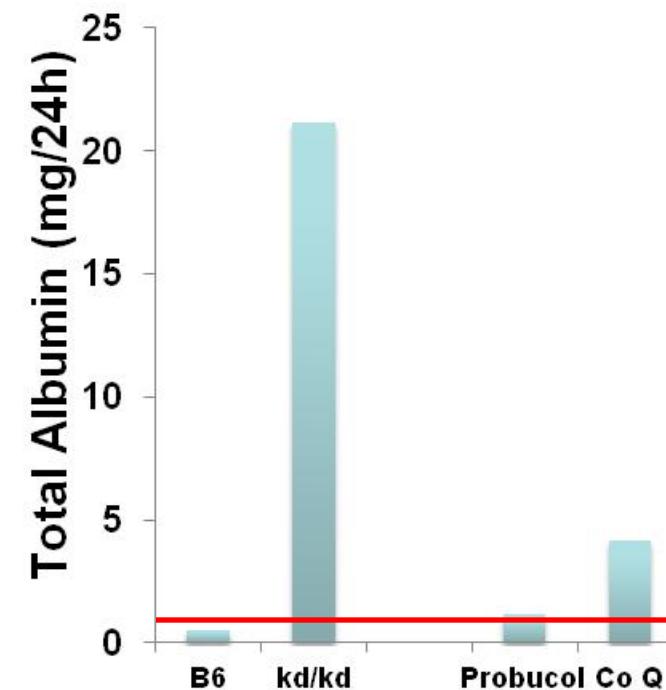
*B6.PDSS2*  $kd/kd$  mutant mice



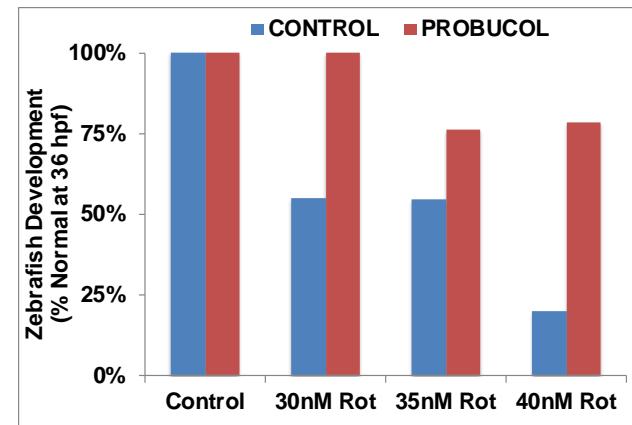
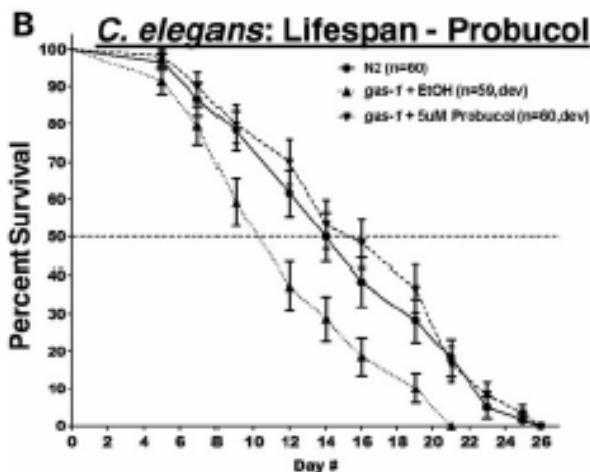
A



B



Falk MJ et al,  
*EMBO Mol Med*, 2011



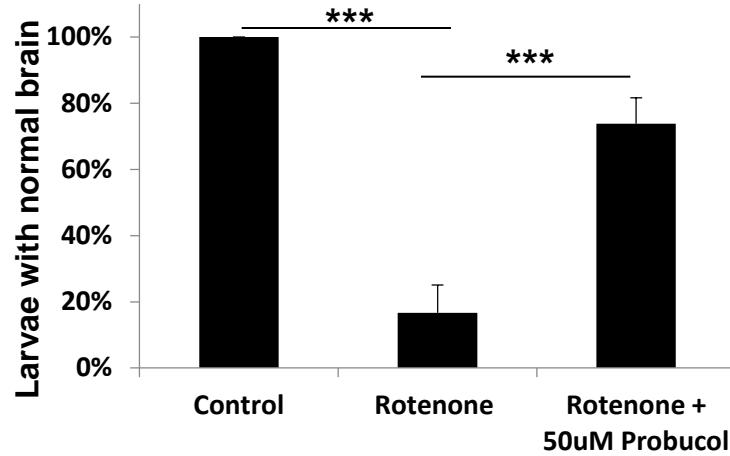
40nM Rotenone  
5uM Probucol



Control, 7dpf



100 nM Rotenone, 7 dpf



Peng M et al, *Hum Mol Gen*, 2015;  
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
- Probuclol

## PPAR Agonists

- Probuclol
- Rosiglitazone
- Fenofibrate

## AMPK Agonists

- AICAR

## Translation Inhibitors

- Cycloheximide
- Actinomycin
- Anisomycin

## Autophagy Inhibitors

- Lithium chloride
- 3-methyladenine

## Nutrients

- Glucose

## Antioxidants

- Vitamin E
- N-acetylcysteine

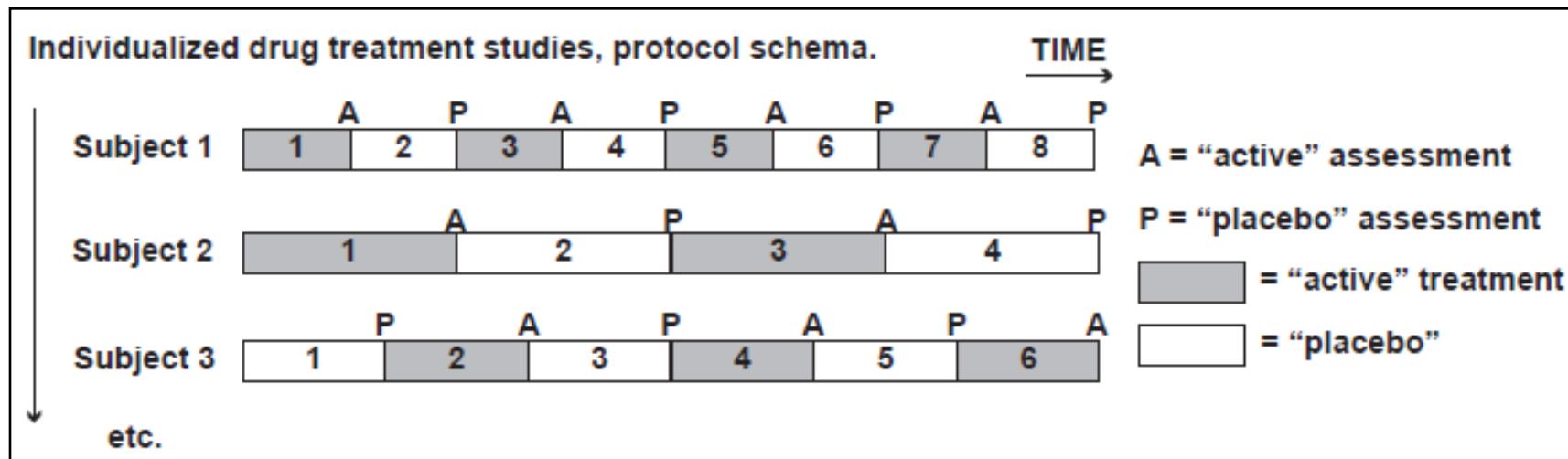
# New Paradigm for Rare Disease:

## 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





# Rare Disease Precision Medicine: Needs and Opportunities

**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
    - Alzheimers, Parkinsons, 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