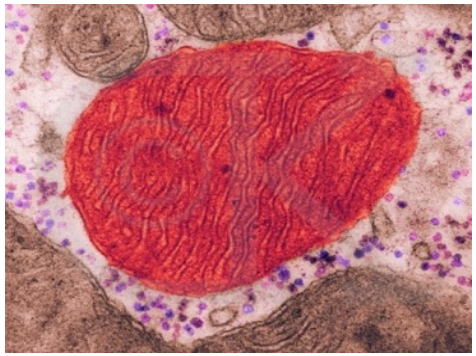
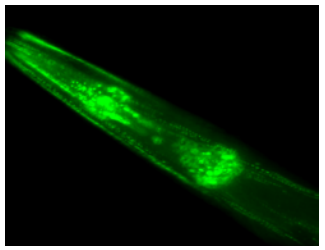
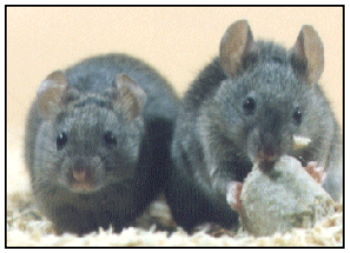
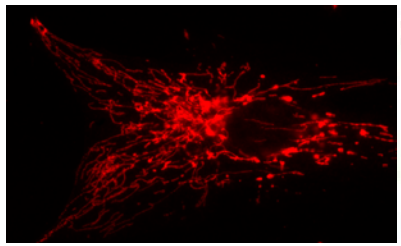
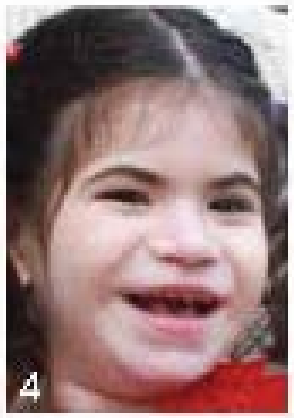
The Children's Hospital of Philadelphia®
RESEARCH INSTITUTE

Using Genetics in Clinical Trials for Mitochondrial Disease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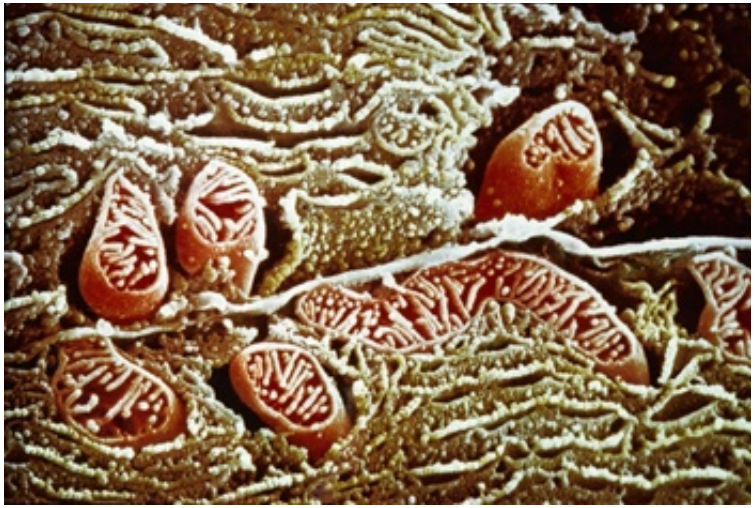
What are Mitochondria?

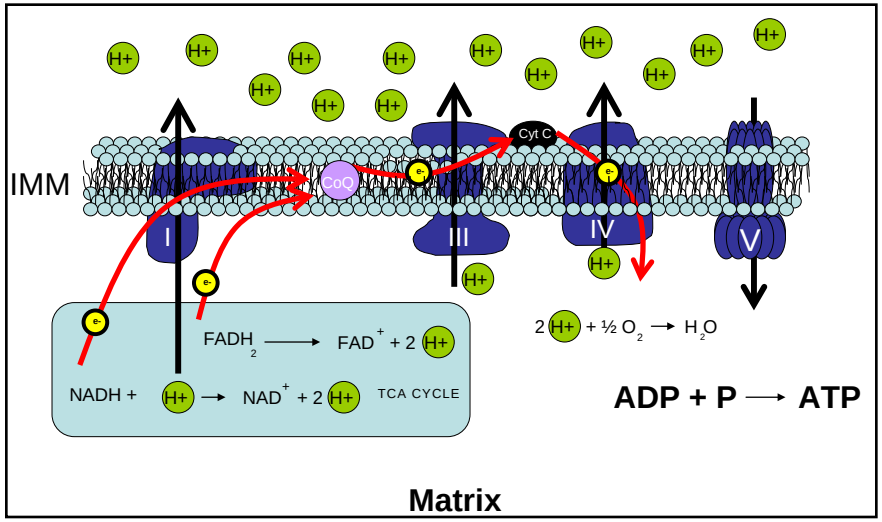
- **Subcellular, cytoplasmic organelles**



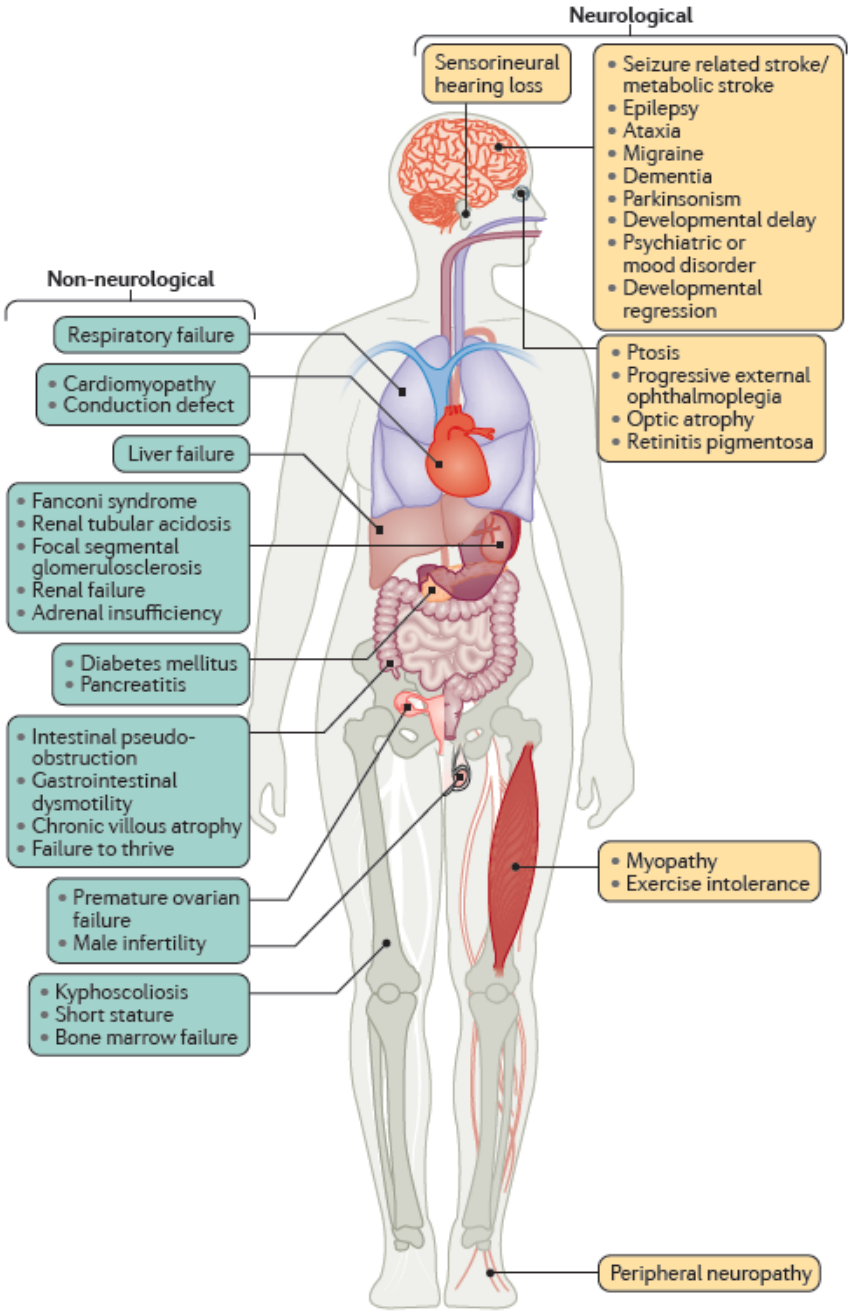
- **Arose from ancient symbiont ancestor: purple sulfur bacteria that could handle oxygen**

- **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:

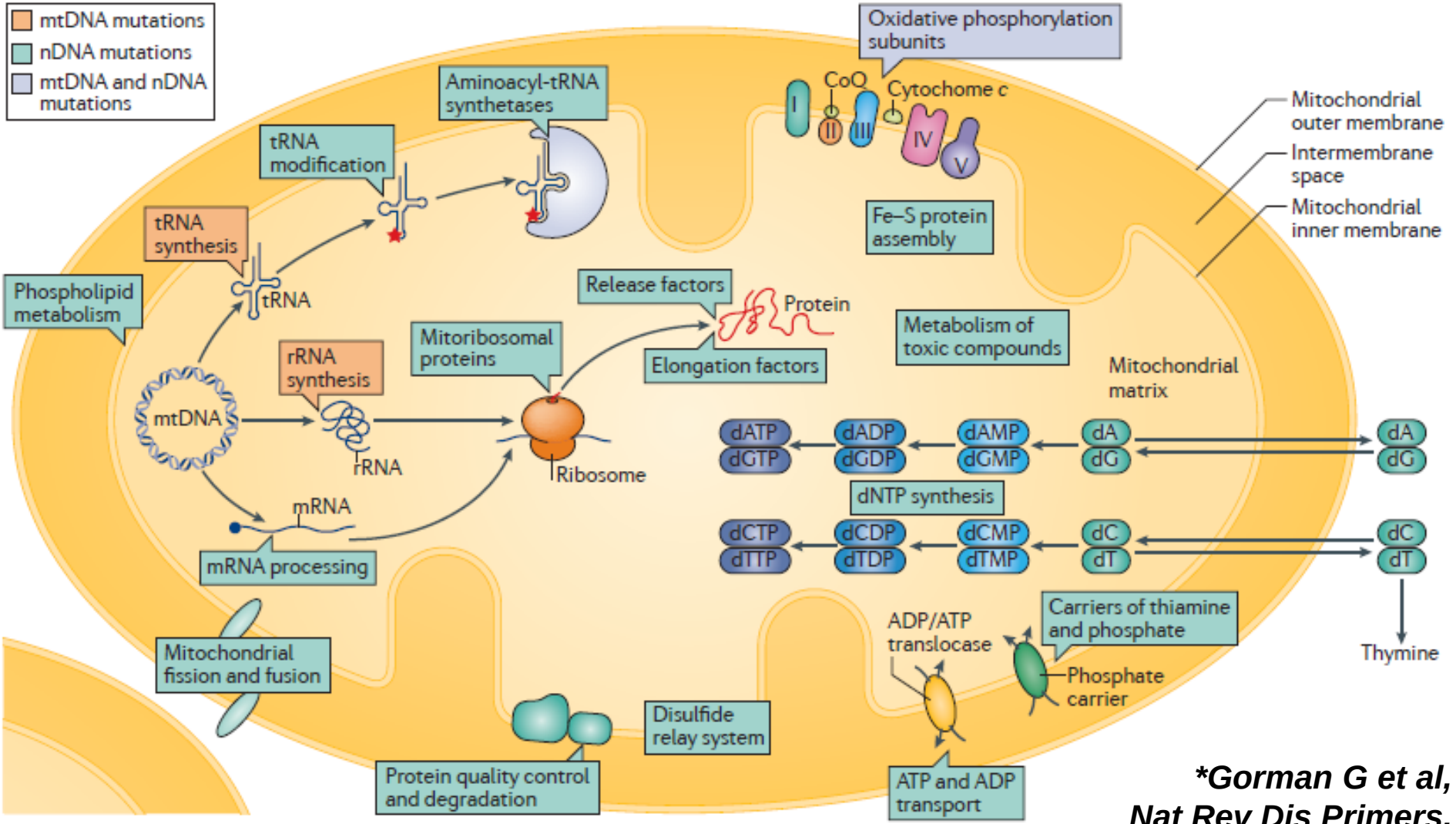
Rapidly changing molecular understanding

"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

*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**

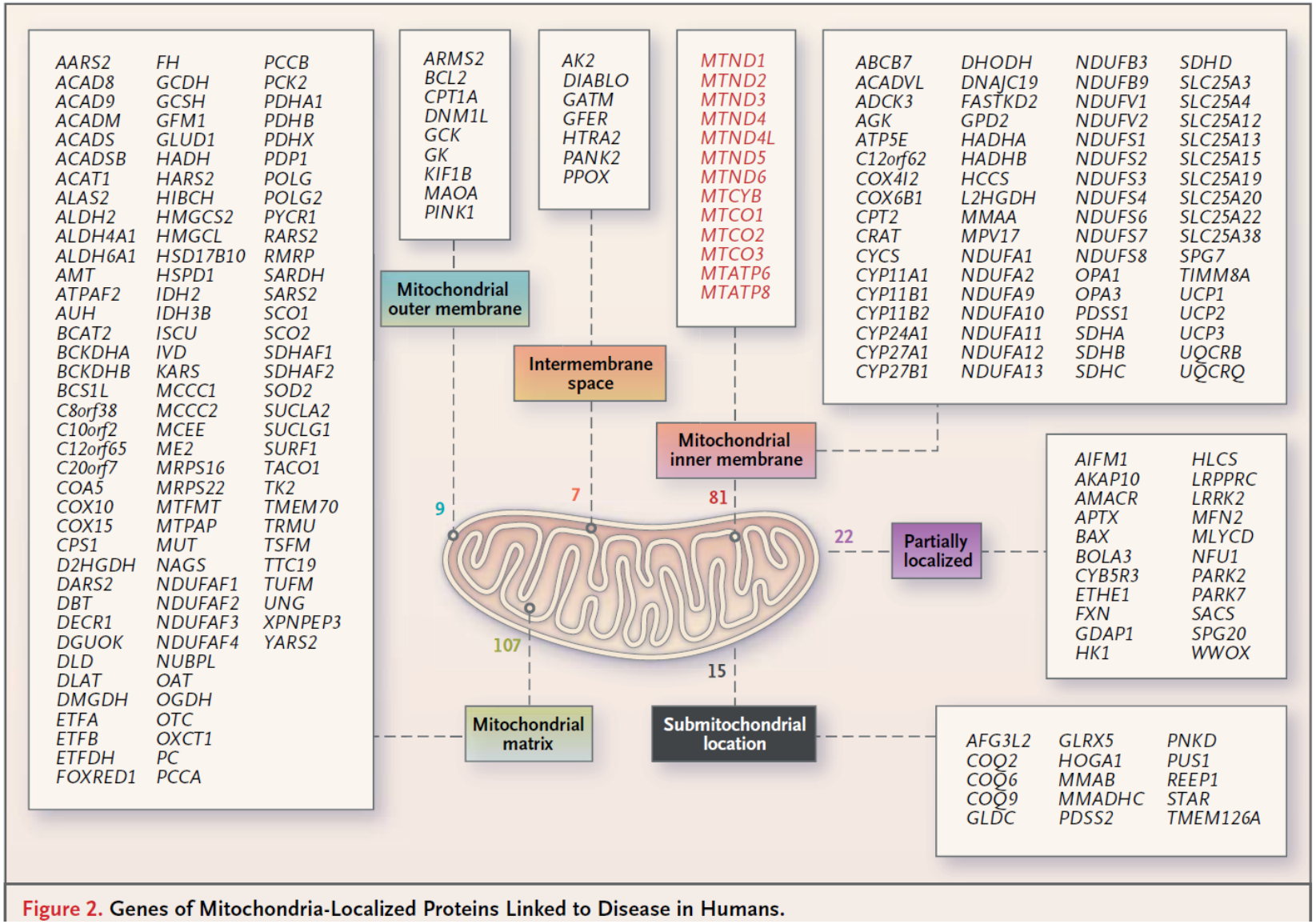


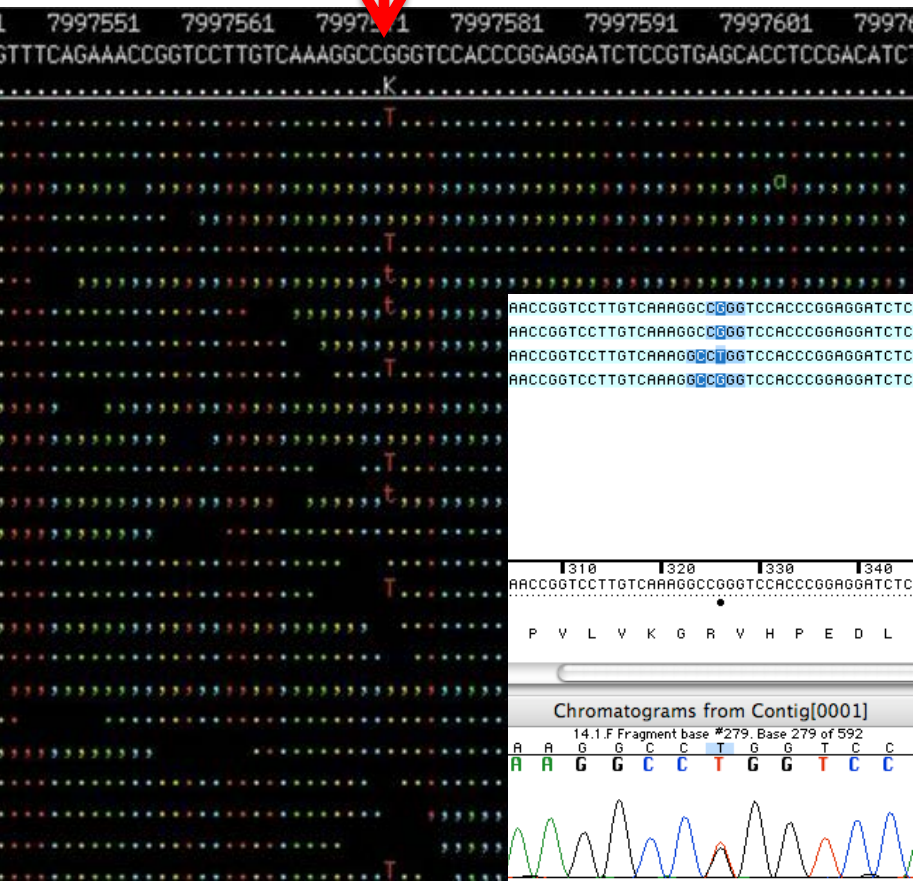
Figure 2. Genes of Mitochondria-Localized Proteins Linked to Disease in Humans.

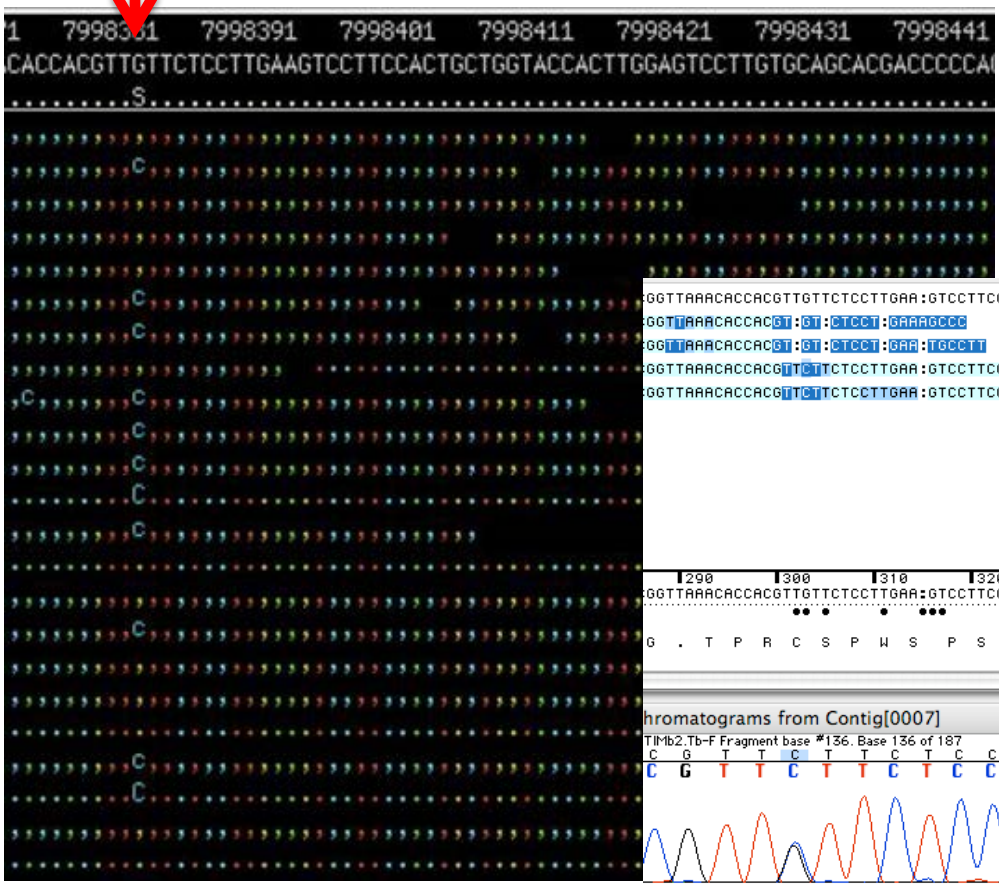
N Engl J Med 2012;366:1132-41.

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)

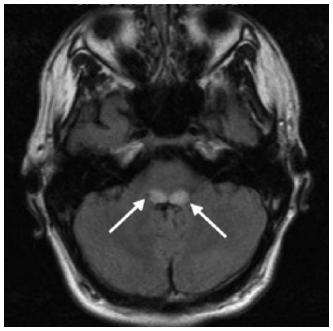
Mutation #2:
G>C transversion (p.N251K)
112 of 231 reads
(maternally inherited)



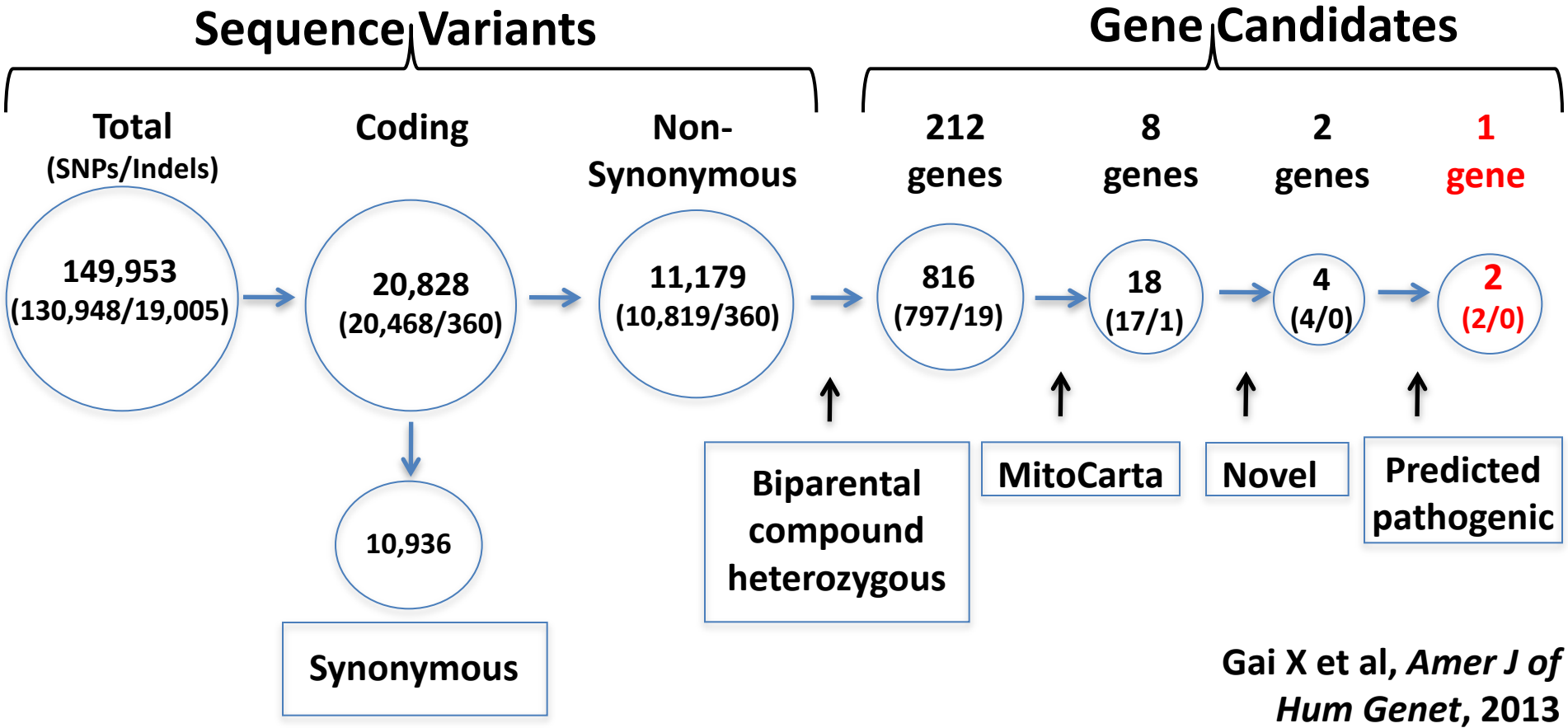


Xiaowu Gai, Stephen Dingley

Whole exome sequencing:
Disease diagnosis relies on computer algorithms

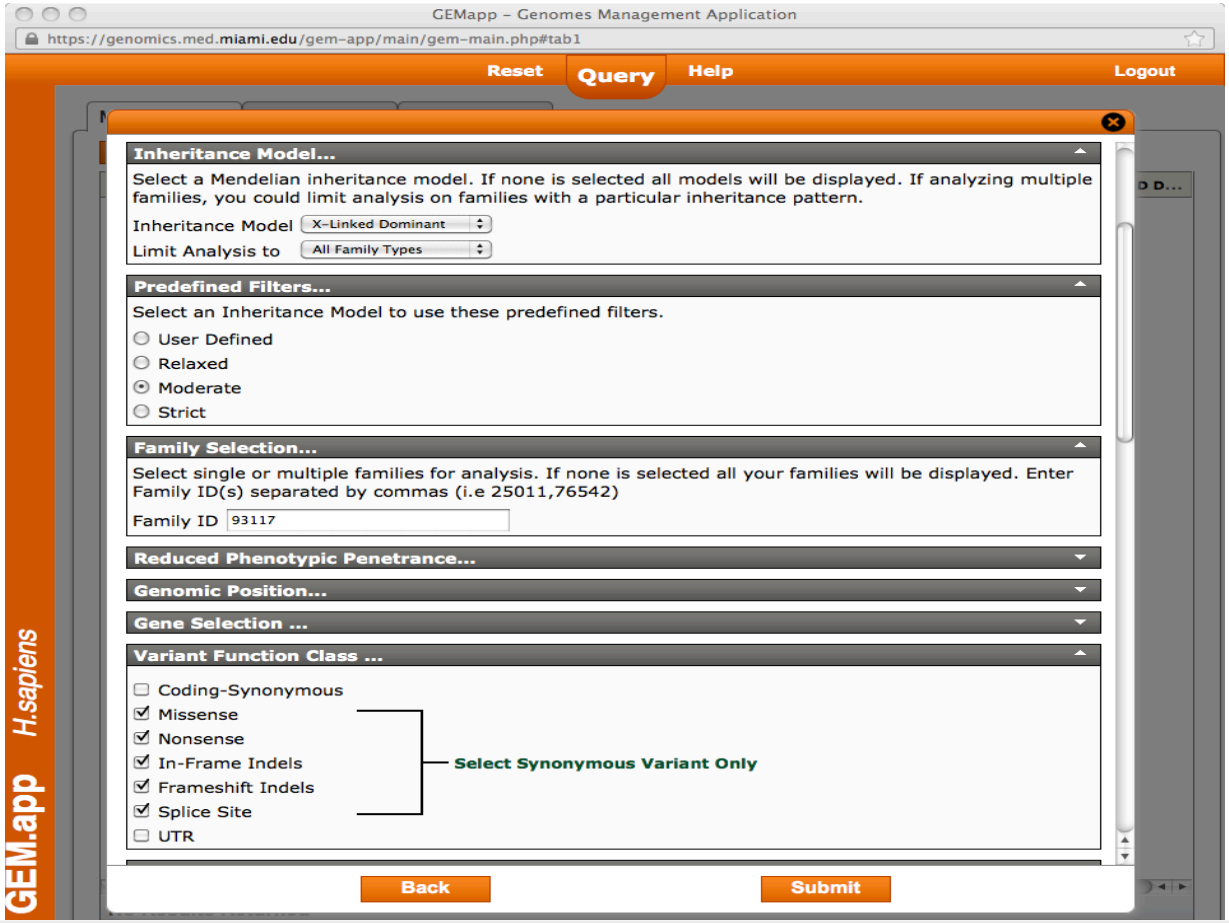


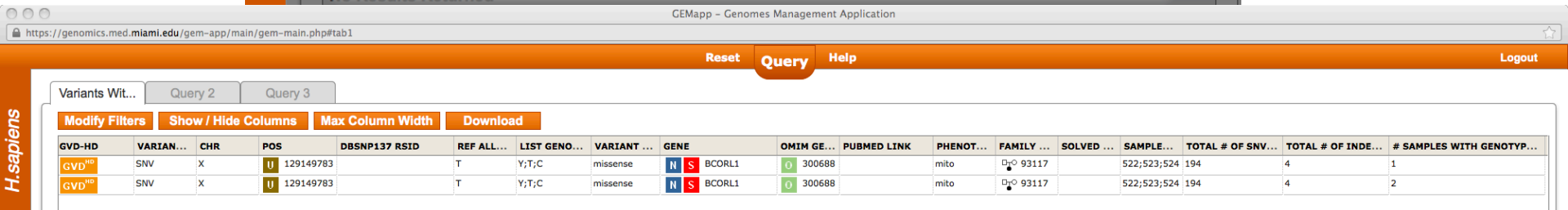
Young girl with Leigh syndrome, chronic lactic acidosis, OXPHOS deficiency. Normal prior testing for 18 individual genes. Only child in otherwise healthy family.



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

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)**

***Parikh S et al, *Curr Treat Options in Neurol*, 2009**

Mitochondrial Disease Community Partners

NAMDC / RDCRN

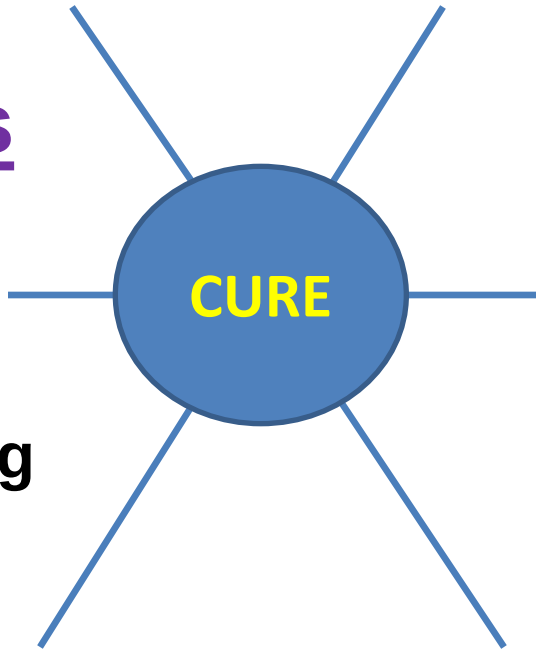
- Physician-entered registry
- Natural history Studies
- Biorepository

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:

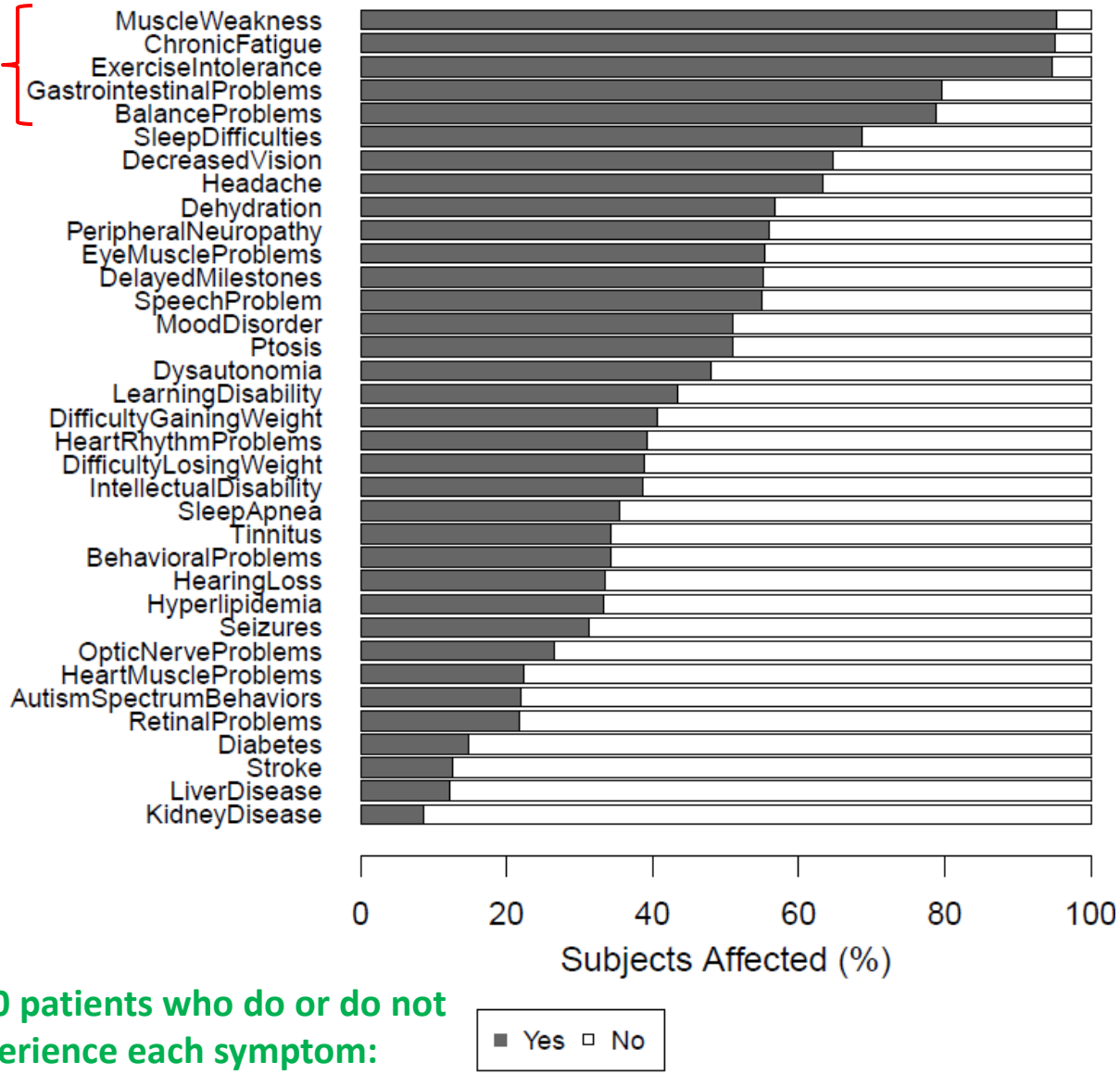
- **Coenzyme Q10 – trial never filled/completed**
- **Idebenone – approved in Europe in 2015 for LHON**
- **EPI-743 (Edison) – failed primary outcome in Leigh disease**
- **RP-103 (Cysteamine bitartrate, Raptor)-phase II, Leigh disease, discontinued**

OTHER MECHANISMS:

- **Elamipratide (Stealth BioTherapeutics) – phase II, myopathy/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



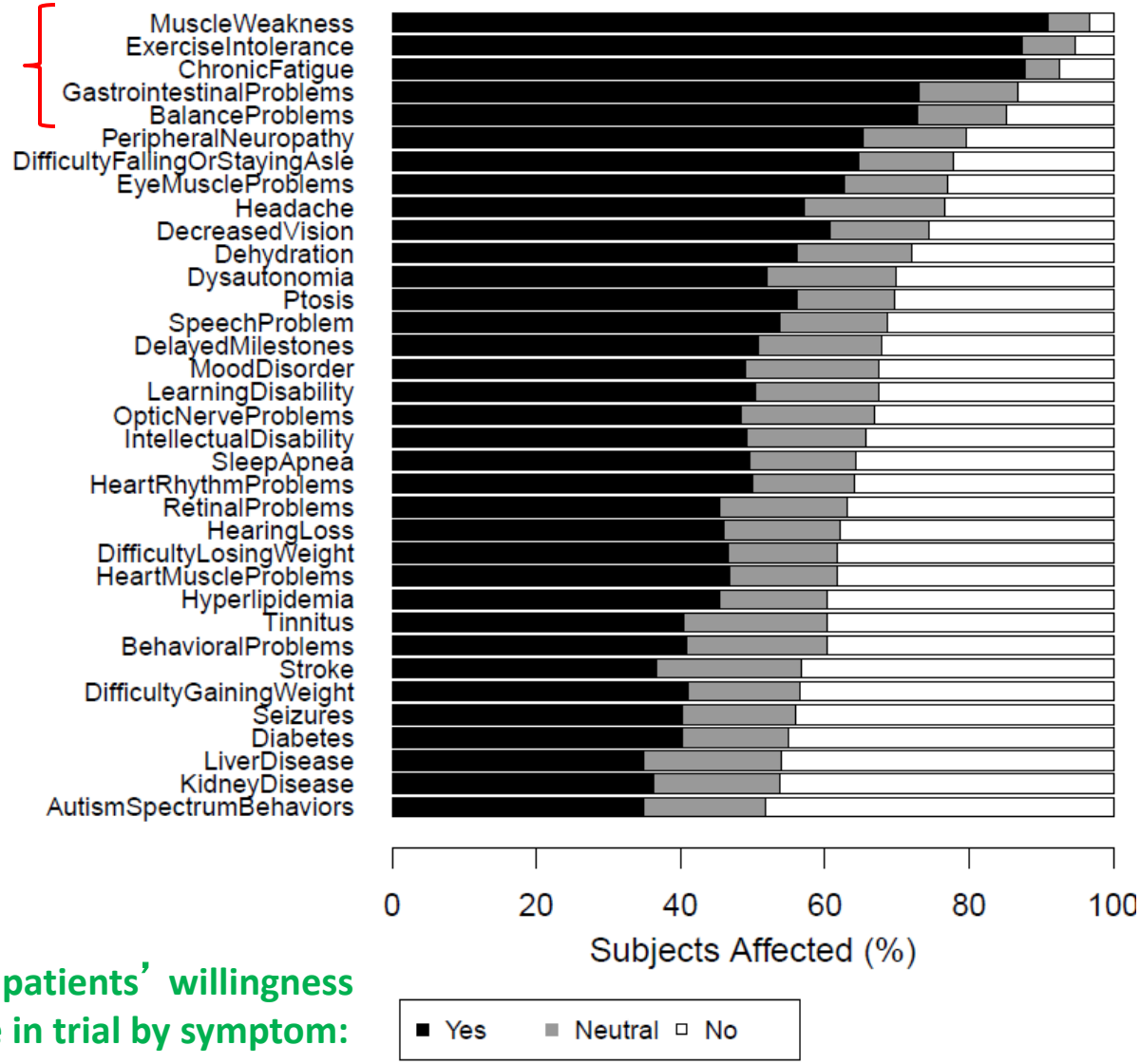
Survey of 290 Mitochondrial Disease Patients*

% 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

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



Survey of 290 Mitochondrial Disease Patients*

Symptomatic patients' willingness to participate in trial by symptom:

*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

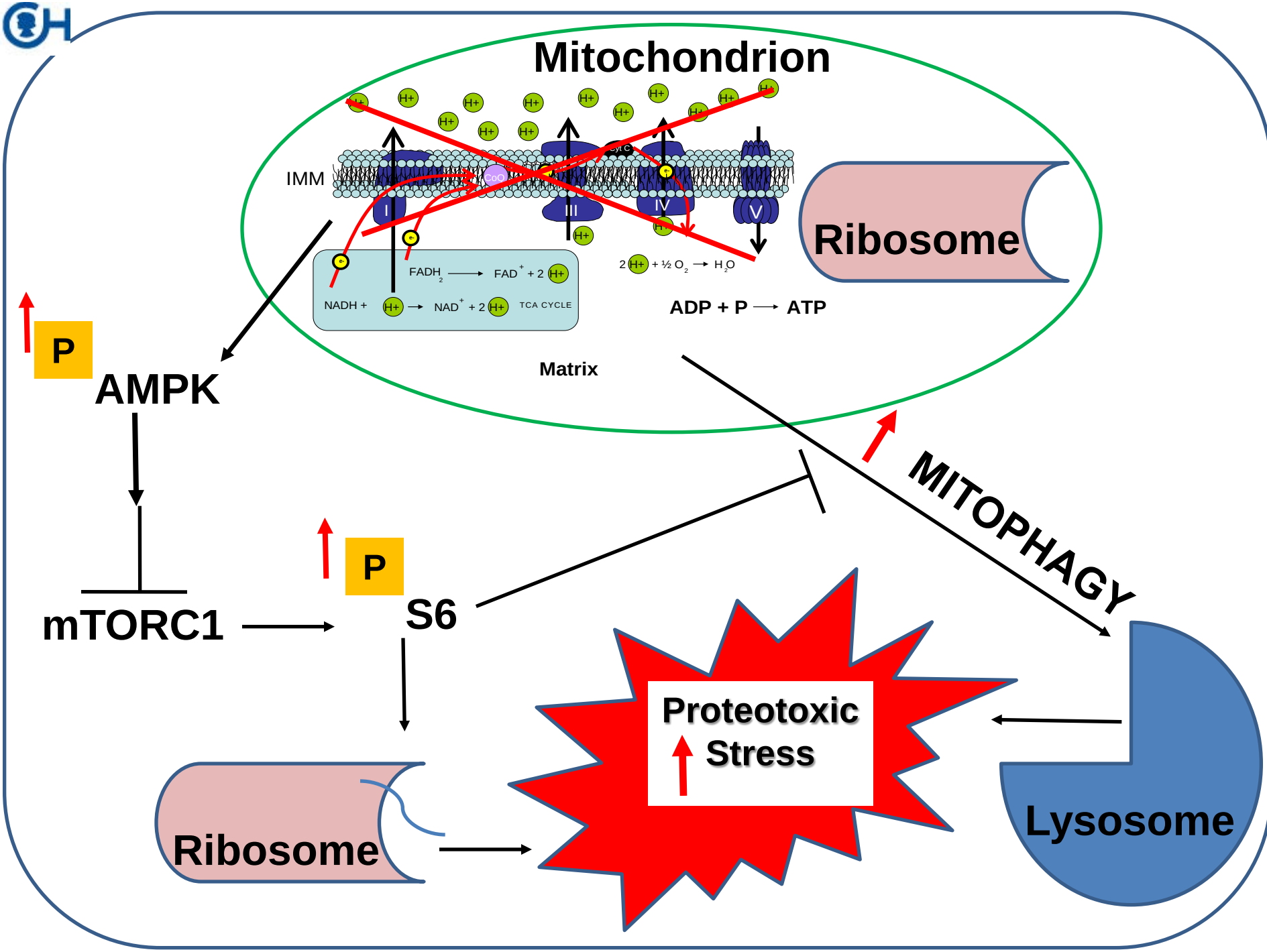
-Off-purpose FDA drugs
-Medical Foods
-Dietary Supplements
-Vitamins
-New drugs from Pharma

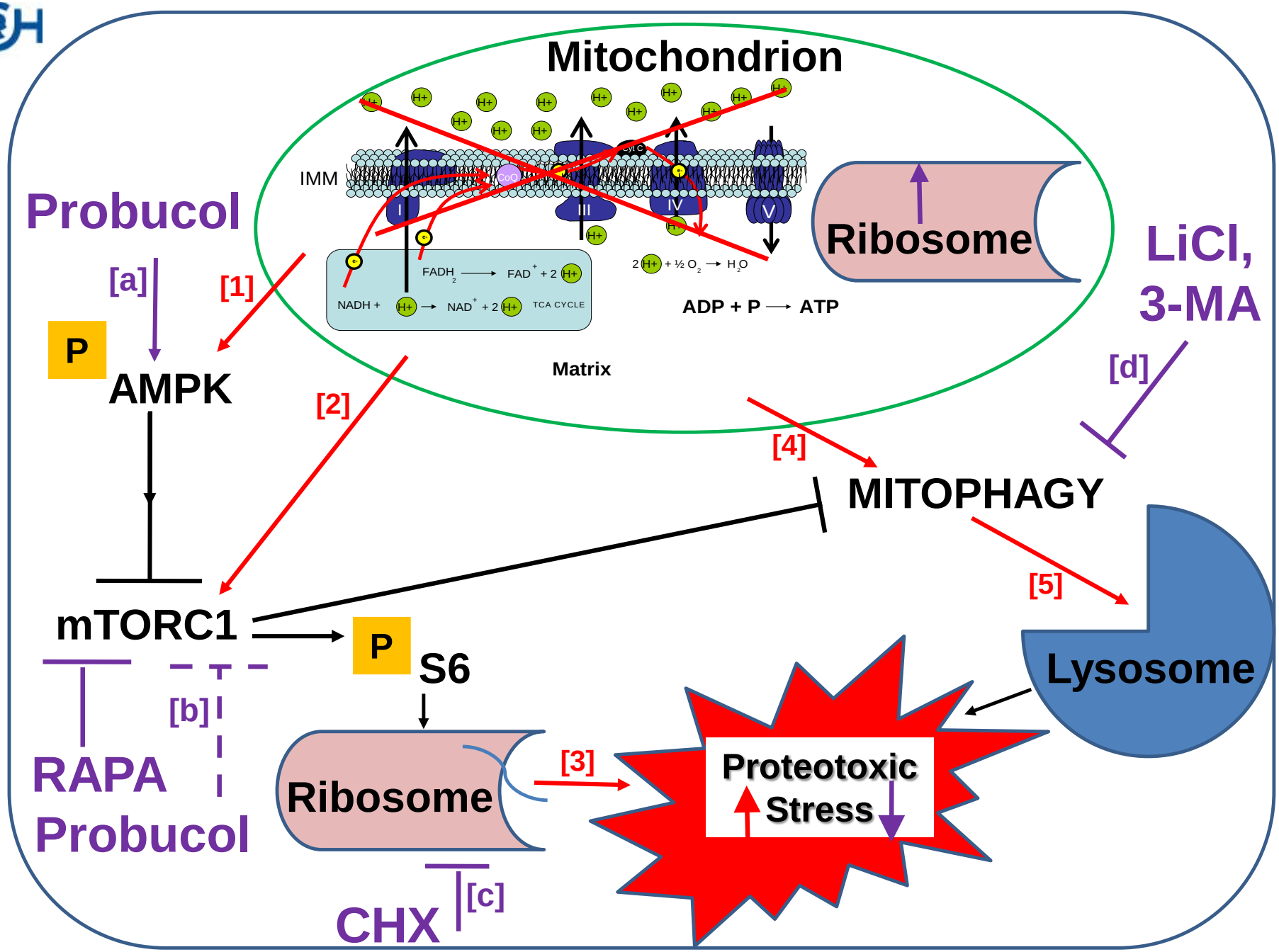
In Vitro Laboratory Drug testing in Mito Disease

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

Clinical Trials

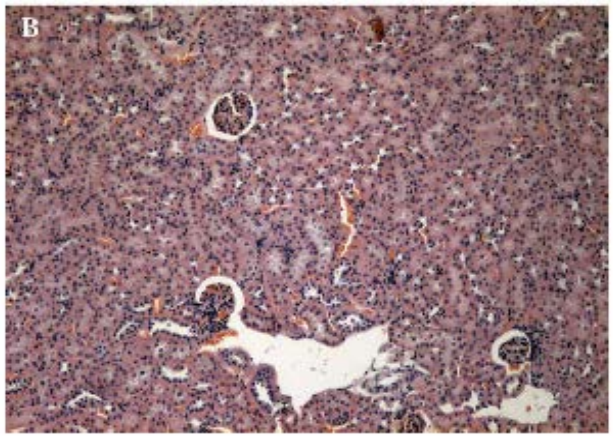
Standard of Care

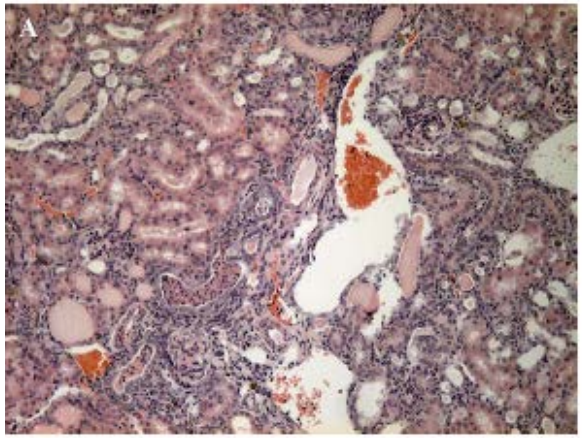

Mitochondrion
IMM
Matrix
Ribosome
ADP + P ⟶ ATP
P
AMPK
mTORC1
P
S6
MITOPHAGY
Proteotoxic
Stress
Ribosome
Lysosome
TCA CYCLE



Peng M et al, *Human Molecular Genetics*, 2015

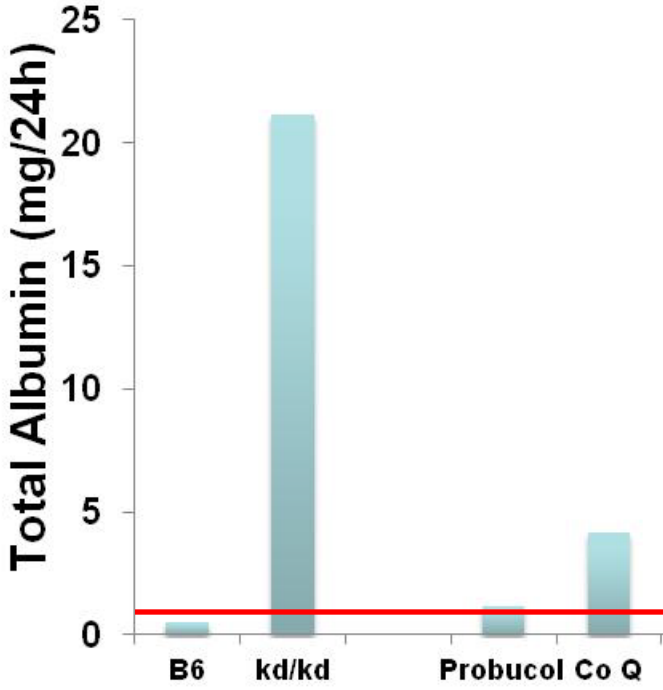
Probucol rescues Coenzyme Q deficient *Pdss2* mice

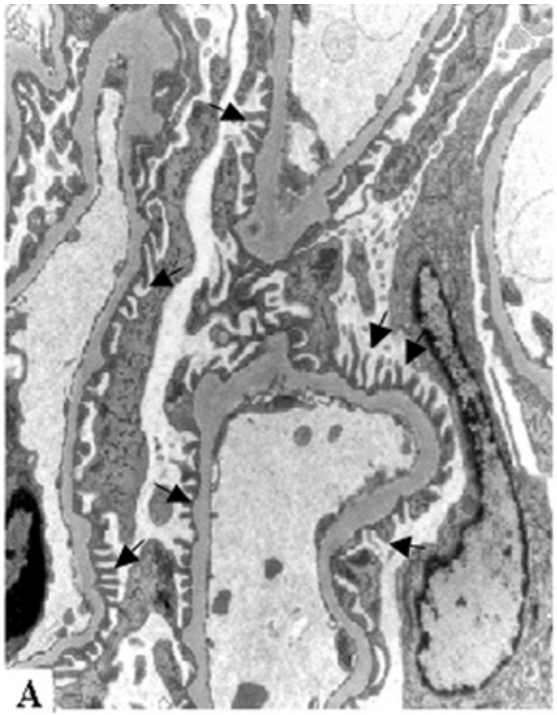


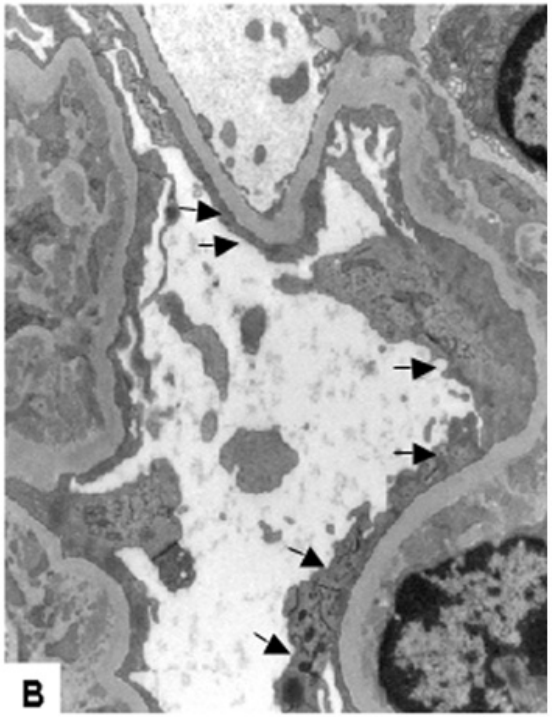


B6 wild-type mice

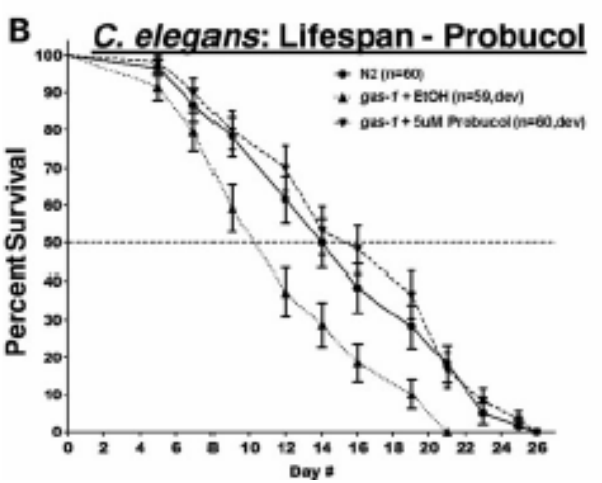
B6.PDSS2 $^{kd/kd}$ mutant mice

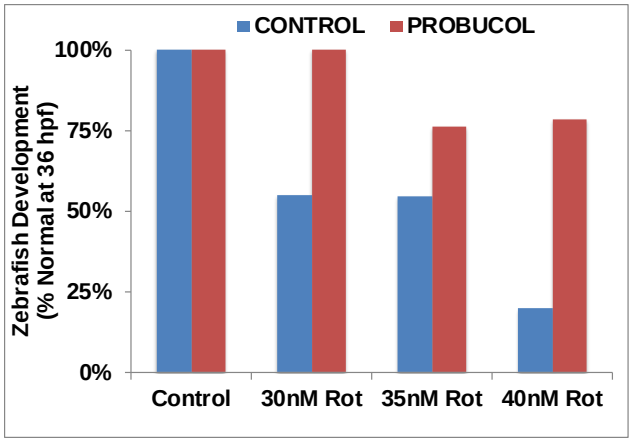


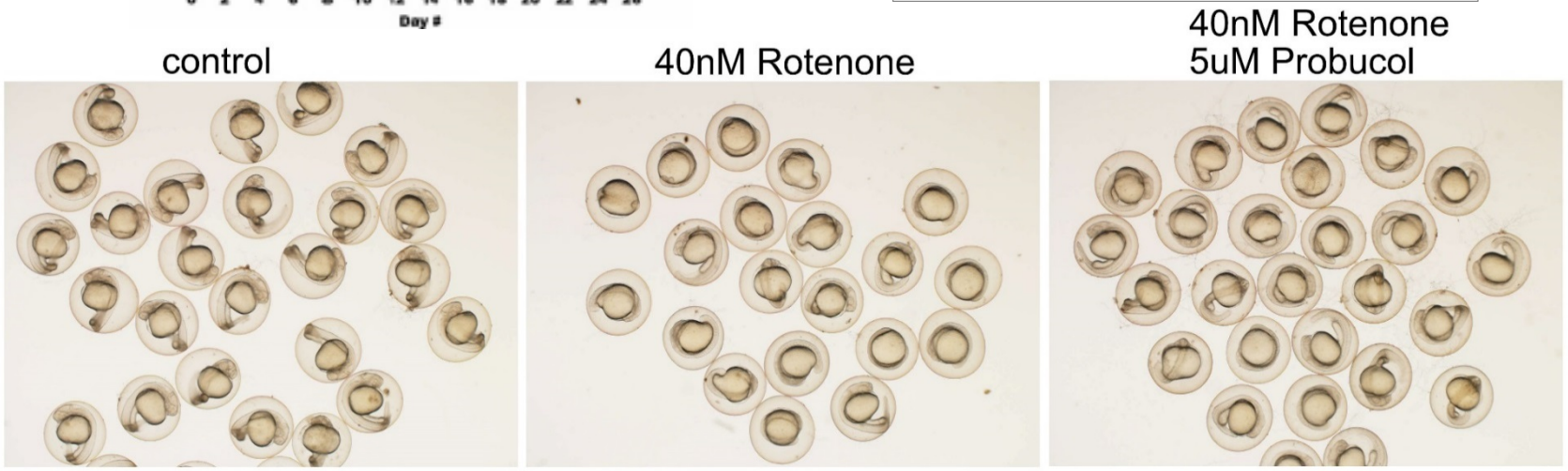


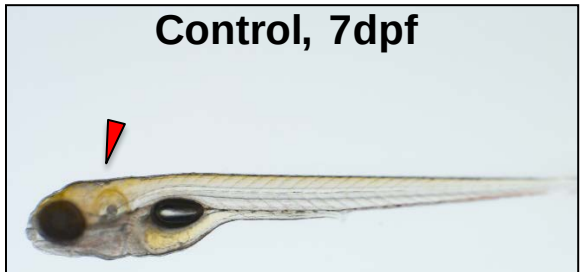


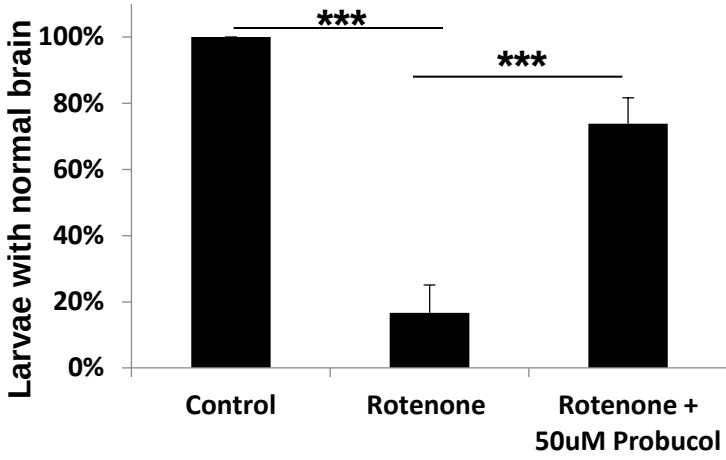
Falk MJ et al,
EMBO Mol Med, 2011











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
- 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

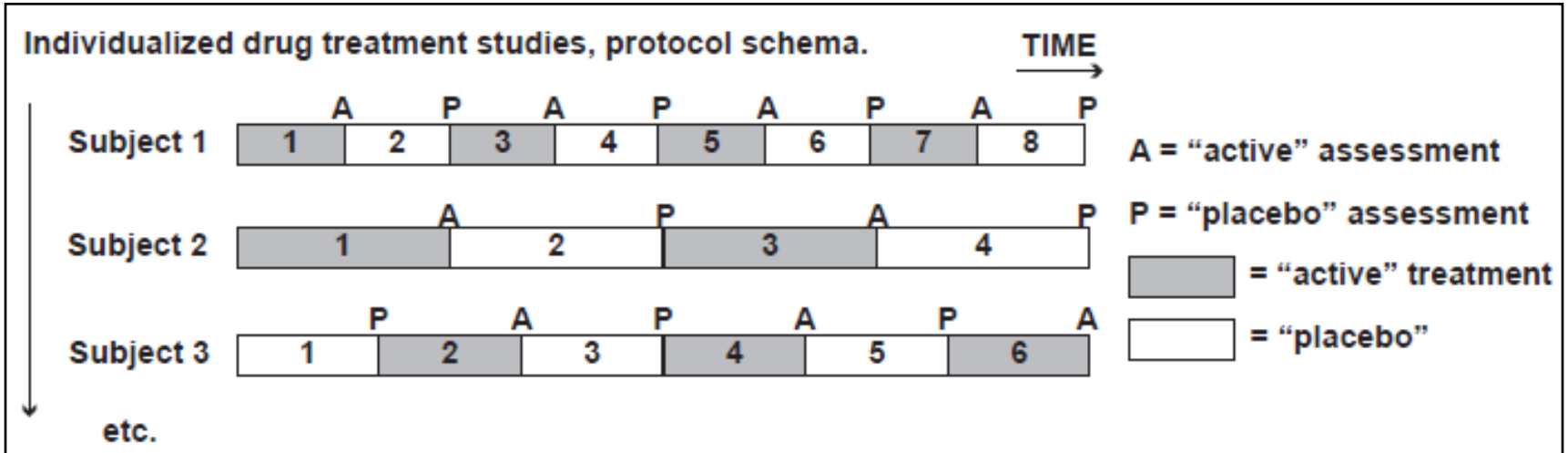
New Paradigm for Rare Disease:

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**



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