Integrating primary mitochondrial disease biology into designing clinical trials

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FDA Mitochondrial Drug Development Workshop

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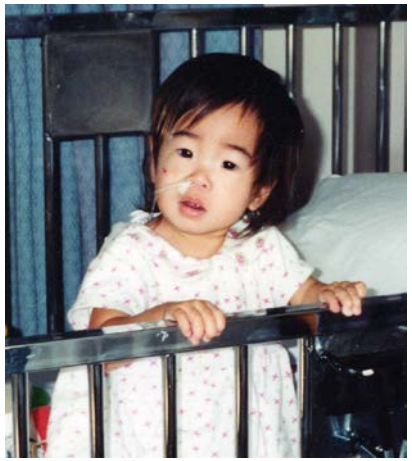
Disclosures

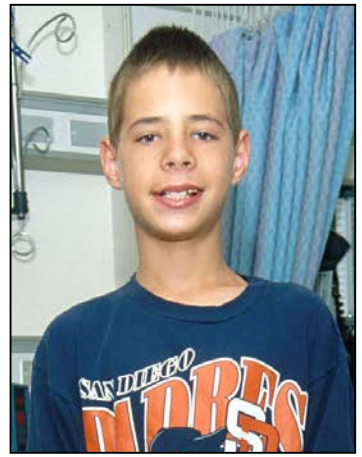
- Dr. Naviaux is an unpaid scientific advisory board member for:
  - The Autism Research Institute (ARI)
  - The Open Medicine Foundation (OMF)
  - Yuva Biosciences
- There are no approved uses of suramin in the United States. It is illegal to import suramin for human use without FDA and IRB approval and an IND.

Outline

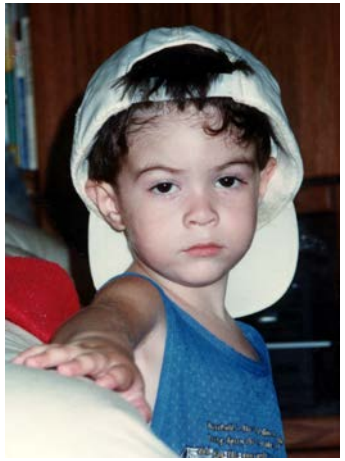
- Definitions: Primary mitochondrial disease (rare) vs secondary dysfunction (common)
  - The Modified Walker definition of PMD
- Genetics
- Symptoms
- Classical functions—steady state vs dynamics
- Emerging functions—Innate immunity and the cell danger response (CDR)
- 5 Practical problems in clinical trial design for primary mitochondria disease
  - Complementary functions *between* organelles
  - Correlated functions *within* organelles
  - Innate immunity and season of enrollment effects—winter infections and neurodegeneration
  - Time's Arrow--Non-reversibility of child development
    - Minimum duration of trial: 2 months for safety, 6 months for efficacy
    - Failure of washout-crossover designs
  - Biomarkers
- Updates from clinicaltrials.gov
  - Outcome metric selection
  - Current trials

The Faces of Mitochondrial Disease—
Over 350 Genetically Distinct Forms are Known

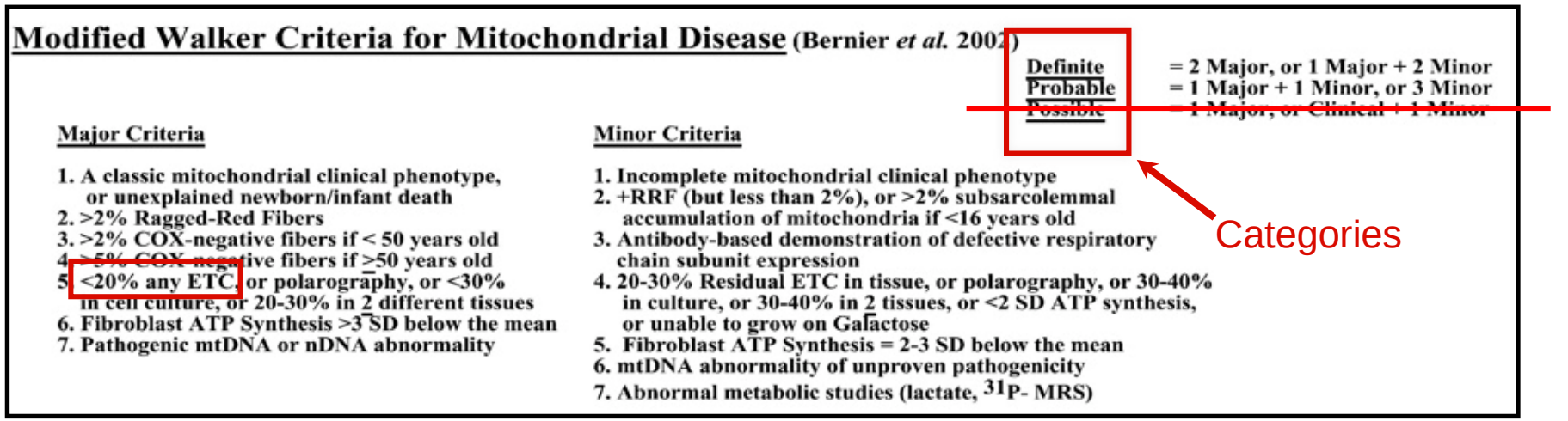




Mitochondrial Disease Diagnosis "Modified Walker Criteria"

Bernier/Thorburn. *Neurology* 2002;59:1406-1411.



Modified Walker Criteria for Mitochondrial Disease (Bernier *et al.* 2002)

Definite	= 2 Major, or 1 Major + 2 Minor
Probable	= 1 Major + 1 Minor, or 3 Minor
~~Possible~~	~~= 1 Major, or Clinical + 1 Minor~~

Major Criteria

1. A classic mitochondrial clinical phenotype, or unexplained newborn/infant death
2. >2% Ragged-Red Fibers
3. >2% COX-negative fibers if < 50 years old
4. ~~>5% COX-negative fibers if ≥50 years old~~
5. <20% any ETC, or polarography, or <30% in cell culture, or 20-30% in 2 different tissues
6. Fibroblast ATP Synthesis >3 SD below the mean
7. Pathogenic mtDNA or nDNA abnormality

Minor Criteria

1. Incomplete mitochondrial clinical phenotype
2. +RRF (but less than 2%), or >2% subsarcolemmal accumulation of mitochondria if <16 years old
3. Antibody-based demonstration of defective respiratory chain subunit expression
4. 20-30% Residual ETC in tissue, or polarography, or 30-40% in culture, or 30-40% in 2 tissues, or <2 SD ATP synthesis, or unable to grow on Galactose
5. Fibroblast ATP Synthesis = 2-3 SD below the mean
6. mtDNA abnormality of unproven pathogenicity
7. Abnormal metabolic studies (lactate, ^{31}P- MRS)

Categories

Published Criteria

1. Modified Walker, 2002 (PMID: 12427892)
2. Nijmegen, 2002 (PMID: 12427891)
3. Morava, 2006 (PMID: 17130416)

Blood DNA 1st, then
Muscle Biopsy if needed → **DNA**

A "definite" diagnosis now requires a confirmed pathogenic DNA mutation.

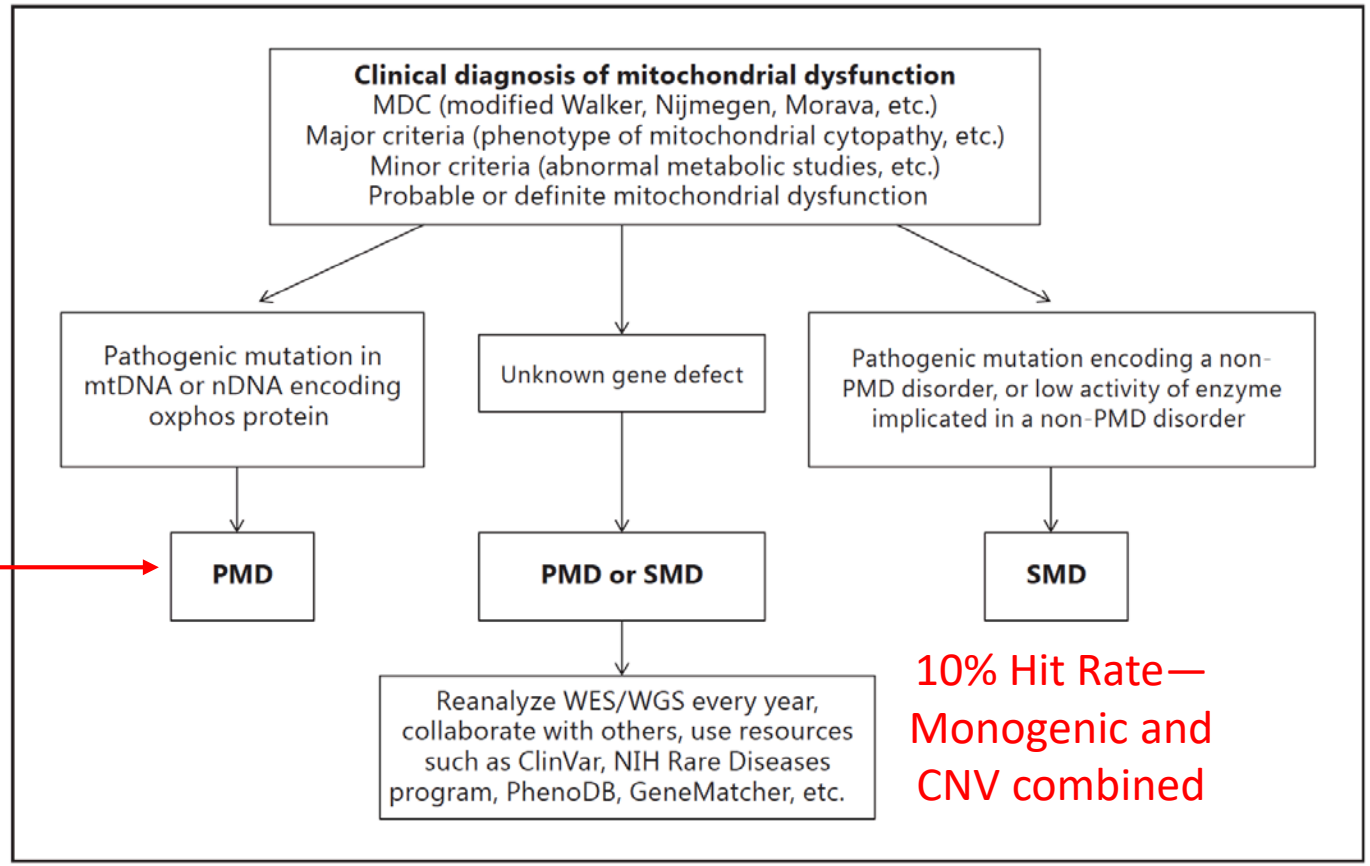
Primary Mitochondrial Disease and Secondary Mitochondrial Dysfunction: Importance of Distinction for Diagnosis and Treatment

Mol Syndromol 2016;7:122–137. PMID: 27587988

Dmitriy M. Niyazov[a] Stephan G. Kahler[b] Richard E. Frye[b]

50-60% Hit Rate

NAMDC, Harvard, CHOP →



10% Hit Rate—Monogenic and CNV combined

Published Criteria

1. Modified Walker, 2002 (PMID: 12427892)
2. Nijmegen, 2002 (PMID: 12427891)
3. Morava, 2006 (PMID: 17130416)

Genetic Causes—5 major groups

- Over 350 mtDNA and nDNA causal genes of PMD identified
  - 15-20 new genes each year in the past decade

1. ETC Subunits and Assembly
2. Cofactors
3. Mt-tRNA Charging
4. MtDNA
5. Dynamics

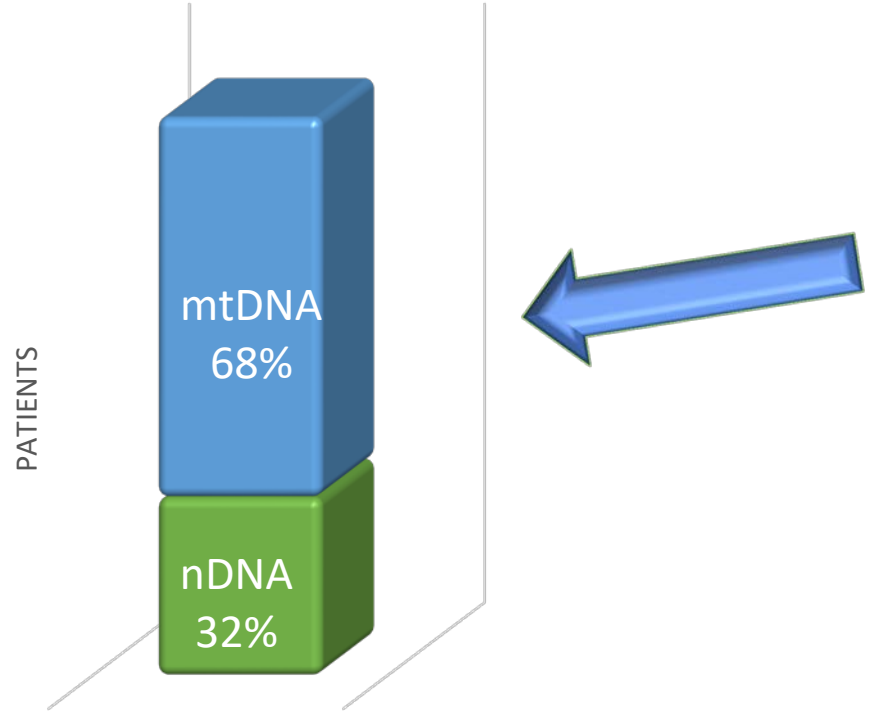
Component	Causal Genome	Gene Mutation Effects	Disease Examples
Electron transport chain enzyme subunits	Nuclear orMitochondrial	Decreased functioning of electron transport chain complex	• Complex I deficiency • Complex II deficiency • Complex III deficiency • Complex IV deficiency • Complex V deficiency
Electron transport chain assembly factors	Nuclear	Decreased assembly of electron transport chain enzyme complex	
Eelectron transport chain cofactors	Nuclear	Decreased functioning of electron transport chain	• Coenzyme Q10 deficiency • Iron sulfur cluster defect • Lipoyltransferase deficiency
mtDNA translation	Nuclear or Mitochondrial	Decreased translation of protein-coding mitochondrial DNA genes leading to decreased functioning of electron transport chain enzymes	• Combined oxidative phosphorylation complexes deficiency
mtDNA maintenance	Nuclear	Increased errors in mitochondrial DNA leading to increased presence of point mutations and deletions, resulting in decreased translation ofelectron transport chain subunits	• Mitochondrial DNA depletion syndromes • Mitochondrial DNA multiple deletion disorders
Mitochondrial membrane fission and fusion	Nuclear	Increased mtDNA point mutations and deletions; clumped and fragmented mitochondria	• *OPA1*-related conditions • *MFN2*-related conditions

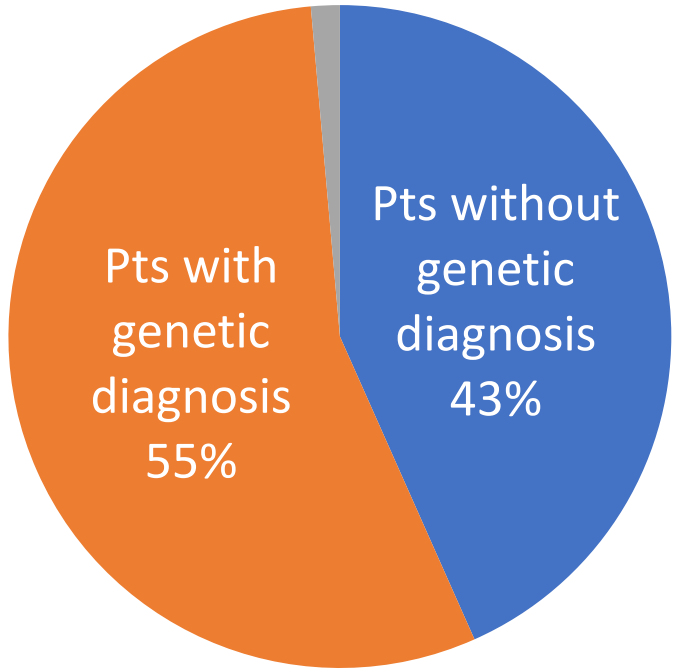
McCormick, et al. *Curr Genet Med Rep* 6:52, 2018.

The Distribution of Genetic Causes from NAMDC

Overview of 999 patients in the NAMDC Registry, 2017

https://www.rarediseasesnetwork.org/cms/NAMDC

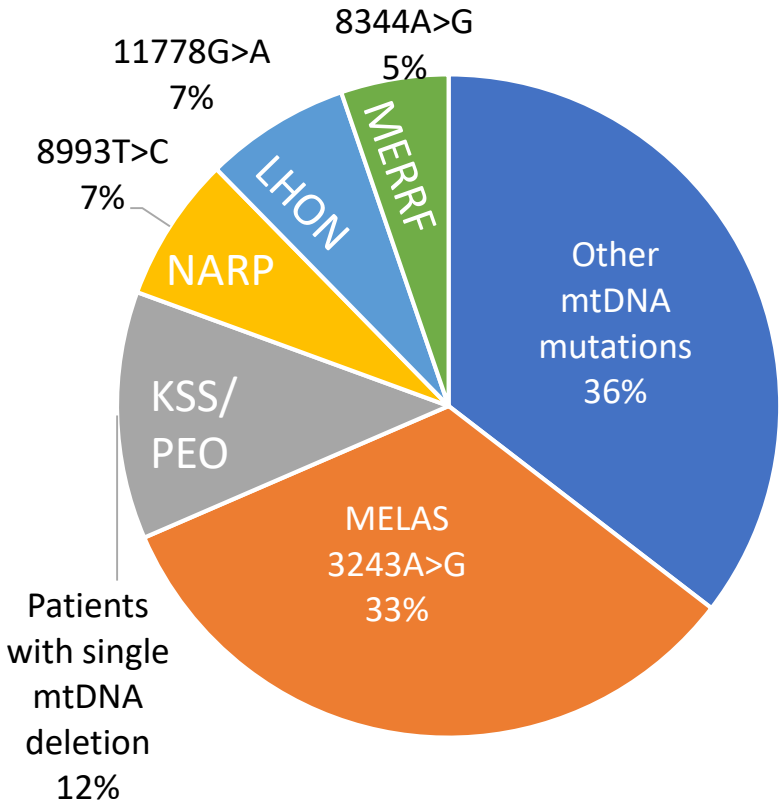




Rare Diseases Clinical Research Network
National Institutes of Health
NIH National Institute of Neurological Disorders and Stroke
NIH Eunice Kennedy Shriver National Institute of Child Health and Human Development
NIH National Institutes of Health Office of Dietary Supplements
United Mitochondrial Disease Foundation
HOPE. ENERGY. LIFE.

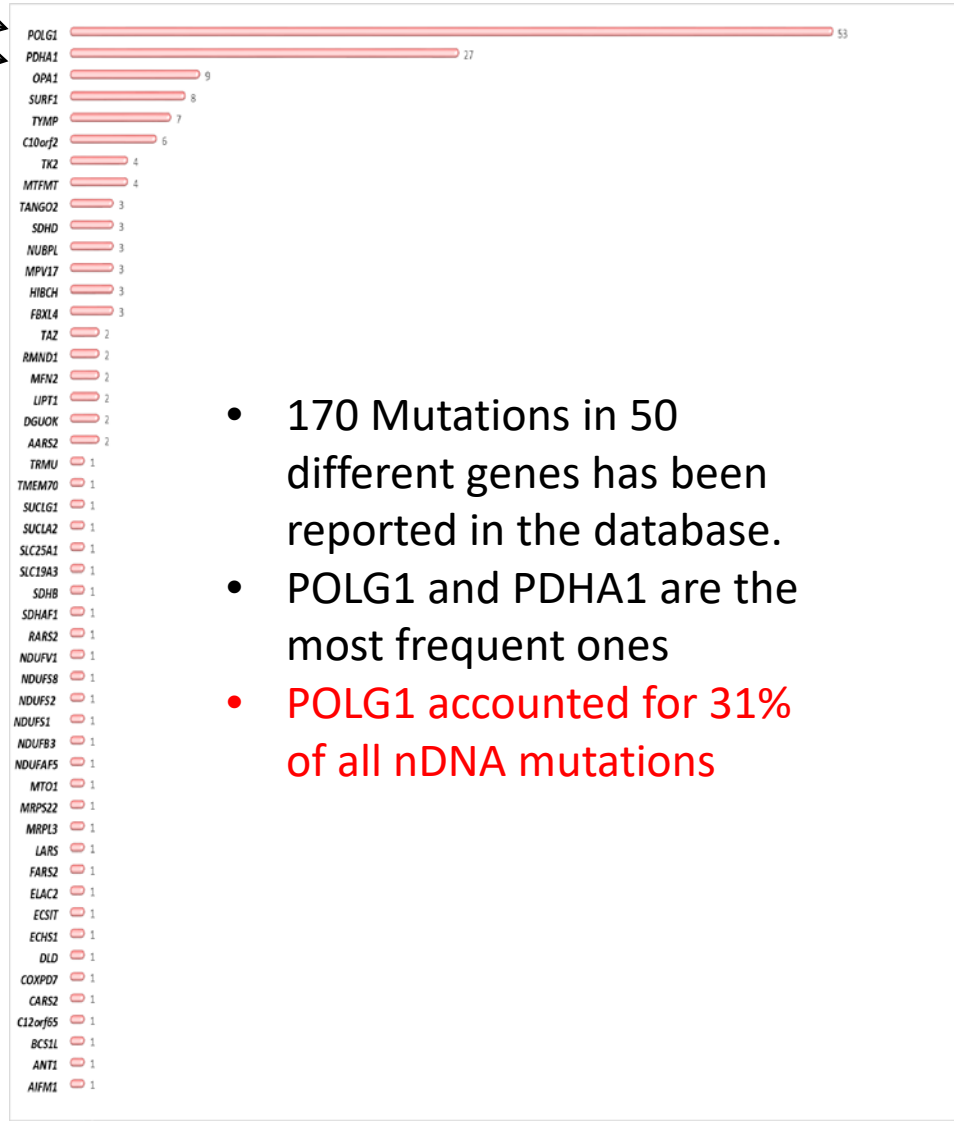
NAMDC Data

mtDNA mutations



Nuclear gene mutations

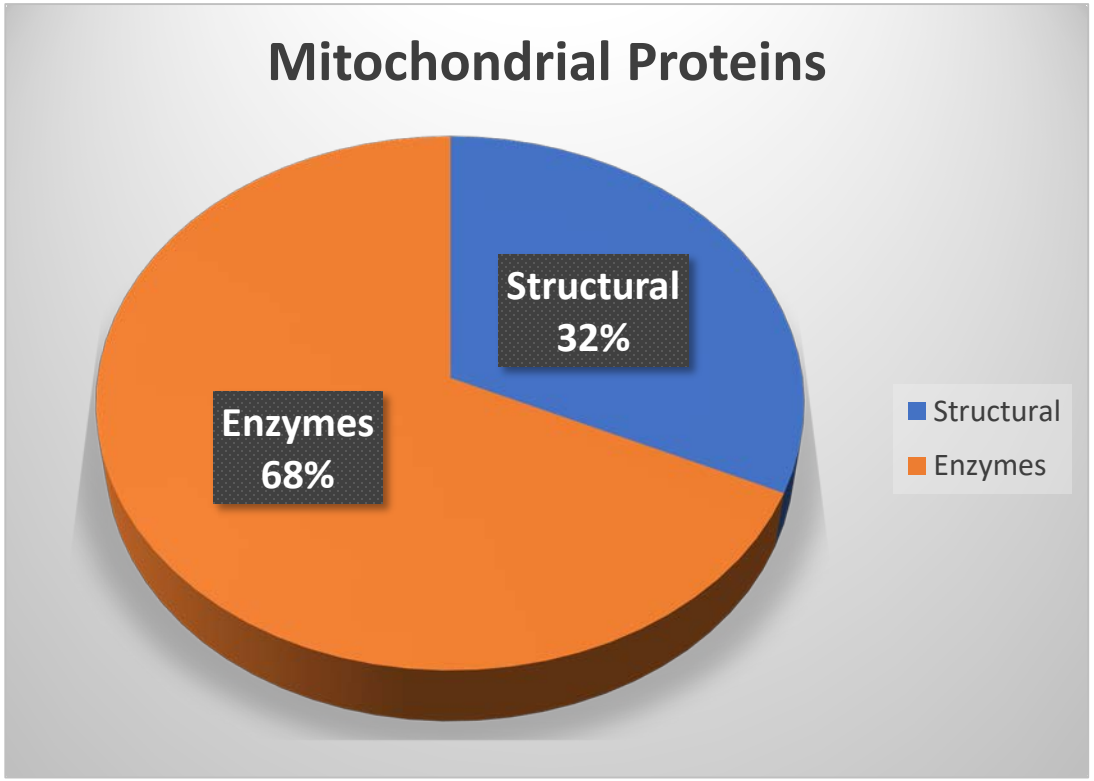
POLG1
PDHA1



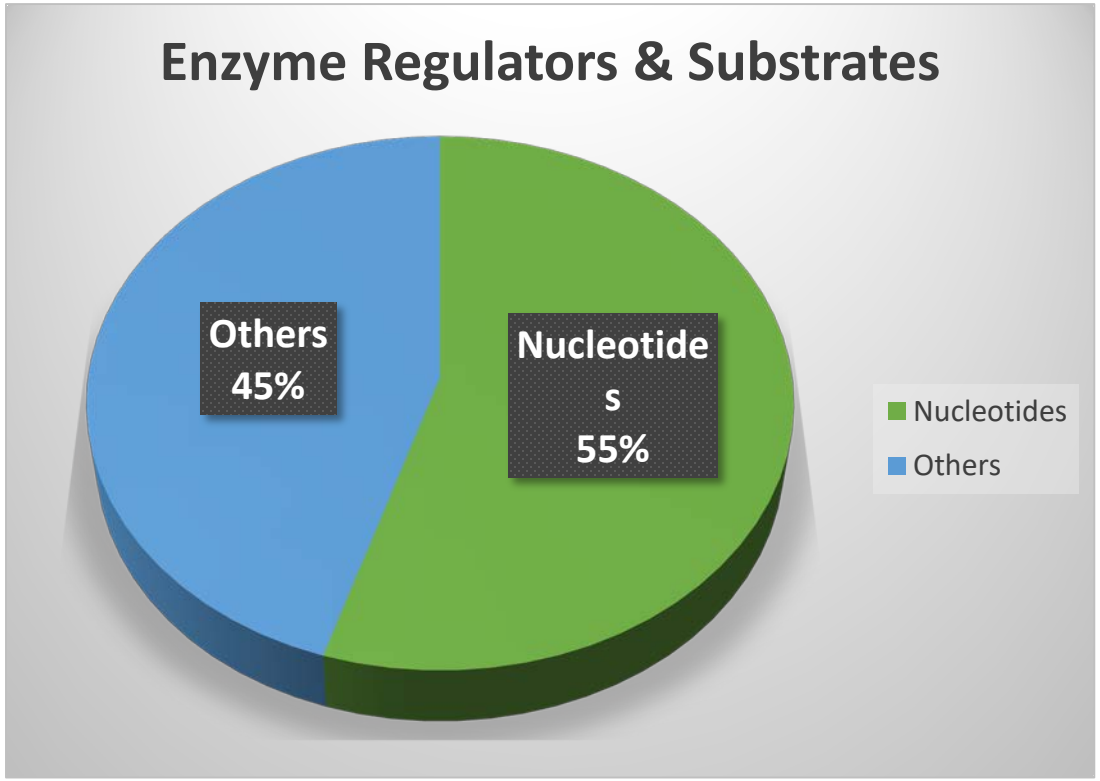
- 170 Mutations in 50 different genes has been reported in the database.
- POLG1 and PDHA1 are the most frequent ones
- POLG1 accounted for 31% of all nDNA mutations

70% of Mitochondrial Proteins are Enzymes, and 55% of the Enzymes are Regulated by Nucleotides

MitoCarta v2.0 N = 1158 Proteins



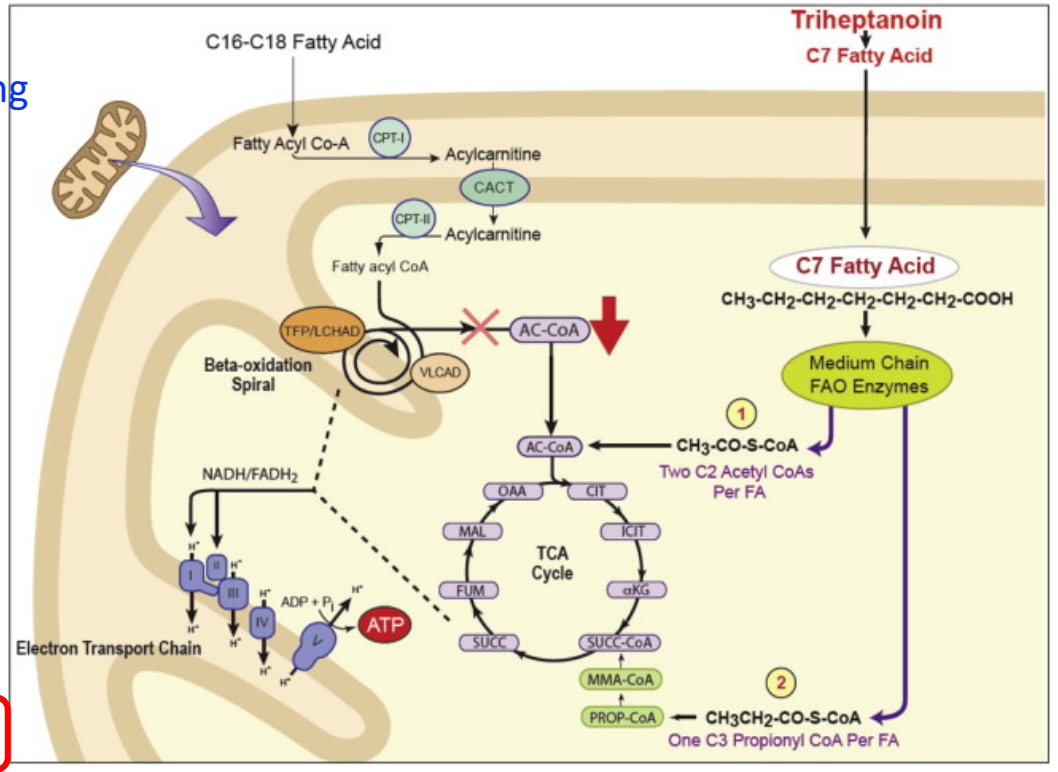
Nucleotide Regulation (ATP, GTP, UDP, etc)



Naviaux. Incomplete healing as a cause of aging. *Biology*. 2019 (New analysis of data mined from Calvo, Mootha, et al. *Nucleic Acids Res*. 2016, 44, D1251–D1257)

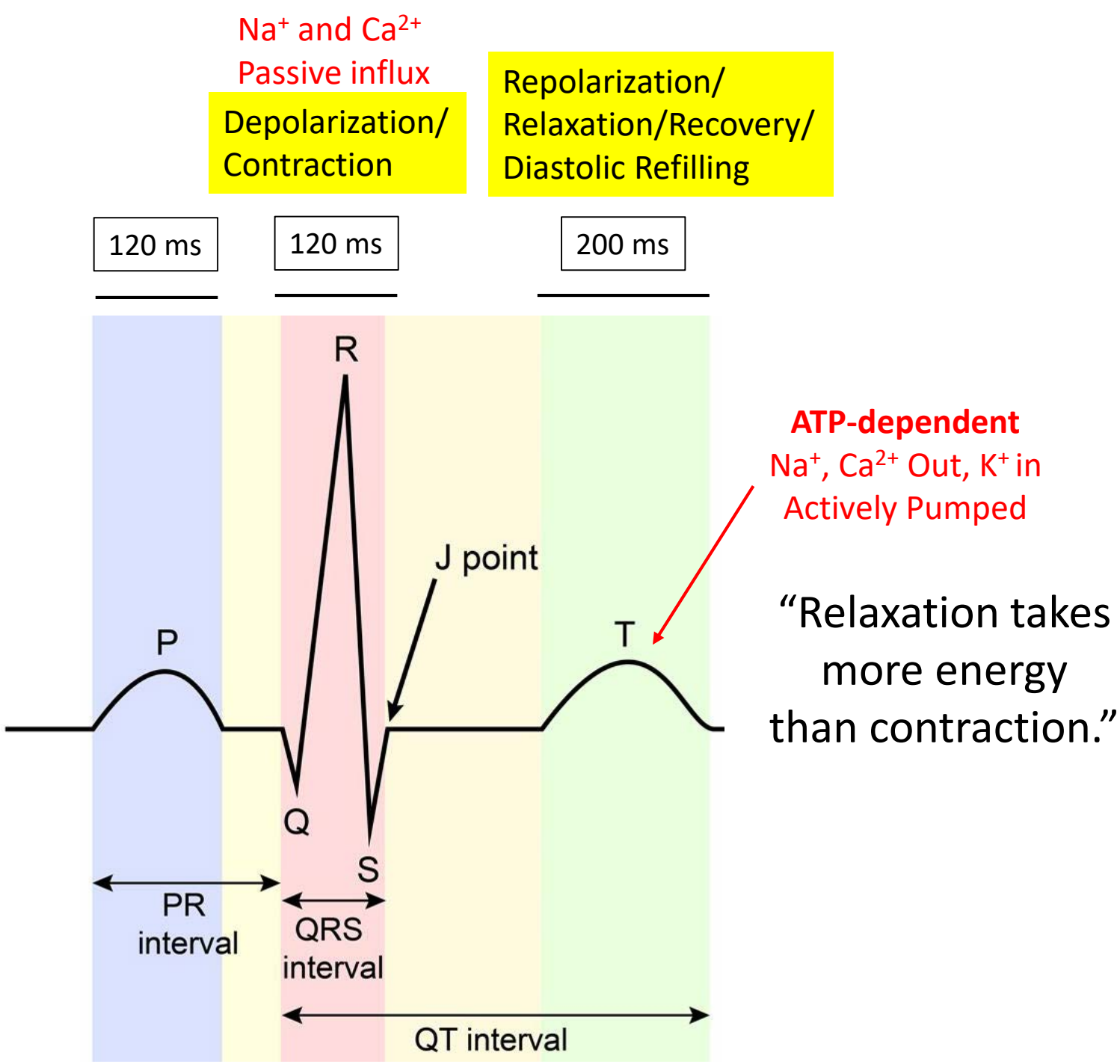
Classical Mitochondrial Functions—
of over 500. The Importance of Metabolic Cooperation (Protein-Membrane Social Networks)

- **Constitutive** oxygen consumption
- **Regulated** ATP Synthesis and heat production
- Folate, B12, SAM, Methionine, Cysteine, Taurine, and Glutathione metabolism
- Cellular Redox and ROS control
- Stress monitoring and apoptosis
- Purine and pyrimidine nucleotide synthesis (ATP, GTP, UTP, etc) and signaling
- Cholesterol, Cortisol, Bile acids, and Steroid hormone metabolism
- Vitamin D Activation and Inactivation
- Glycolate and Oxalate Metabolism
- Eicosanoid inactivation
- Porphyrin, Fe-S Cluster, and Heme Biosynthesis
- Ca^{2+}, Fe^{2+}, Cu^{2+} metabolism
- Meiosis
- Production of the metabokines needed to regulate the healing cycle
  - Choreography of transitions from CDR1 to CDR2 to CDR3
- **Differentiation, Development, Injury Recovery, Healing, and Regeneration**



The EKG Window into Mitochondrial Function

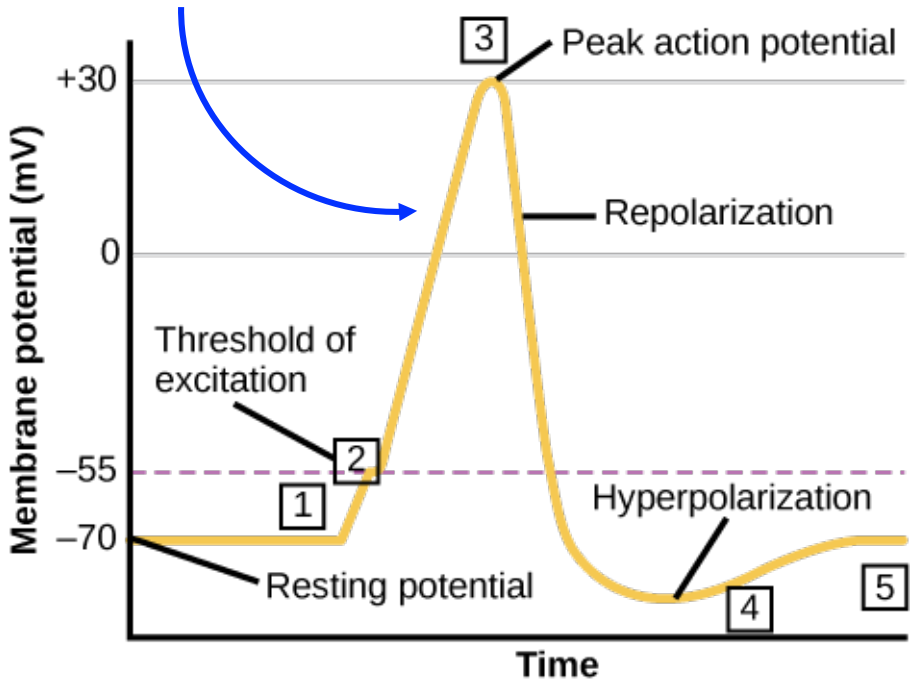
"It's harder to relax than to react."

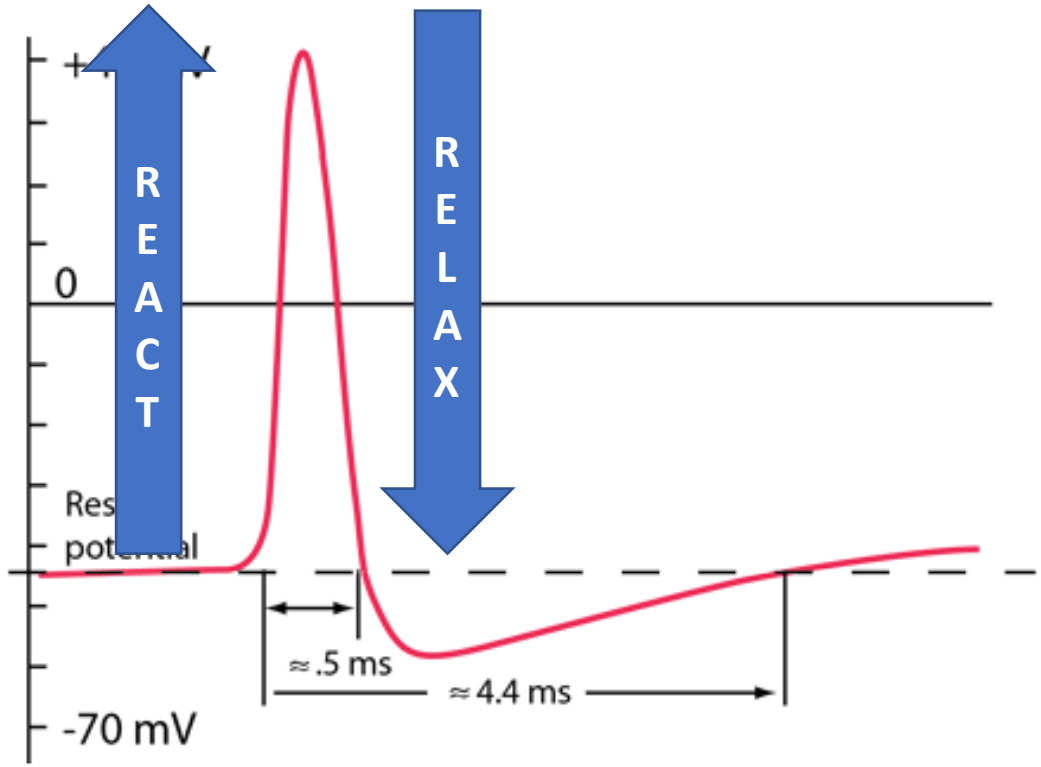


"Relaxation takes more energy than contraction."

Reaction-Relaxation Coupling—storing potential energy (coiling the spring) for the next stimulus-response

Calcium influx activates mitochondrial
Dehydrogenases and oxphos

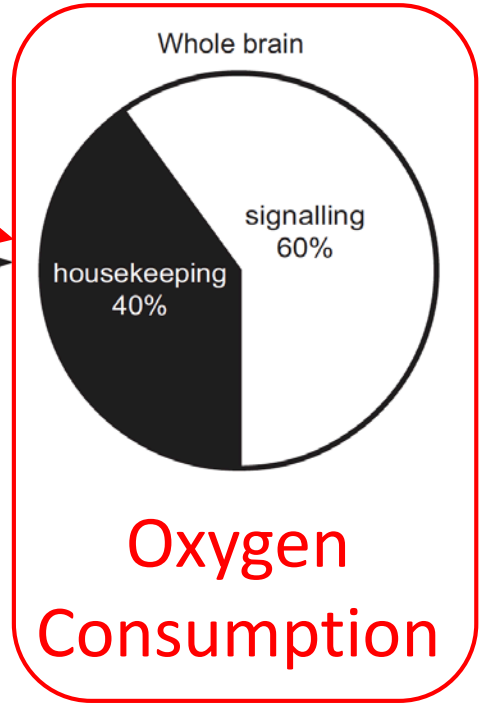




Energy Budget of the Brain—ATP and GTP synthesis and turnover

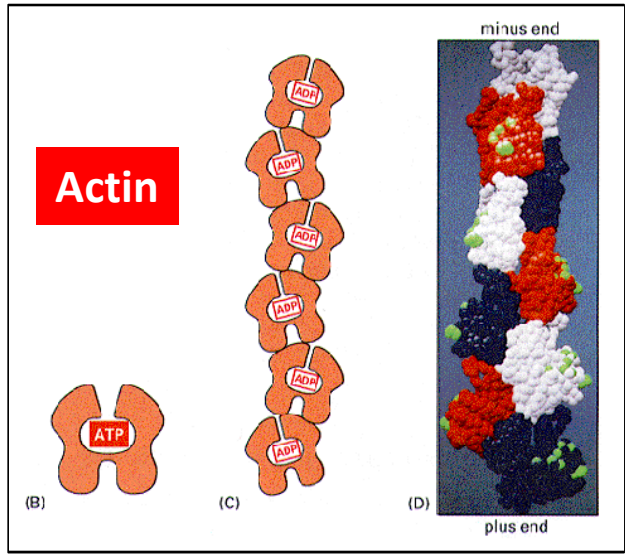
'Housekeeping' process	% of total brain energy use
Actin treadmilling	<1% (M) - 50% (E)
Microtubule dynamic instability	<< 1% (M)
Protein synthesis	1.3% (M)
Lipid turnover	13% - 25% (E+M)
Microglial motility	?
Mitochondrial proton leak	20% (E+M)

Energetic cost:	Estimate obtained by:
Undecided	Experiment (E)
Likely 'cheap'	Modelling (M)
Likely 'expensive'	Modelling (M)

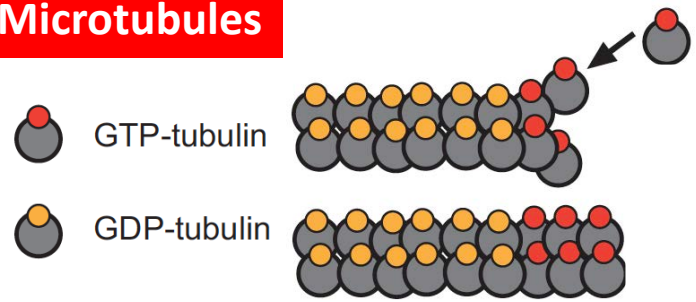


Engl and Attwell. *J Physiol* 593:3417, 2015

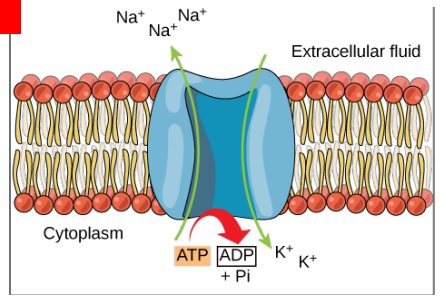
Actin



Microtubules

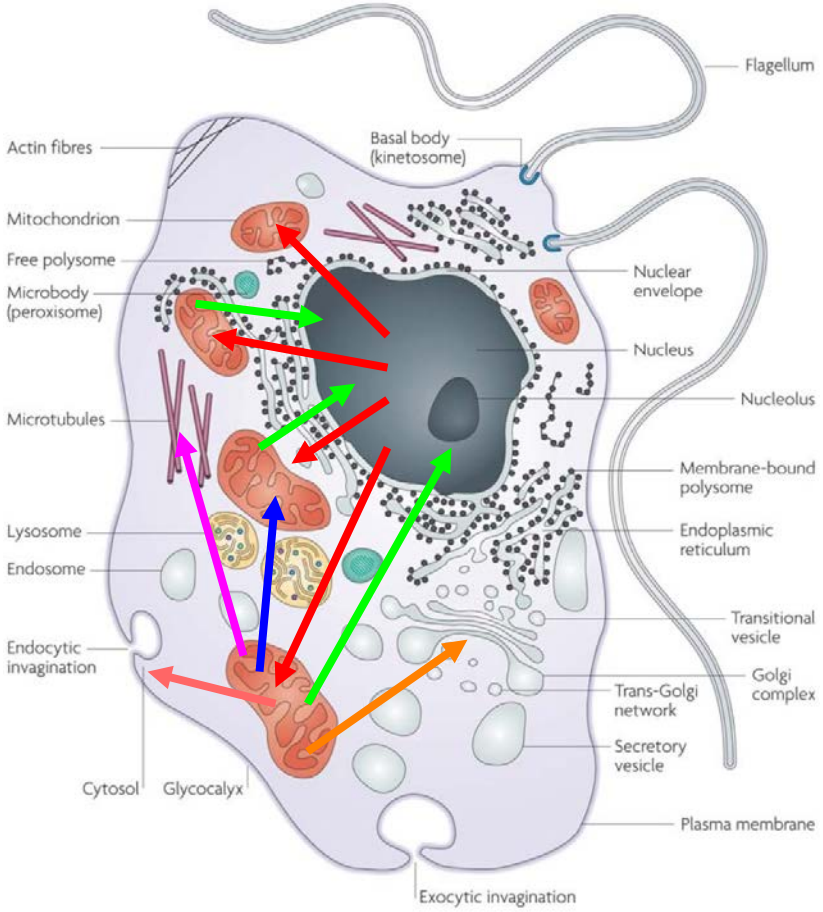


Na-K ATPase



Only intermediate filaments assemble without the need for ATP or GTP.

Primary Mitochondrial Disease—are all disorders of intra- and intercellular communication



- The causes are genetic
- The symptoms are metabolic

Mitochondrial medicine: A metabolic perspective on the pathology of oxidative phosphorylation disorders **Minireview**

Jan A. Smeitink,[1,*] Massimo Zeviani,[2] Douglass M. Turnbull,[3] and Howard T. Jacobs[4,5]

Cell Metabolism, 2006

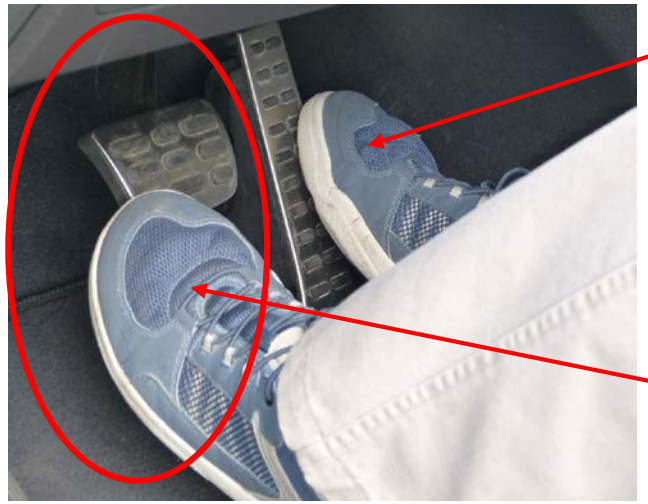
Metabolic Symptoms of Primary Mitochondrial Disease. Q: Should single symptoms be the target of clinical trials in primary mitochondrial disease?

- Developmental delay
- Seizures
- Liver failure
- Stroke-like episodes
- Renal tubular acidosis
- Vision loss
- Hearing loss
- Diastolic dysfunction—diastolic hypertension
- Headache
- Heart block
- Heart failure
- GI dysmotility
- Pseudoobstruction
- Microbiome dysfunction
- Ptosis
- Ophthalmoplegia
- Dysarthria
- Muscle weakness
- Ataxia/Imbalance
- Muscle pain
- Neuropathy
- Speech delay
- Chronic Fatigue/Poor Endurance
- Dysautonomia
- Immune dysfunction
- Sleep disturbances

Very General Conclusions from > 50 Clinical Trials—
The mechanism of all successful developmental therapies:

Disease puts pressure on the brakes of development

This



Maximum speed is an intrinsic property of child development—this is not druggable

Catch-up development occurs for a few months then settles back to a sustainable rate, eg PKU

Not This

Effective treatment lifts the pressure on the brakes

Sustainable treatments don't add pressure to the gas pedal

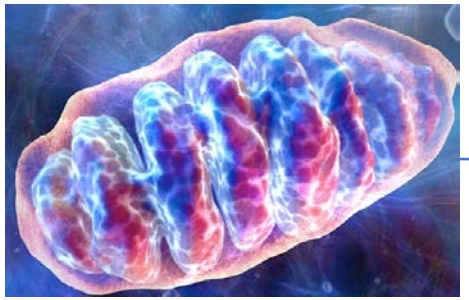
Complications at the Crossroads of Primary and Secondary Mitochondrial Dysfunction

Immunomitochondrial Biology

The “Secret Life of Mitochondria” leads to regulated changes in mitochondrial function

Mitochondrial function changes to fight infection, to learn, and to heal

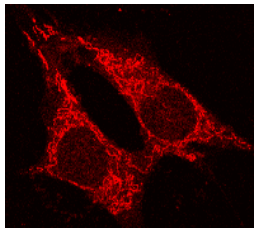
Health and Fitness



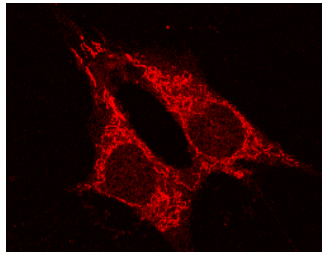
Mitochondria

Injury → Healing

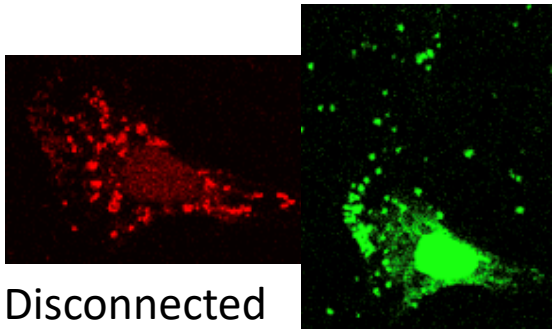
Effector Release



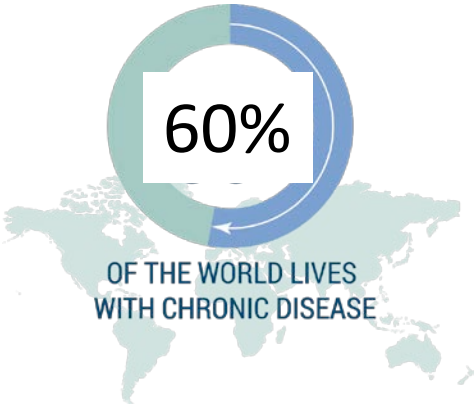
Reconnected Mitochondria



Connected

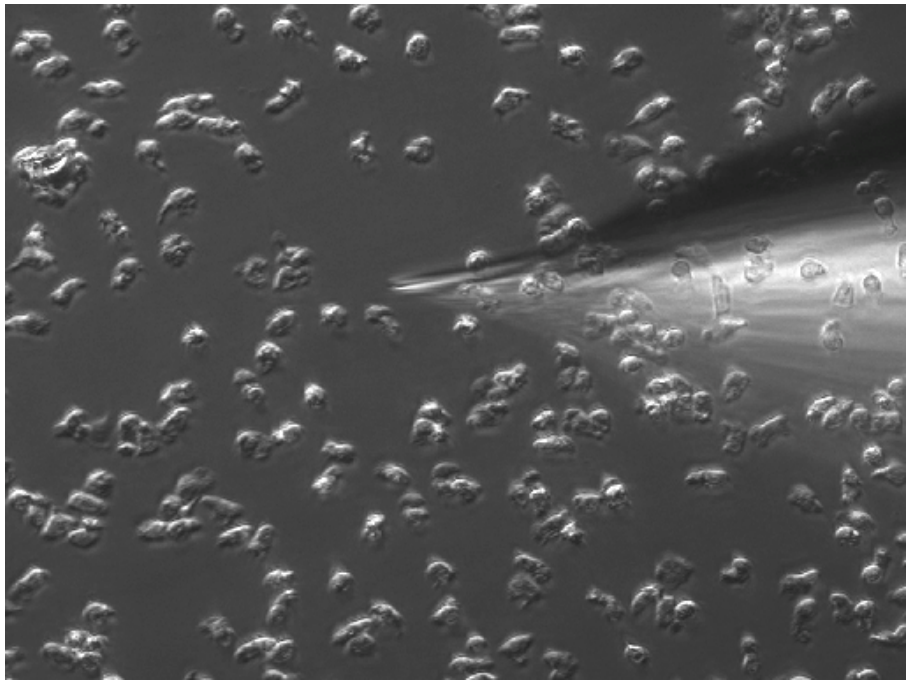


Disconnected

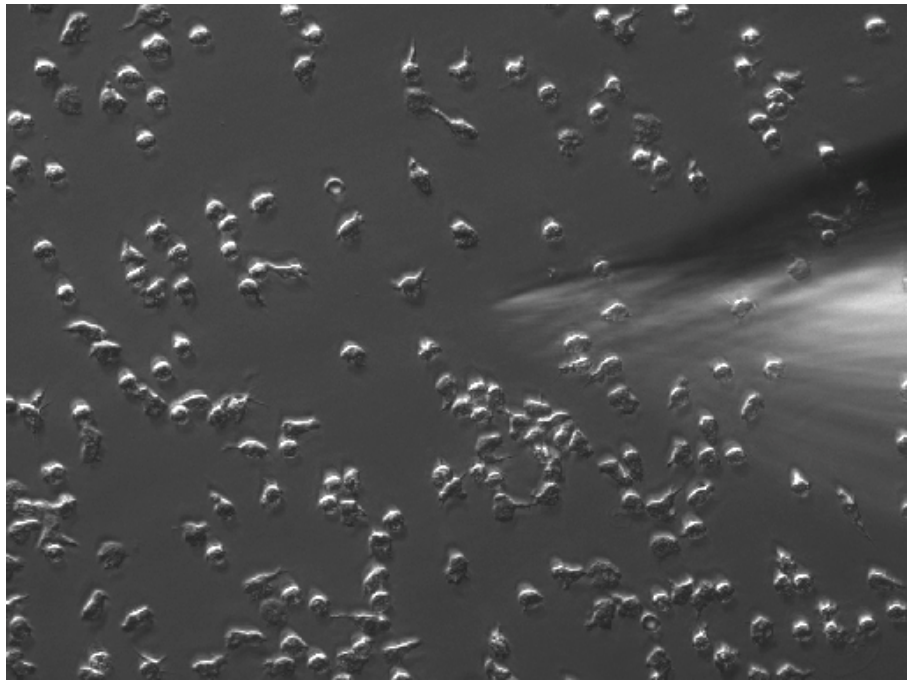


Chronic Disease

(40% of Children in US)



Pipet with mtDNA



Pipet with mtDNA +
α-Formyl Peptide Receptor Ab

Free mtDNA, ATP, and mitochondrial peptides act as effectors of the cell danger response (CDR) and are chemotactic for neutrophils, T-cells and macrophages.

From Zhang/Hauser. Circulating Mitochondrial DAMPs Cause Inflammatory Responses to Injury. *Nature* 464:104, 2010

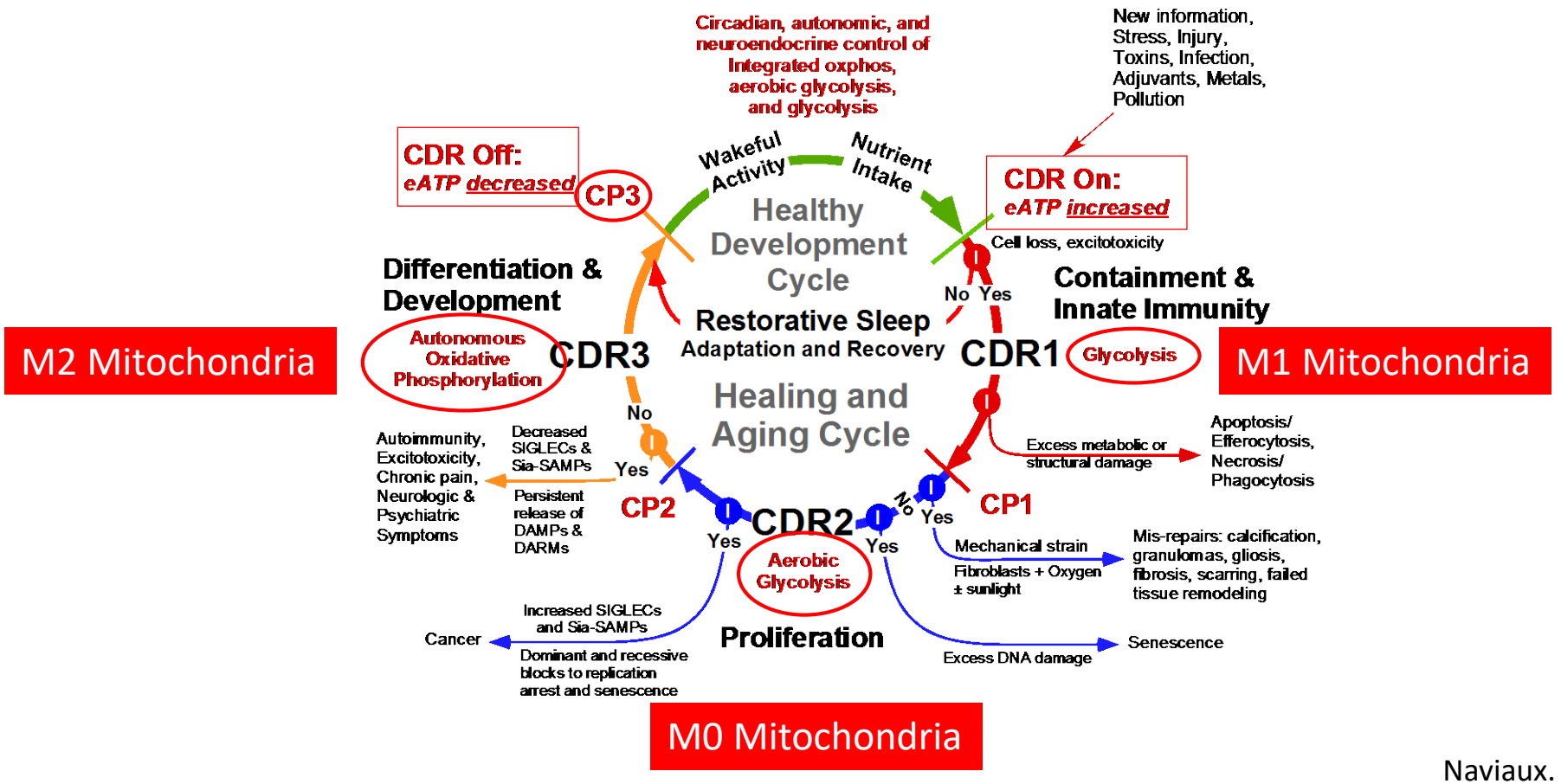
Three programmed forms of mitochondria are needed to heal—M1, M0, and M2

No.	Trait	Mitochondrial Phenotype[40,41,46] M1	M0	M2
1	Cellular energy metabolism	Glycolysis	Aerobic glycolysis	Oxidative phosphorylation
2	Mitochondrial DNA copy number	Low	Intermediate	High
3	Predominant morphology	Punctate	Intermediate	Filamentous
4	Cell replicative potential	Intermediate	High (Warburg)	Low
5	Cell multilineage regenerative potential	Low	High	Low
6	Cell differentiation potential	Intermediate	Low	High
7	Cell cancer potential	Intermediate	High	Low
8	Inflammatory potential	High	Intermediate	Low
9	Cell susceptibility to killing by apoptosis	Intermediate	Low	High
10	Inducible organellar quality control	Low	Intermediate	High
11	Baseline oxygen consumption	Low	Low	High
12	Stressed (uncoupled) oxygen consumption above baseline (spare respiratory capacity)	Low	Intermediate	High
13	ROS production	High	Intermediate	Low
14	NLRP3 inflammasome assembly	High	Low	Low
15	Lactate release from cells	High	Intermediate	Low
16	Pentose phosphate pathway (PPP)	Intermediate—NADPH for NOX	High—NADPH for biosynthesis and cell growth	Intermediate—NADPH for redox
17	Use of fatty acid oxidation (FAO)	For ROS and NLRP3 activation	Fatty acid synthesis for growth > FAO	For oxphos
18	Use of glucose	Glycolysis and lactate release	Glycolysis and PPP	PPP and pyruvate for oxphos
19	Use of glutamine	Low	High: citrate for ATP citrate lyase and Acetyl-CoA	High: oxphos via alpha-ketoglutarate
20	Stage of greatest use in the healing cycle and cell danger response	CDR1	CDR2	CDR3

These are normal states of mitochondrial function required for healing.

Naviaux, *Biology*, 2019. doi: 10.3390/biology8020027

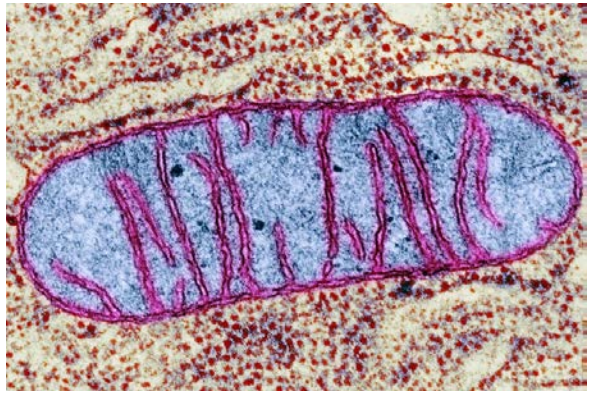
The Problem of Programmed States of Mitochondrial Function: the Healing Cycle and the *Choreography of Complementarity*

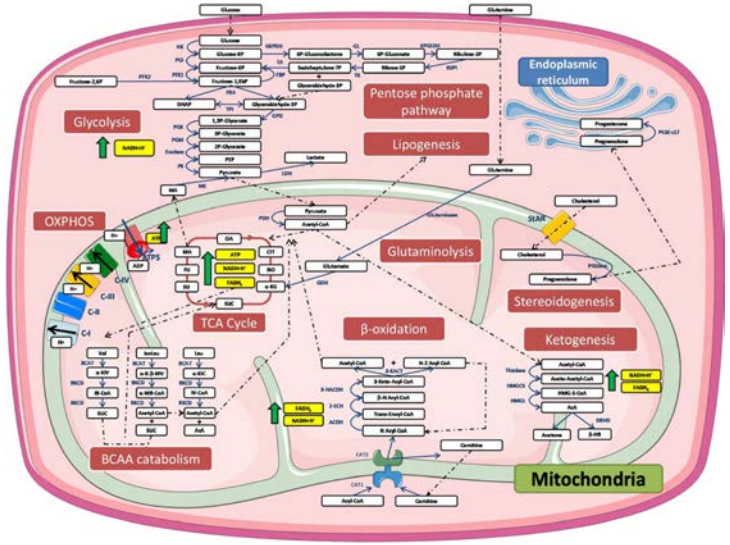


Naviaux. *Mitochondrion,* 2019

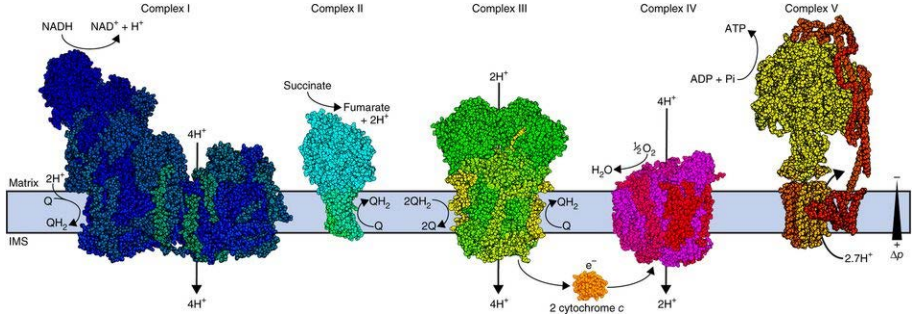
The Problem of Multiple, Correlated Functions

- No effective drug for mitochondrial disease will have a single action
- Improved mitochondrial function will improve many symptoms





Oxphos creates the gradients



Oxygen and electron gradients create the Potential energy that drives the reactions of life.

Mitochondria are semi-solid state bioreactors. Hydration = 50%.

Metabolically related proteins are tightly packed to facilitate substrate-product channeling.

The Problem of Season of Enrollment Effects

The Otolaryngological Manifestations of Mitochondrial Disease and the Risk of Neurodegeneration With Infection

Joseph L. Edmonds, MD; Daniel J. Kirse, MD; Donald Kearns, MD; Reena Deutsch, PhD; Liesbeth Spruijt, MD; Robert K. Naviaux, MD, PhD

Arch Otolaryngol Head Neck Surg, 2002.

PMID: 11926907

Results:

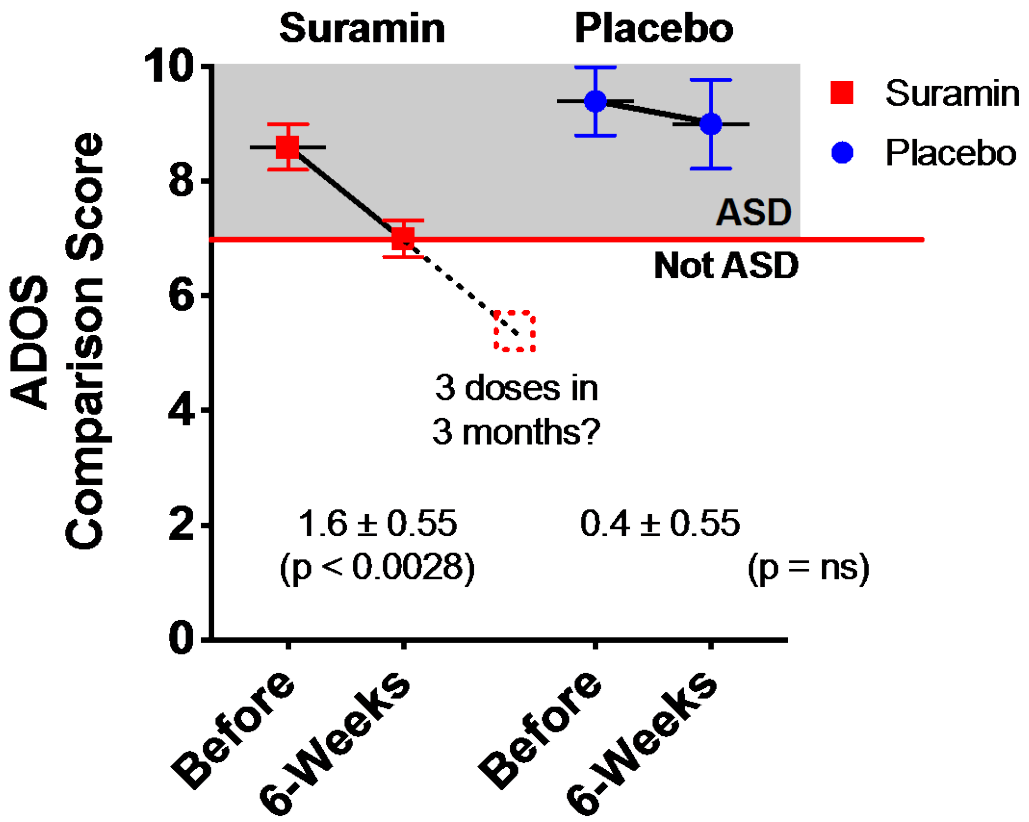
1. 60% of children with primary mitochondrial disease have an episodic course
2. 72% suffered neurodegeneration events with seasonal intercurrent infection

Conclusion:

Children enrolled for 3 months in the winter will have worse outcomes and more adverse events than children enrolled for 3 months in the summer.

The Time's Arrow Problem—Non-reversibility of development after Washout—Crossover challenges

Maximum Possible Developmental Improvement Rate = 1.6 ADOS2 Points in 6 Weeks. This is 0.25 points/week



When can pediatric studies use patients as their own controls?

Placebo first, then treatment—OK.
Treatment first, then placebo—Not OK.
Asymmetry creates statistical challenges.

Minimum time to observe a 2.0 point improvement at 0.25/week in the suramin group is 8 weeks.

If 6 weeks is the outcome time, then the study will only detect a 1.5 point (0.25 per week x 6) Improvement, and the Subject # must increase from N = 36 to N = 50 for adequate power.

Surrogate Biomarkers of Mitochondrial Dysfunction—Molecules on the Horizon

Serine decreased
Alanine increased

Old

- Lactate
- Lactate/Pyruvate ratio
- Alanine/Lysine ratio
- 3-OH Butyrate/Acetoacetate ratio
- FGF21
- GDF15

New (still being validated)

- 1-Deoxyceramides (m18:1/22:0)
- 1-Deoxydihydroceramides
- 3-OH-Long Chain (C12-C18) Acyl-carnitines
- 2-OH Butyrate/2-Ketobutyrate ratio
- Combinations of “old” and “new” markers

Updates from Clinicaltrials.gov—Past Studies in Mitochondrial Disease

- 44 Phase 1 and 2 registered trials have been completed
- 12 Phase 3 trials completed—no drugs approved/no NDAs
  - CoQ10 x 1 (N = 24)
  - IFNγ1b in Friedreich ataxia x 3 (N = 216)
  - Idebenone in Friedreich ataxia x 5 (N = 529)
  - Pioglitazone in Friedreich ataxia x 1 (N = 40)
  - Curcumin in LHON x 1 (N = 70)
  - ND4-AAV gene therapy in LHON x 1 (N = 37)
- 36 Phase 1-3 trials actively recruiting
  - 28 observational studies
  - 8 interventional clinical trials, but only 1 Phase 3 (DCA for PDH)

Primary Outcome Metric Selection

- Global Scales—History, Physical, Quality of Life
  - Observer reported outcome (ObsRO, 2018)—PMID: 29129554
  - International Pediatric Mitochondrial Disease Scale (2016)—PMID: 27277220
  - Newcastle Pediatric Mitochondrial Disease Scale (2006)—PMID: 17123819
  - Modified Friedreich Ataxia Rating Scale (MFARS)—PMID: 21805332
  - McMaster Gross Motor Function (GMFM-88)—PMID: 23802141
- Functional Scales
  - 6-minute walk test (6MWT)
  - Primary Mitochondrial Myopathy Symptom Assessment (PMMSA)

Clinicaltrials.gov—8 trials still recruiting (R), plus 4 others (C and NR)

Drug	NCT	PMD	Phase	MOA or Target	Primary Outcome
EPI-743	NCT02352896 NR	Leigh	II	Vitamin E-like	NPMDS
Elamipretide	NCT03323749 NR	Myopathy	II	Cardiolipin+	6MWT, PMMSA
Dichloroacetate (DCA)	NCT02616484 R	PDH	III	PDH disinhibition	ObsRO
Nucleosides	NCT03639701 R	TK2	I/II	Pool recovery	Safety (LFTs, EKG, etc)
KL1333	NCT03888716 R	MELAS	I/II	NAD+	Safety (LFTs, EKG, etc)
REN001	NCT03862846 R	Myopathy	I/II	PPAR β/δ agonist	Safety (LFTs, EKG, etc)
Nicotinamide Riboside	NCT03432871 R	Myopathy, PEO, MELAS	I/II	NAD+, biogenesis	Safety, Mito biogenesis
Resveratrol	NCT03728777 R	Myopathy	II	Sirtuins, mitophagy	Exercise heart rate
Resveratrol	NCT03933163 R	Friedreich ataxia	II	Sirtuins, mitophagy	MFARS
ND4-AAV Gene therapy	NCT02161380 R	G11778A-LHON	I/II	ND4 complementation	Safety and Toxicity
KH176	NCT02909400 C	MELAS, Leigh	II	NAD+	Motor deficits
Suramin	NCT02508259 C	Autism spectrum disorder	I/II	ATP and UTP signaling	ADOS (ASD severity)

Christini Fund

...Give a Child Hope and Smiles

UC San Diego Health Sciences

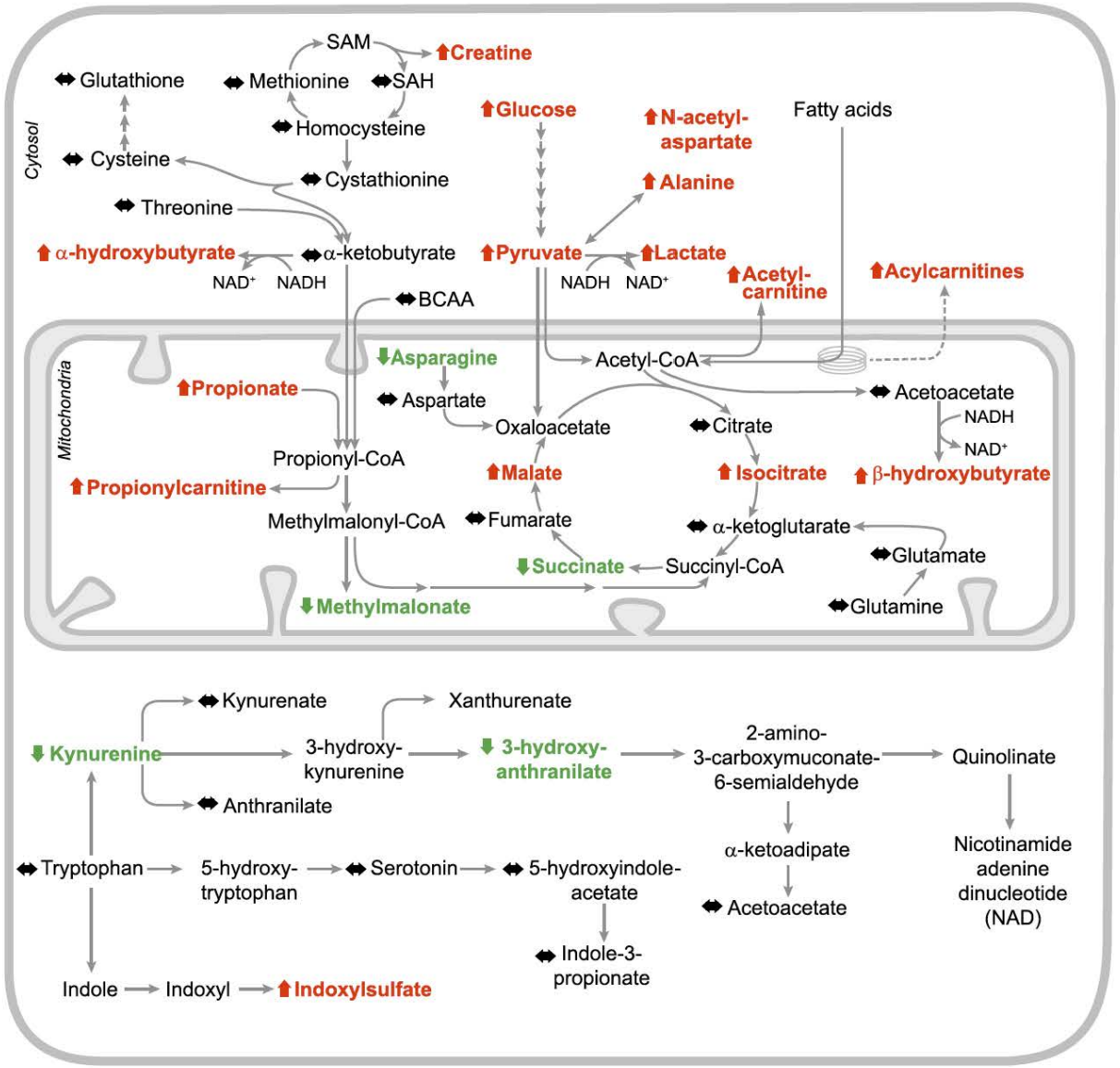
Thank you

Kefeng Li, PhD

Lin Wang, MD, PhD

Jane Naviaux, MD, PhD

NAVIAUX lab



Legault, et al. *Cell Reports* 13:981, 2015. (LRPPRC mutations)