MITOCHONDRIAL DISORDERS

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Children's Hospital of Philadelphia®
Mitochondrial Medicine

DISCLOSURES

Marni J. Falk, M.D. is

- **Co-Founder and Chief Scientific Advisor**
  - **Rarefy Therapeutics LLC**
- **Scientific Advisory Board Member**
  - **United Mitochondrial Disease Foundation (UMDF)**
  - **Pharma Companies: Khondrion, Larimar Therapeutics, RiboNova Inc**
- **Research Collaborator**
  - **AADi, Astellas (Mitobridge), Cyclerion, Epirium Bio, Khondrion, Imel Therapeutics, Neurovive, Minovia Therapeutics, Mission Therapeutics, Raptor Pharmaceuticals, RiboNova Inc, Saol Therapeutics, Stealth BioTherapeutics**
- **Consultant**
  - **Agios Therapeutics, Abliva (formerly Neurovive), Astellas (Mitobridge), Autobahn, Casma Therapeutics, Cyclerion, Epirium Bio, GenoMind, HealthCap, Hibiscus Bio, Imel Biotherapeutics, Minovia Therapeutics, Mission Therapeutics, Neurovive, Precision BioTherapeutics, Primera Therapeutics, Taysha Gene Therapy**
- **PI, CHOP site**
  - **North American Mitochondrial Disease Consortium (NAMDC, RDCRN)**
  - **RTA-408 (Reata), SPIMM-301 (Stealth), IW-6463 (Cyclerion) Clinical Trials (*completed*)**
  - **DCA in PDH (FDA); REN-001 (Reneo); Astellas; SPIMD-301 (Stealth) Clinical Trials (*active*)**

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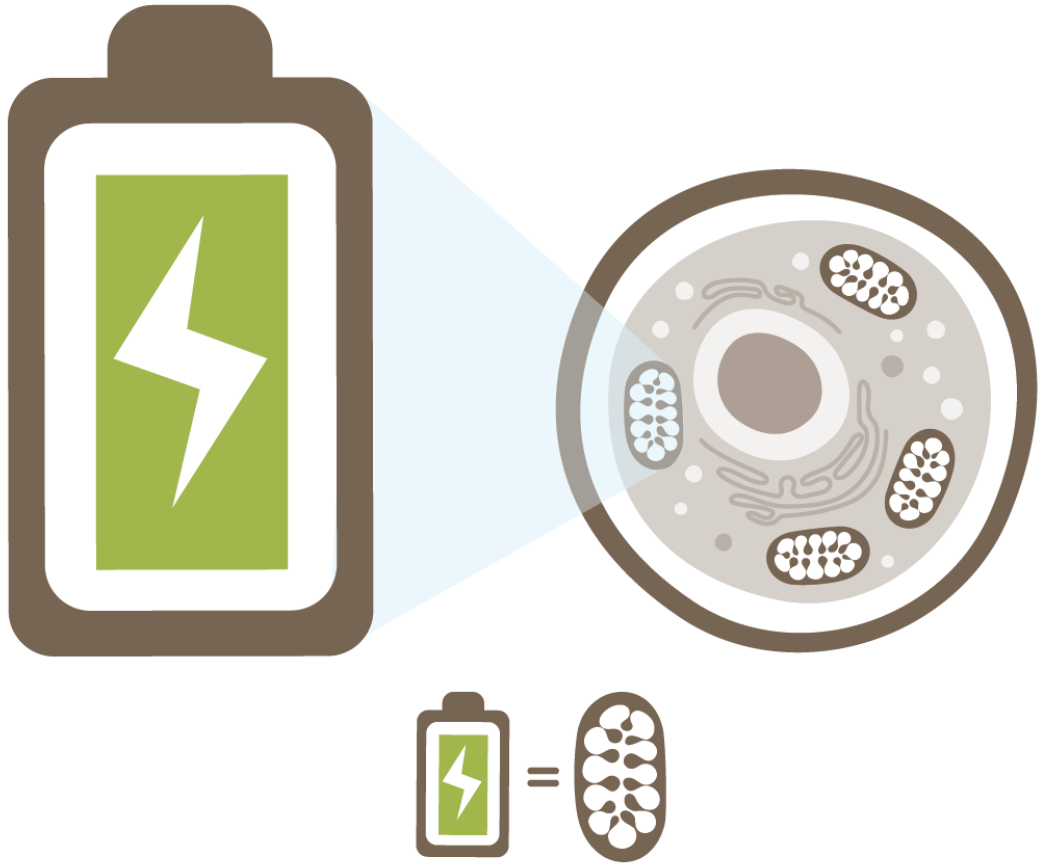
MITOCHONDRIAL DISEASE
DEFINITION AND CLINICAL FEATURES

WHAT DO MITOCHONDRIA DO?

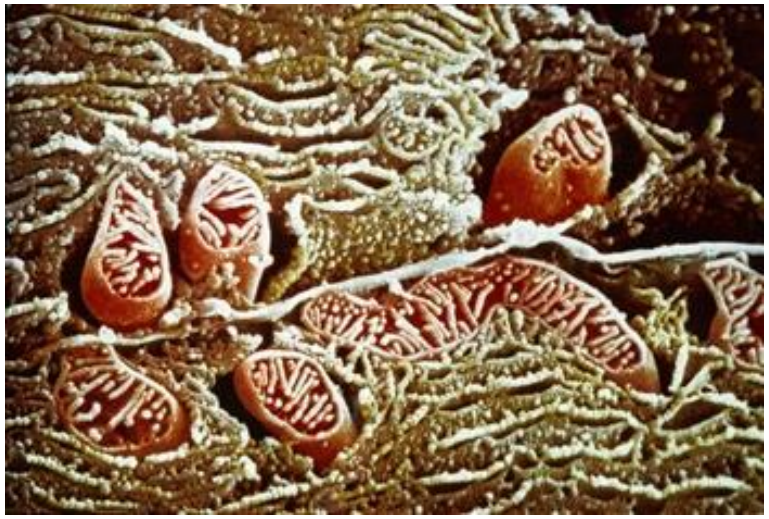
Mitochondria function as batteries that produce energy in the body's cells.

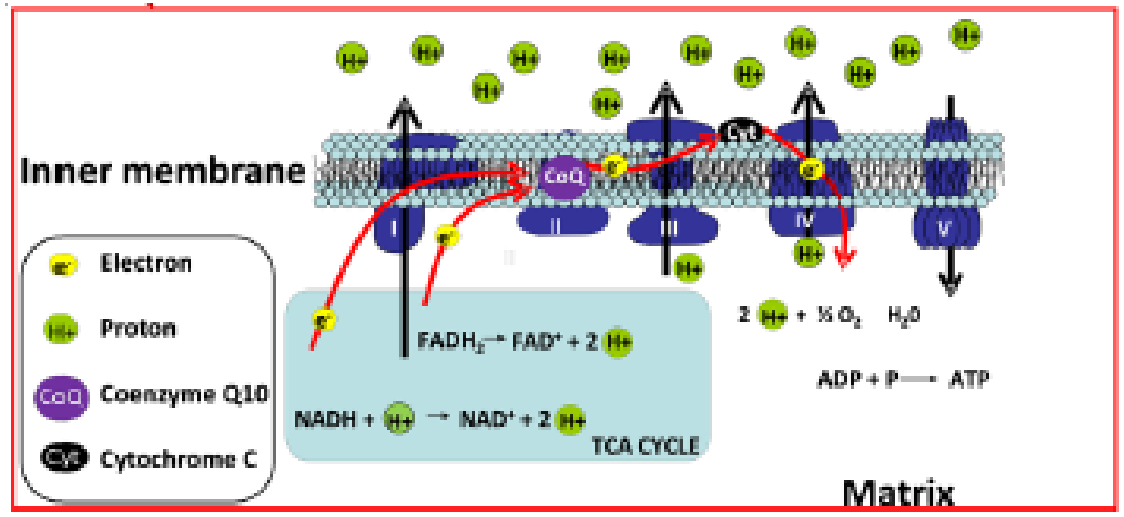
They are particularly important in high-energy demanding organs such as the heart, liver, muscles and brain.



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**

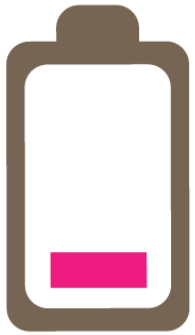




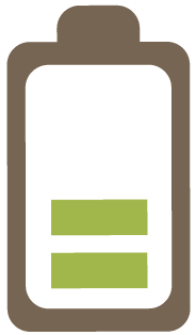
Mccormick E et al, *Current Genetic Med Reports*, 2018

MITOCHONDRIAL ENERGY SPECTRUM

Mitochondria create more than 90% of the energy needed by the body. When they fail, less and less energy is generated within cells. This can lead to cell damage and sometimes result in chronic diseases.



Mitochondrial Disease



Chronic Disease

General Population

Extreme Athletes

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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**
- **Over 350 different gene disorders in 2 genomes***
  - **Mitochondrial DNA:** 37 genes
  - **Nuclear DNA:** >300 genes
- **Collectively affect > 1 in 4,300 people**

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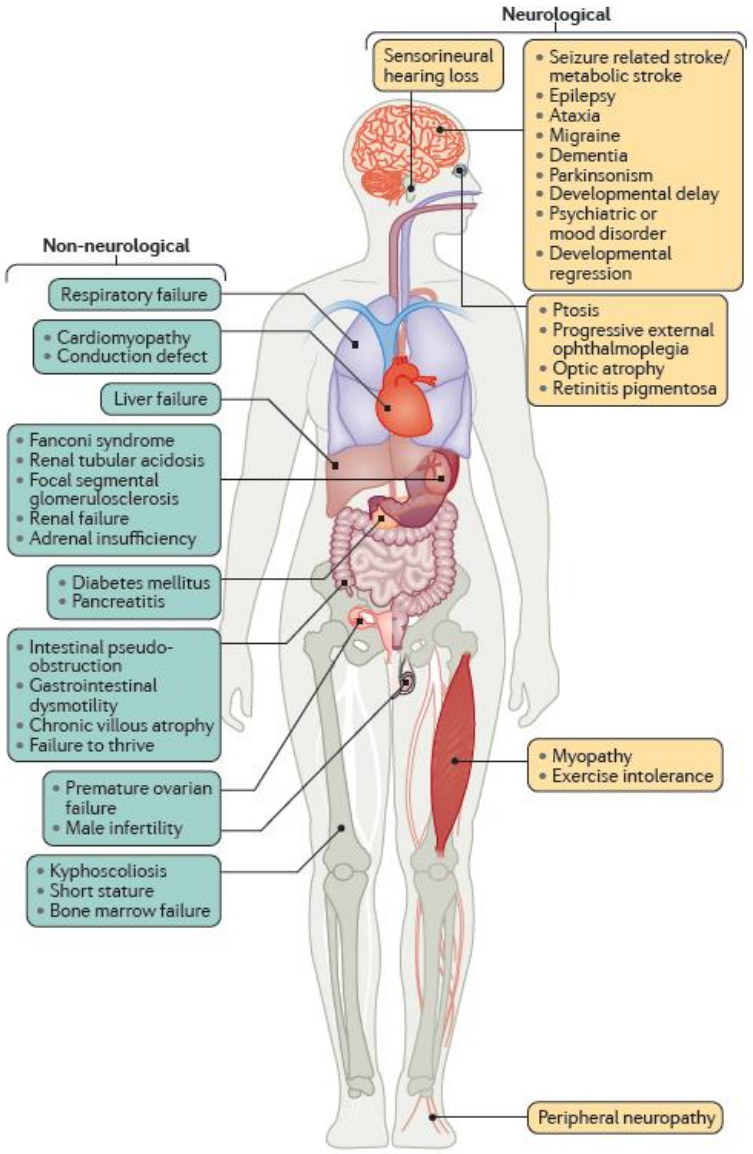
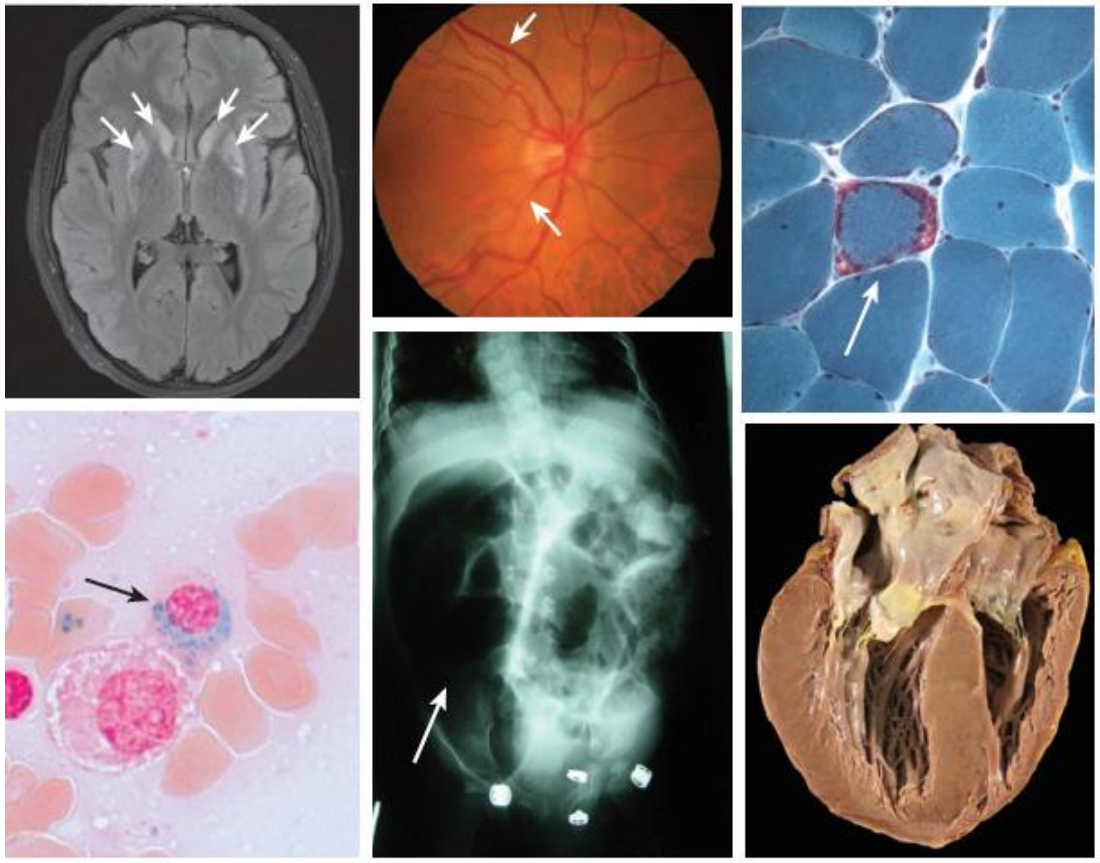
**McCormick et al, Neurotherapeutics, 2013; McCormick et al, Curr Genet Med Rep, 2018*

CLINICAL FEATURES OF MITOCHONDRIAL DISEASES





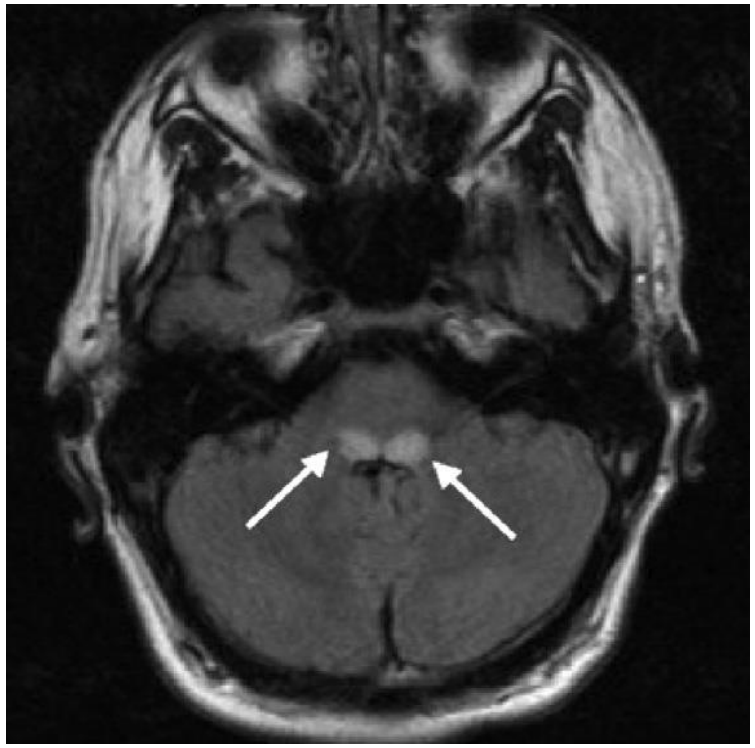
~16 SYMPTOMS PER PATIENT!

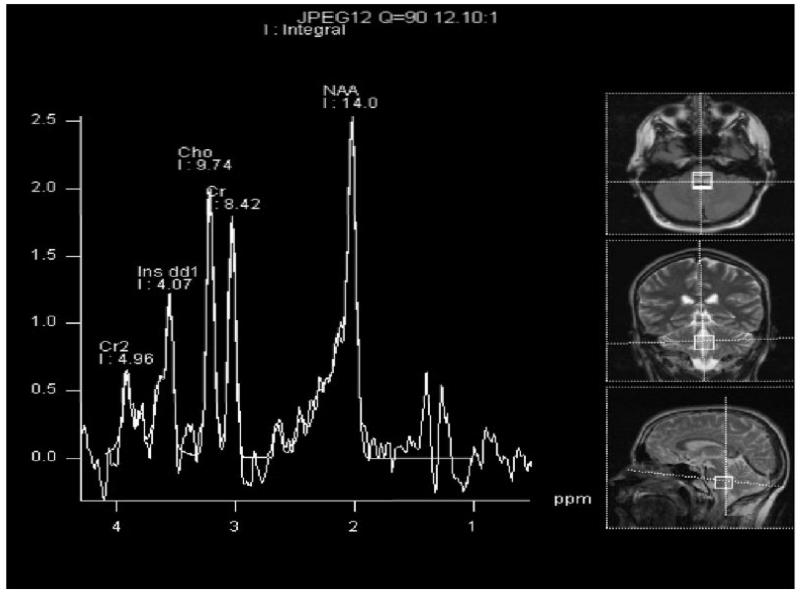
Gorman G et al, *Nat Rev Dis Prim*, 2016; Vafai and Mootha, *Nature*, 2012

LEIGH SYNDROME

- **Most common mitochondrial disease pediatric presentation**
  - **Neurodevelopmental regression**
  - **Metabolic strokes on brain MRI**

- **113+ genetic causes**
  - **All inheritance patterns occur**
  - **NICHD U24 expert-panel curation effort***
    - **ClinGen & ClinVar integration**
    - **MSeqDR: https://mseqdr.org**





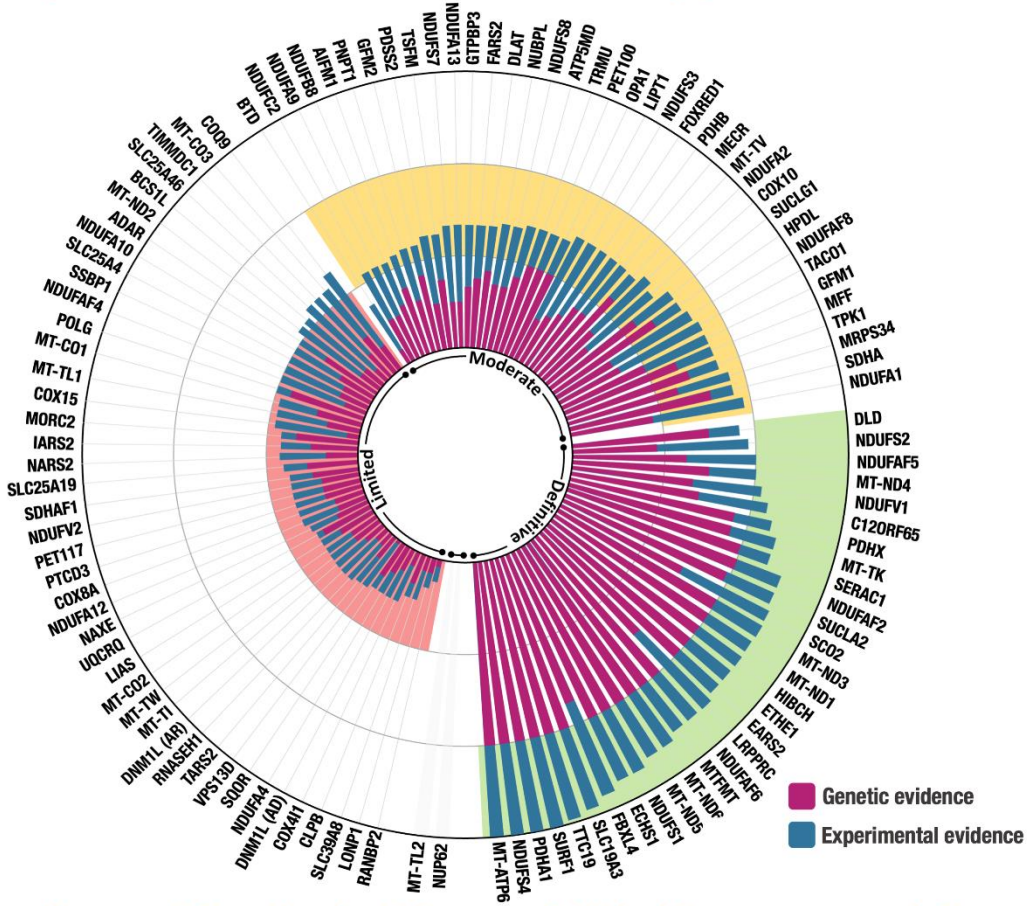
Lake et al, 2016

*McCormick E et al, *Annals Neurol*, 2023; U24-HD086984

Expert panel curation of 113 primary mitochondrial disease genes for the Leigh syndrome spectrum

McCormick, Keller, … Rahman (2023)

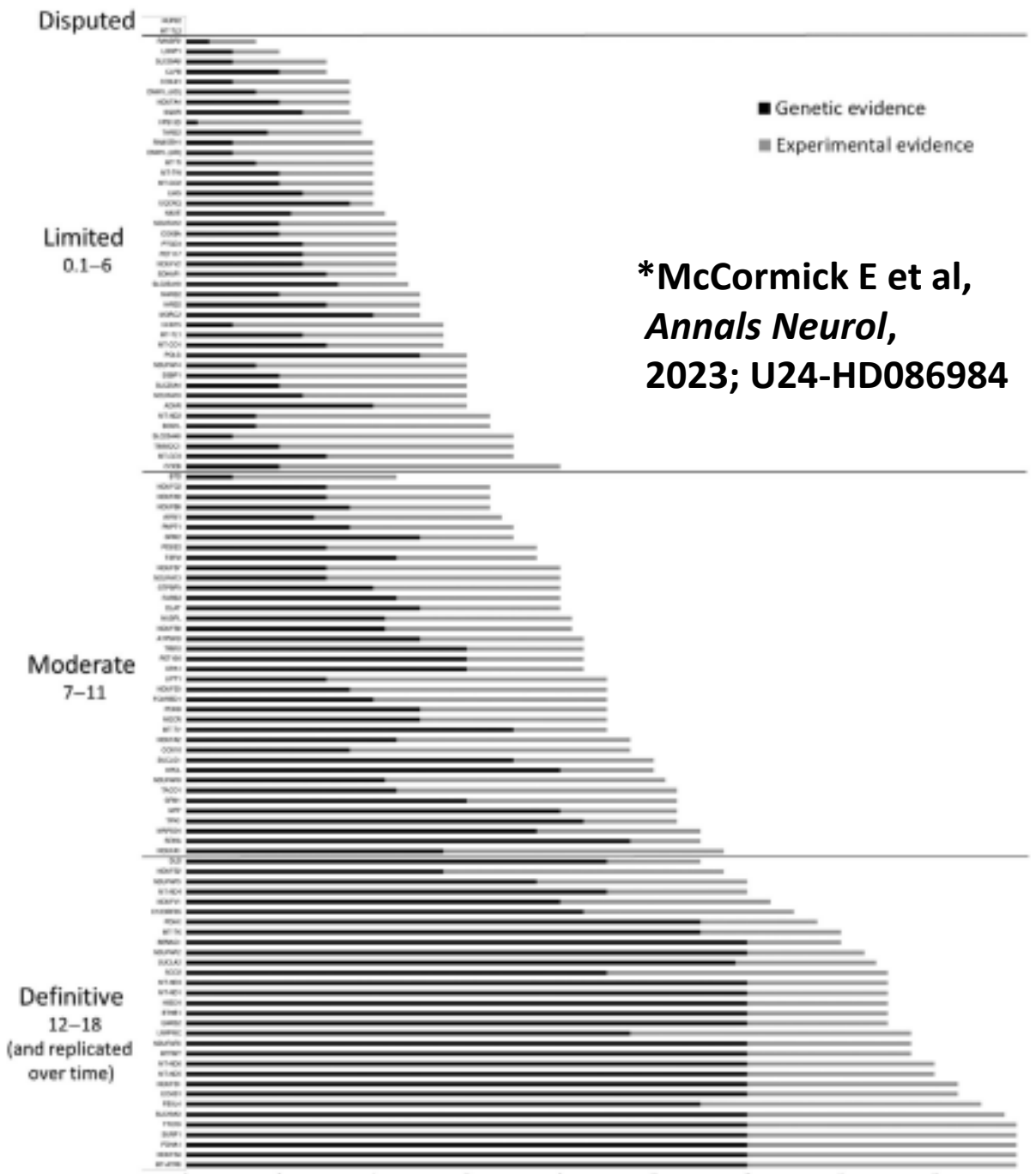
Leigh syndrome spectrum (LSS) is the most common manifestation of Primary Mitochondrial Disorder in children and may present in adults too.



Gene-disease relationships for LSS were established for genes across both nuclear and mitochondrial genomes.

This will allow improved diagnostics and facilitate disease surveillance, reproductive counselling, natural history studies and study design.

AMERICAN NEUROLOGICAL ASSOCIATION
WILEY
ISSN 0364-5134

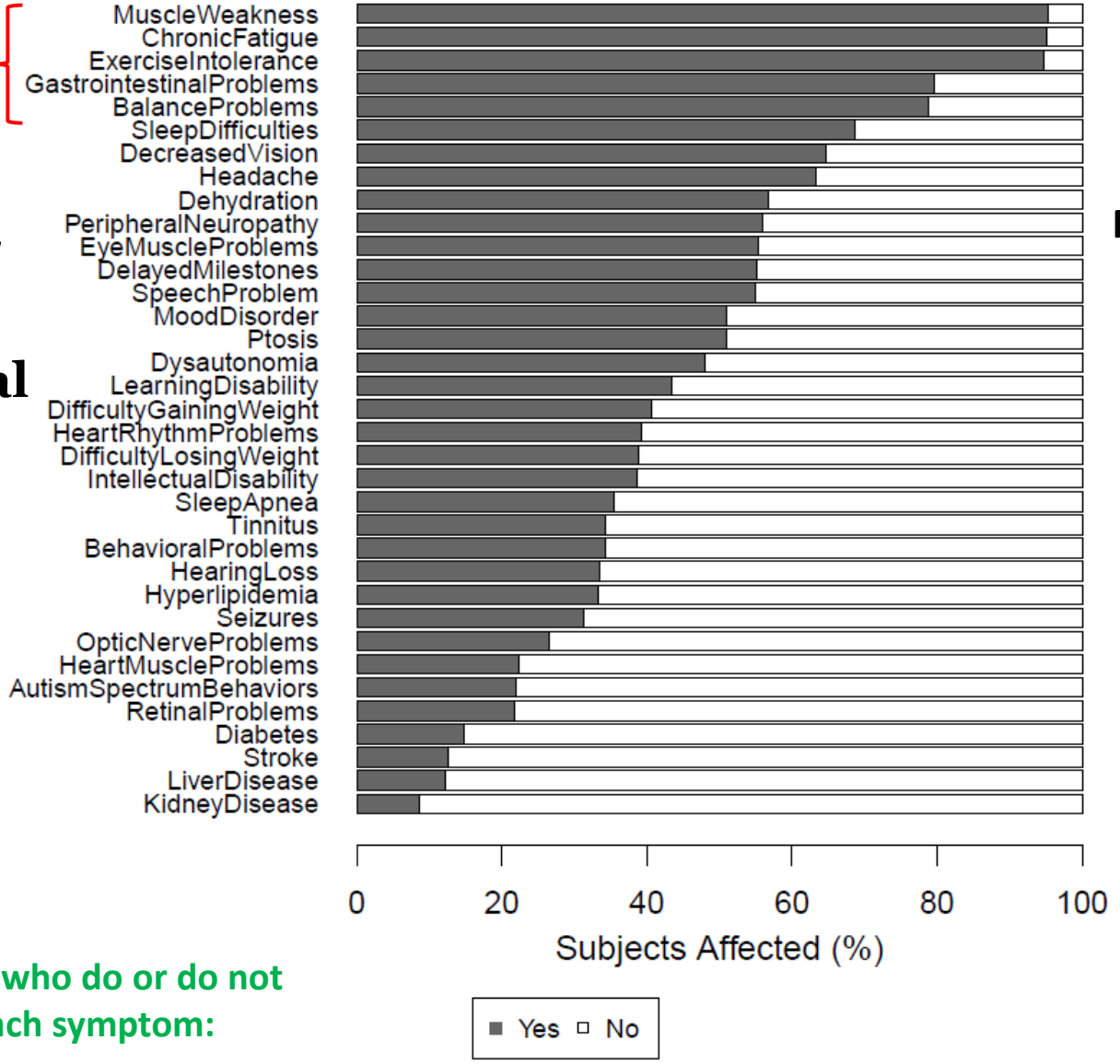


***McCormick E et al, *Annals Neurol*, 2023; U24-HD086984**

FIGURE 3: Scores for each curated gene–disease relationship with Leigh syndrome spectrum by the Mitochondrial Disease Gene Curation Expert Panel. The default scoring range for a definitive classification is 12–18, moderate is 7–11, and limited is 0.1–6.

Mitochondrial disease symptom frequency

Top 5 symptoms *experienced* by mitochondrial disease patients



Survey of 290 Mitochondrial Disease Patients in RDCRN*

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

*Zarazuela Zolkipli-Cunningham, *PLOS ONE*, 2018

ARE THERE CLINICAL DIAGNOSTIC CRITERIA FOR MITO DISEASE?

- **Walker Criteria (1996)**
- **Modified Thorburn Criteria (2002)**
- **Nijmegen Criteria (2006)**
- **Newcastle Criteria (Adult and Pediatric scales)**
  - **All heavily weighted on clinical + biochemical findings**
  - **Genetic etiologies generally not known when criteria established**

www.mitosoc.org

HISTORIC MITO DISEASE CLASSIFICATIONS:

Definite Probable Possible Unlikely

NO COMMON BLOOD OR URINE BIOMARKER EXISTS FOR ALL MITOCHONDRIAL DISEASES

- **Lactate has low sensitivity AND specificity for mitochondrial disease**
  - **FGF-21, GDF-15**
  - **Exercise testing (CPET)**
- **Other analytes may increase suspicion, but neither their detection or absence is diagnostic**

Biochemical Analysis for Mitochondrial Dysfunction		
Amino Acid (plasma/CSF)	**Organic Acid** (urine)	**Acylcarnitines** (plasma)
• Elevated alanine • Elevated glycine, proline, sarcosine or tyrosine	• TCA cycle intermediates • Ethylmalonate • 3-methyl-glutaconate • Dicarboxylic acids	• Low free carnitine • Elevated acyl:free carnitine ratio • Elevations suggestive of disrupted fatty acid oxidation

Haas RH et al, *Molecular Genetics and Metabolism*, 2008

TISSUE BIOCHEMISTRY ROLE IN MITOCHONDRIAL DISEASE DIAGNOSIS

- **OXPHOS disorders are most common inborn errors of metabolism**
  - **Combined prevalence ~1 in 4,300 across all ages**

- **Polarographic OXPHOS analysis of respiratory capacity**
  - **Historically, the diagnostic "Gold Standard"**
  - **Measures integrated mitochondrial function**
  - **Freshly isolated cells or tissues**

- **Electron transport chain (ETC) enzyme activities analysis**
  - **Fresh *vs* frozen tissue**
  - **Widely accessible and utilized**
    - **Not directly concordant with OXPHOS results**

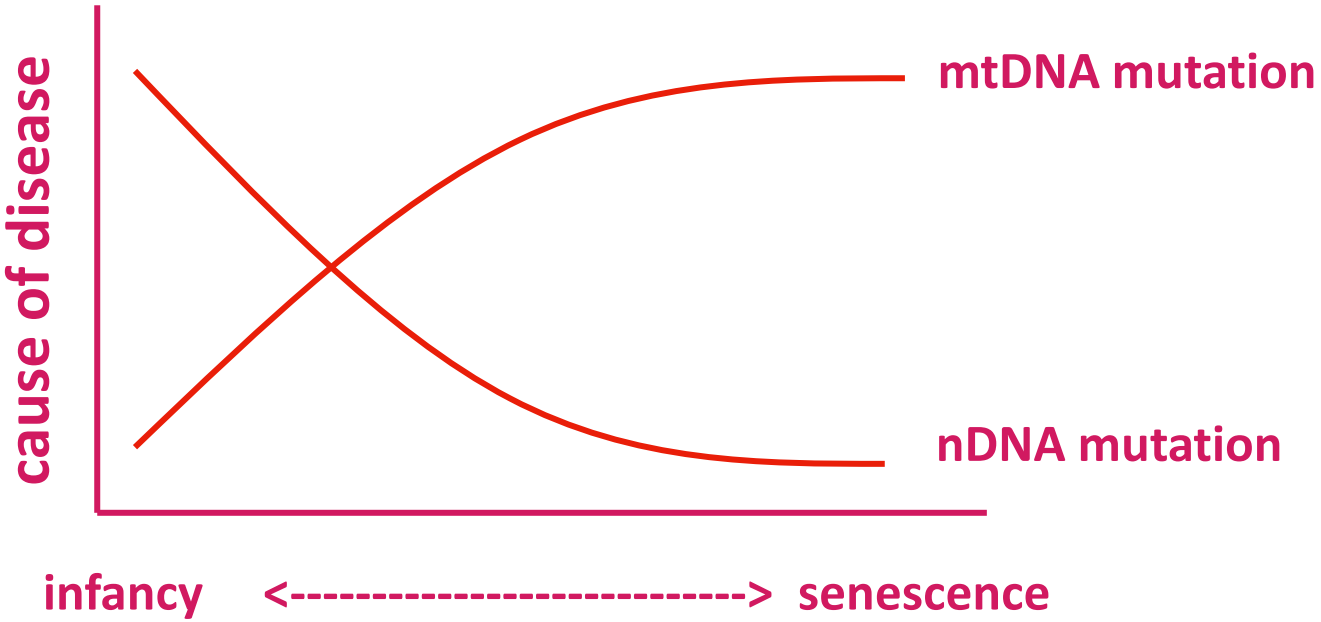
MITOCHONDRIAL DISEASE
CAUSES
AND
DIAGNOSTIC APPROACH

MITOCHONDRIAL DISEASE ETIOLOGIES

- ***PRIMARY*:**
  - **Genetic based mitochondrial dysfunction**
    - **Nuclear or mitochondrial DNA pathogenic variant**
  - **>95% disease genes encode mitochondrial proteins**
    - **Chronic and/or stress-induced disease**

- ***SECONDARY*:**
  - **Mito dysfunction occurs as secondary finding in other disorder**
    - **Acute or chronic mitochondrial impairment**
    - **Gene disorders in which mitochondria are impaired as an "innocent bystander" effect**
    - **Toxic, pharmacologic, or environmental exposure**
    - **Aging**

GENOMIC CONTRIBUTION TO MITOCHONDRIAL DISEASE VARIES ACROSS THE LIFESPAN



- **Diseases with immediate onset after birth (congenital lactic acidosis) are most frequently due to autosomal recessive <u>nDNA</u> defects**

- **Diseases with later (adult) onset more often result from <u>mtDNA</u> mutations**

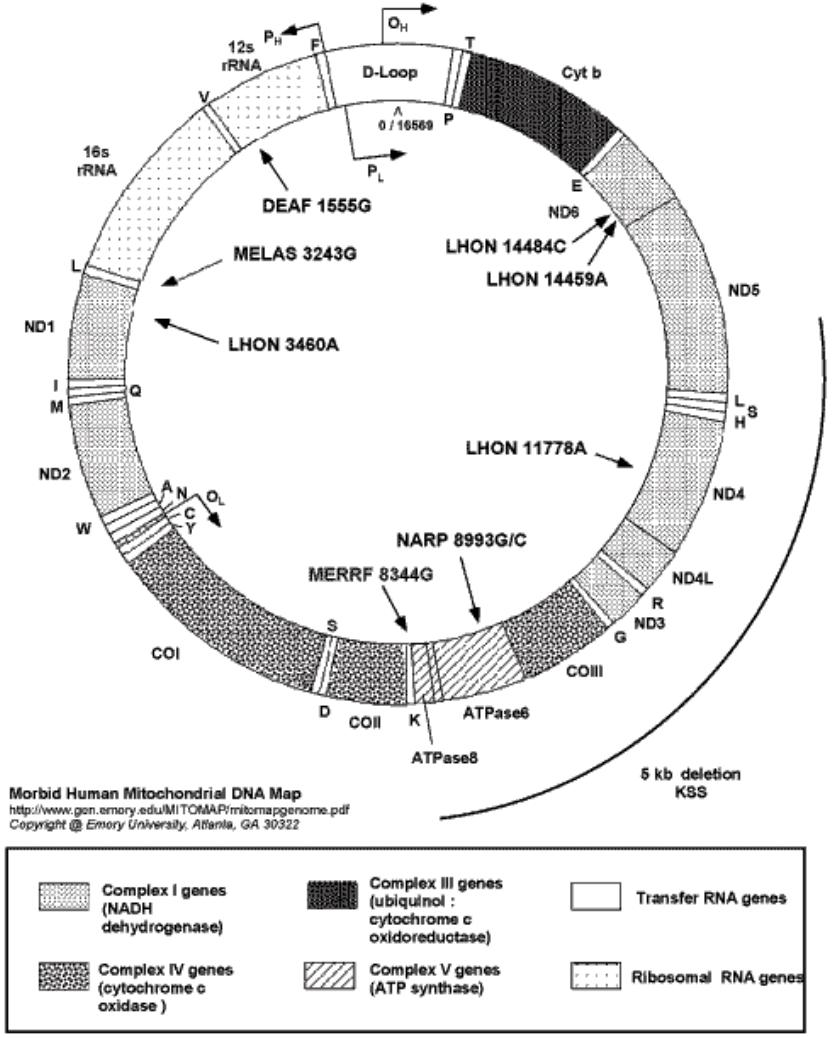
mtDNA DISORDERS

MITOCHONDRIAL DNA (mtDNA)

- **Mitochondrial genome has 16,569 base pairs**
  - **Double-stranded**

<u>Mammalian mtDNA contains 37 genes</u>:

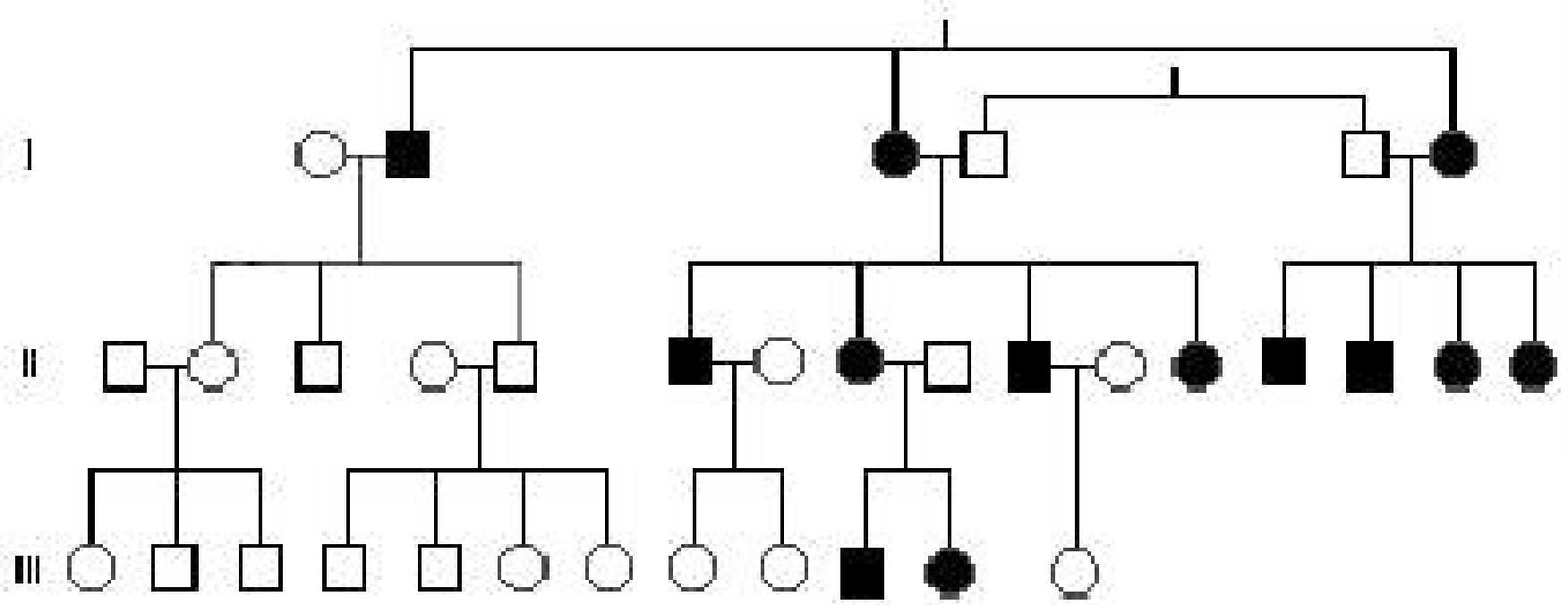
- **13 polypeptides**
  - **Complex I: 7/45 subunits**
  - **Complex II: 0/4 subunits**
  - **Complex III: 1/11 subunits**
  - **Complex IV: 3/13 subunits**
  - **Complex V: 2/12-13 subunits**
- **22 tRNAs**
- **2 rRNAs (12S and 16S)**



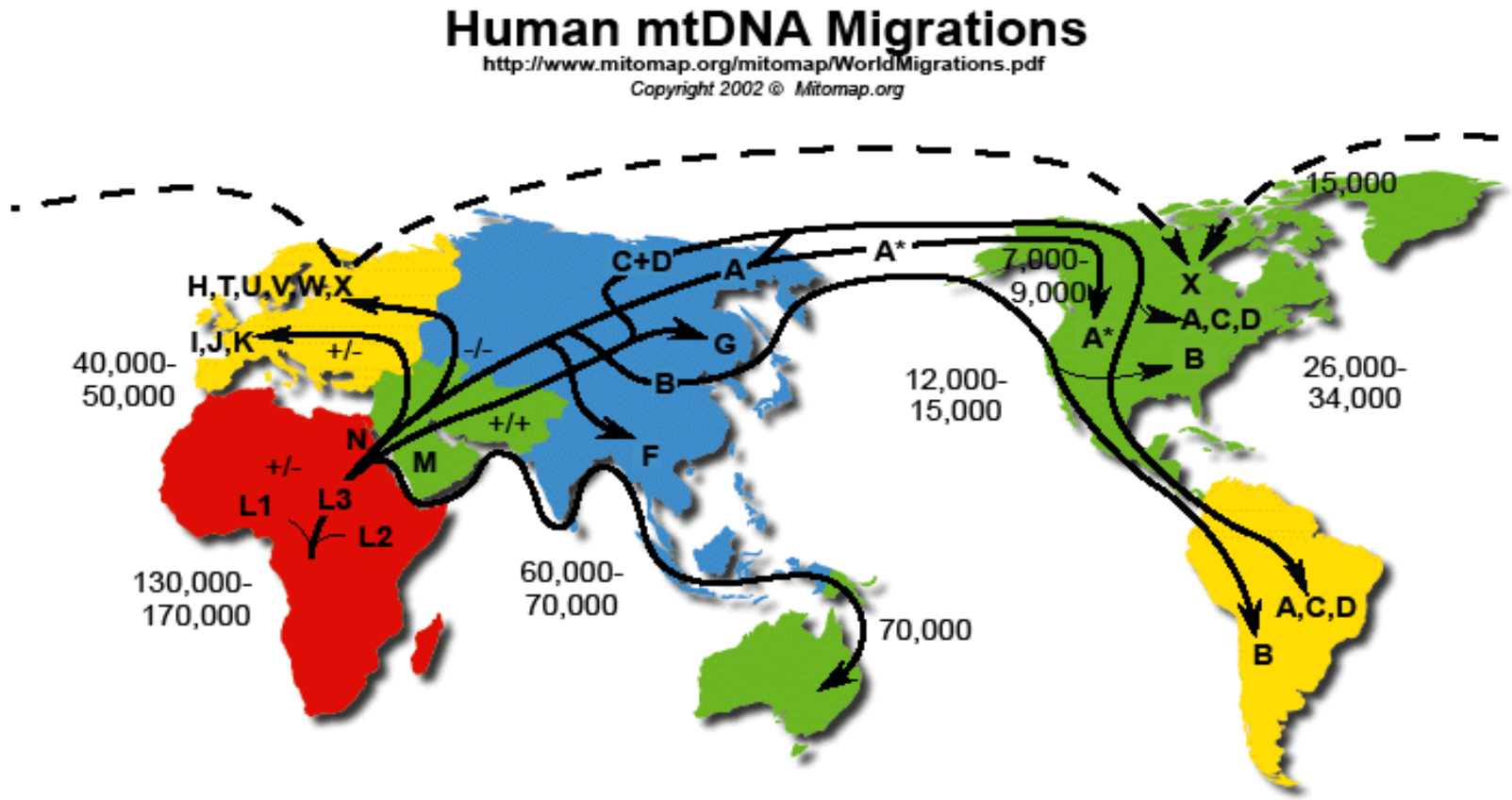
KEY FEATURES OF mtDNA GENOME

- **No introns**
- **No homologous recombination or meiosis**
- **Replication is continuous, not synchronized with cell cycle**
- **Relative to nDNA, mtDNA has a high mutation rate**
  - **mtDNA exists in a "nucleoid" but has no histones**
- **Disease-causing mtDNA mutations occur in tissue-specific fashion**
  - **Point mutations (single or few nucleotide basepairs)**
  - **Deletions or duplications (common 5 kilobase deletion)**
  - **Depletion or proliferation (mtDNA genome copy number change)**

mtDNA IS MATERNALLY INHERITED



NATURAL mtDNA VARIATION DEFINES HAPLOGROUPS AND HUMAN EVOLUTION



"Number of (fixed, homoplasmic) mtDNA differences between any 2 people indicates the time since they shared a common mother" – Douglas Wallace, PhD, CHOP CMEM/Upenn

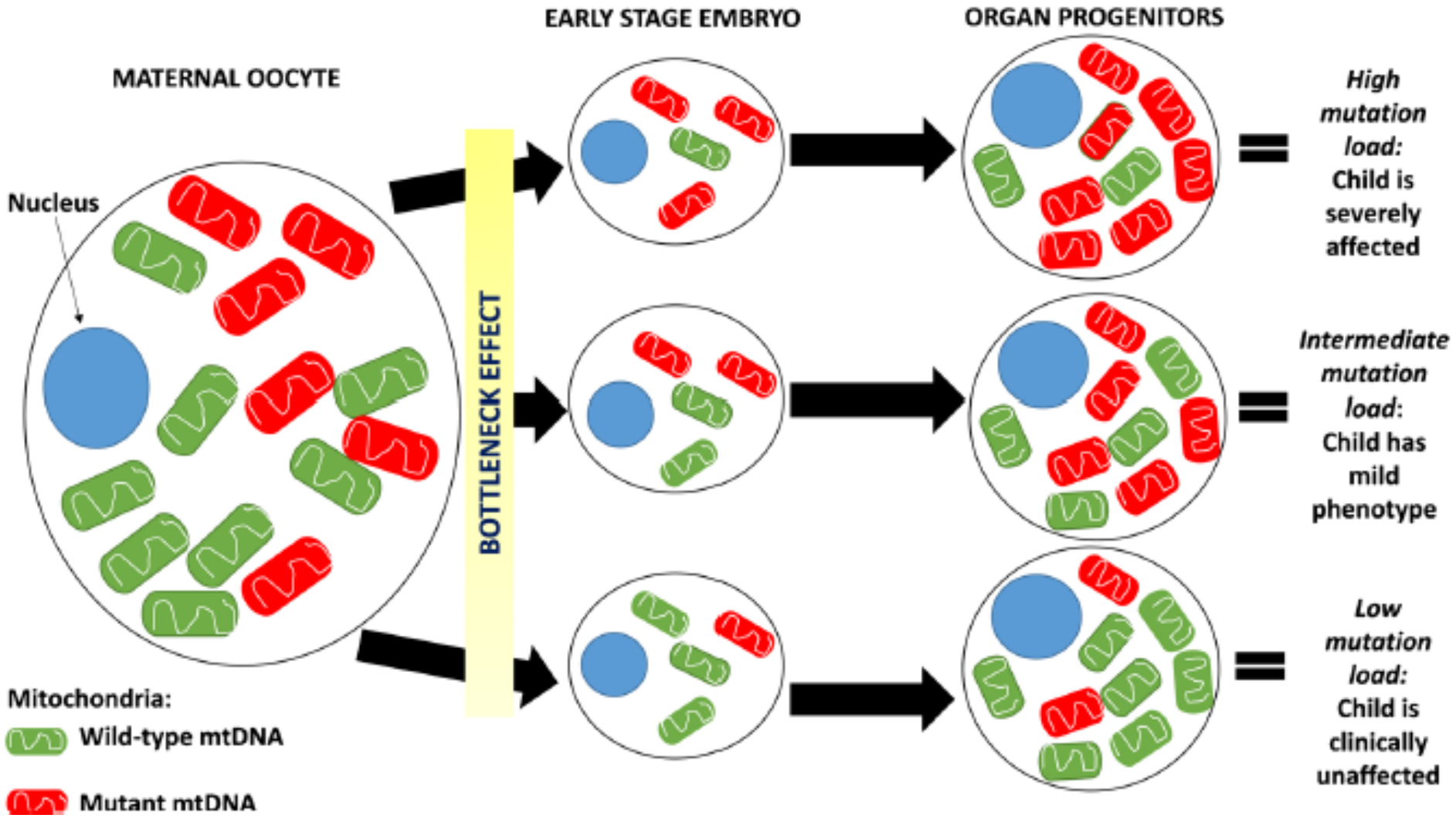
MAJOR mtDNA DISEASE CONCEPTS: HETEROPLASMY & THRESHOLD EFFECT

- **Multiple copies of mtDNA are in every cell/tissue/organ**
  - **2-10 genomes per mitochondrion**
  - **10^2-10^3 mitochondria per cell**

- **"Heteroplasmy" vs. "Homoplasmy" for a mtDNA mutation**
  - **"Homoplasmic wild-type" = only wild-type mtDNA present**
  - **"Homoplasmic mutant" = only mutant mtDNA present**
  - **"Heteroplasmy" = 2 different populations of mtDNA are present in a given cell or tissue (eg, wild-type and mutant)**

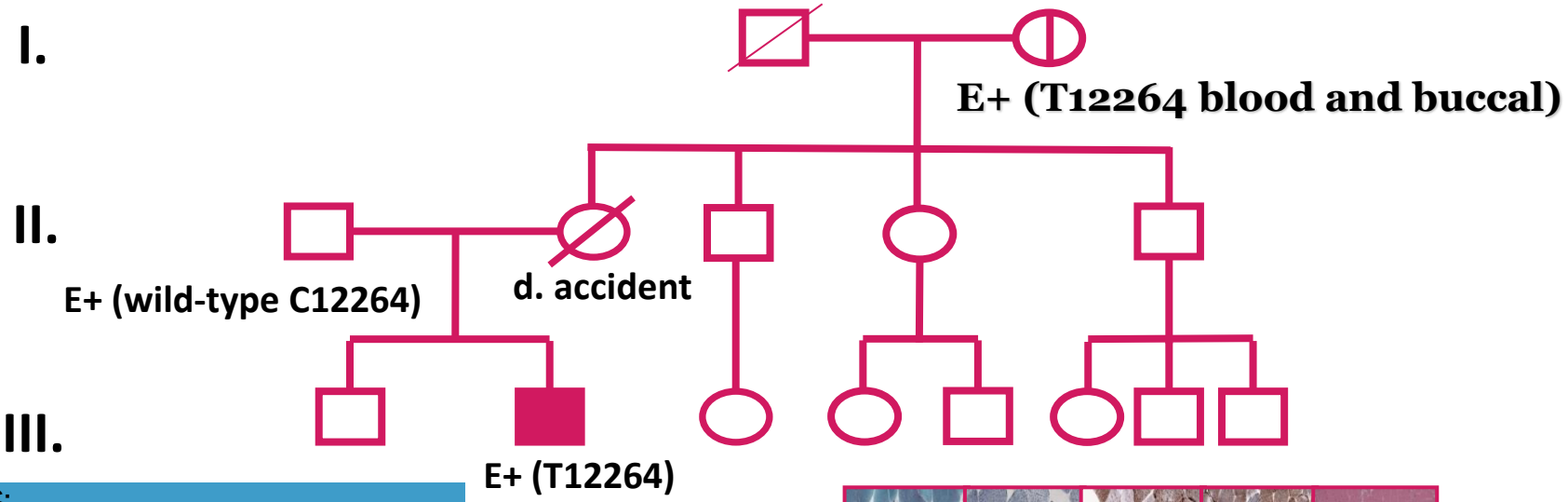
- **"Threshold Effect":**
  - **Specific heteroplasmy load for a specific mtDNA mutation that any given tissue tolerates before it shows signs of pathology**
    - **Different tolerance for the exact level of abnormal mtDNA accumulation that will cause disease**

HETEROPLASMY THRESHOLD EFFECT

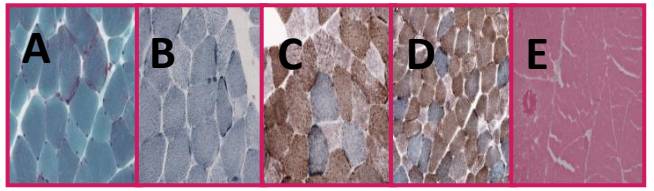


Mccormick E et al, *Current Genetic Med Reports*, 2018

mtDNA MUTATION → MULTI-SYSTEM PROBLEMS



1. CARDIAC:
   - hypertrophic cardiomyopathy
   - WPW arrhythmia
   - congestive heart failure
2. CNS:
   - Developmental delay/ Mental retardation
   - hypotonia
   - bilateral severe mixed hearing loss
   - sleep apnea
3. ENDOCRINE:
   - Truncal obesity
   - Non-insulin dependent diabetes mellitus
   - Short stature (5'2")
   - Hypogonadotropic hypogonadism
4. OPHTHALMOLOGIC:
   - Retinitis pigmentosa
   - Bilateral dense nuclear cataracts (s/p extraction)
5. MUSCULAR:
   - Irregularly thickened bladder wall
6. METABOLIC:
   - Intermittent lactic acidosis (2-3 mM range)
   - Hyperalaninemia



INDIVIDUAL	Sample	Average Wildtype load percentage (C12264)	Average Mutant load percentage (T12264)
Proband	Blood	66%	34%
Proband	Muscle	0%	100%
Proband	Cataract	0%	100%
Proband	Buccal swab	0%	100%
Father	Blood	100%	0%
Maternal Grandmother	Blood	99%	1%
Maternal Grandmother	Buccal swab	82%	18%

Schrier S et al, *Discovery Medicine*, 2012

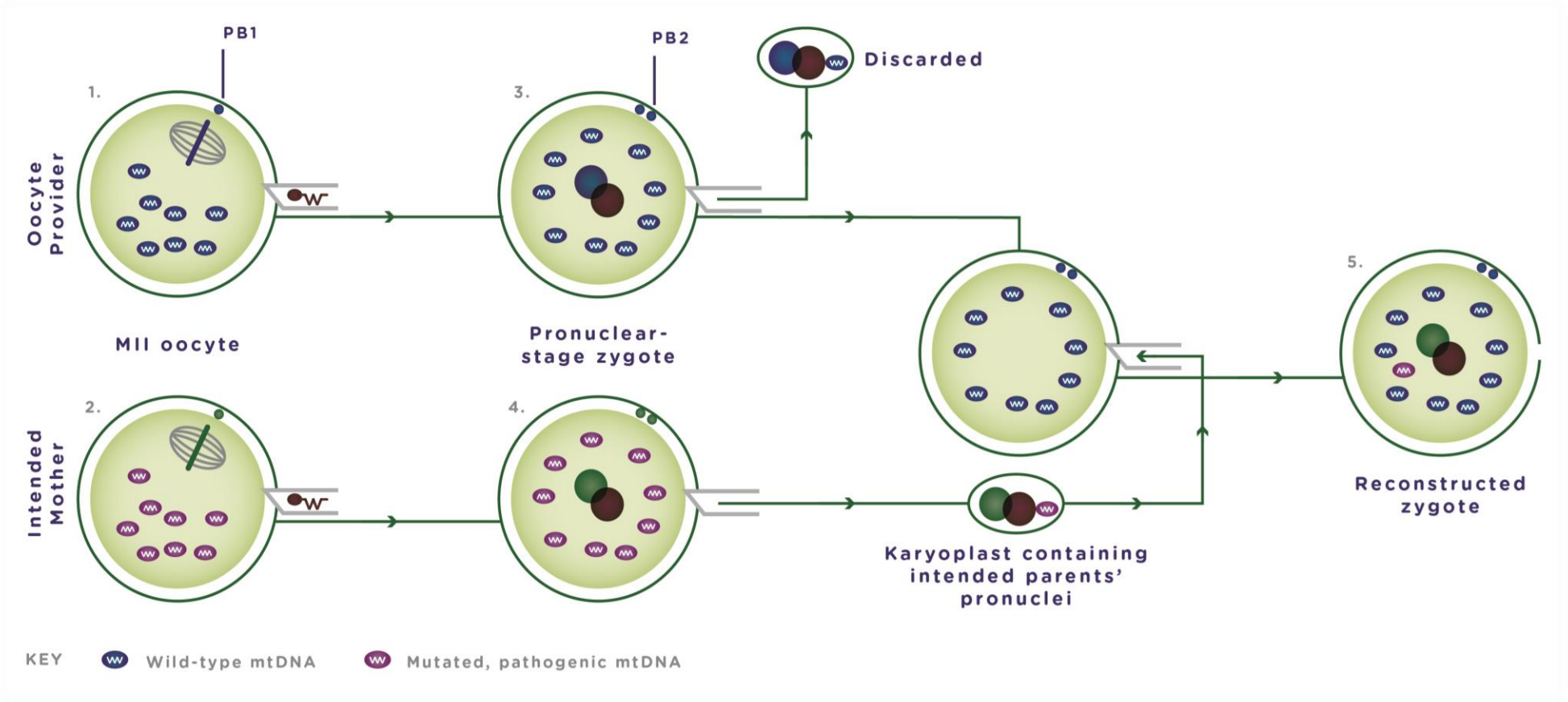
PGD FOR mtDNA DISEASE?

- **Controversial, but current understanding suggests may consider a trial (test in UK lab)**
  - **Perform for only known pathogenic mutations in day 3 embryo**
  - **Genetic bottleneck of mtDNA # in embryogenesis (few thousand mtDNAs)**
  - **<5% heteroplasmy in blastomere "unlikely" to increase to clinically significant levels in child**
    - **MRT results in 1-2% mutant mtDNA heteroplasmy carryover**
- **Children born following PGD**
  - **Likelihood to identify a low-level embryo varies**
    - **Whether mother is/not mtDNA mutation carrier**
    - **Specific mutation (m.8993 NARP mutation tends to be present at very low or very high levels in embryos)**
    - **mtDNA technical analysis method used (NGS preferred) and center experience**
  - **Need to follow child long-term in mitochondrial medicine clinical center**
    - **Blood mtDNA level may not reflect variable tissue levels**
    - **Some mtDNA disorders do not present until later childhood/adult**
    - **Low-level mtDNA mutations often have multi-system findings such as diabetes, hearing loss, headaches, etc**

[Mitochondrial Disease: Nora's Story | Children's Hospital of Philadelphia (chop.edu)]

Mitalipov et al, *Cell Reports*, 2014

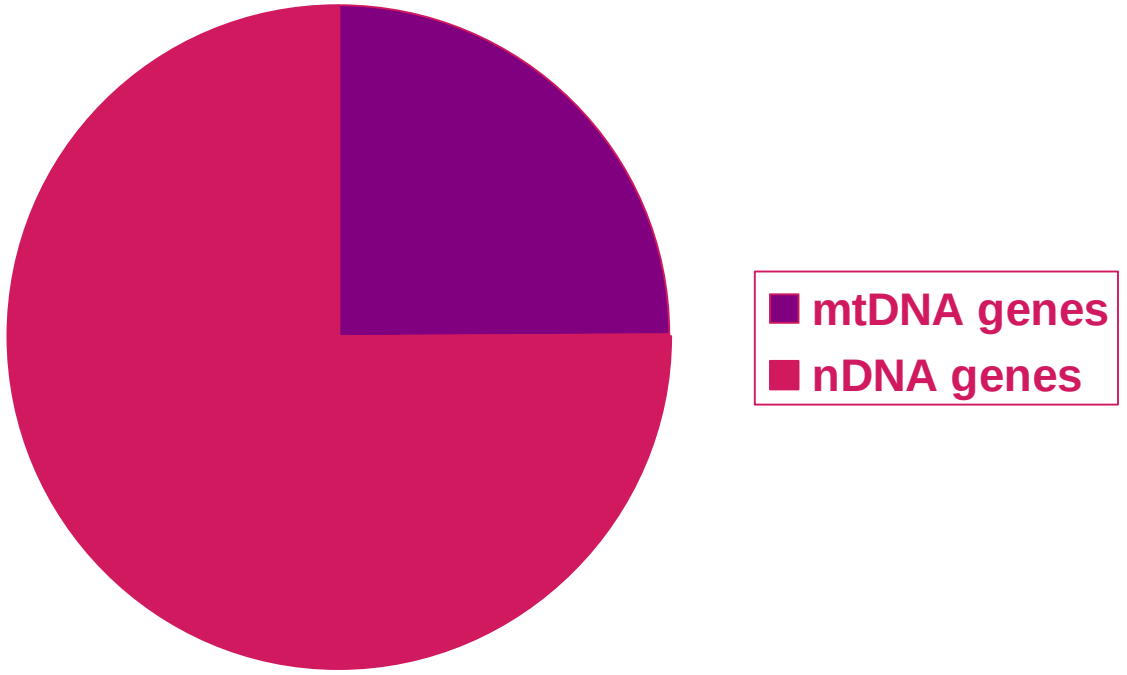
Mitochondrial Replacement Techniques: PRONUCLEAR TRANSFER



SOURCE: Modified figure based on those appearing originally in: Richardson, J., L. Irving, L. A. Hyslop, M. Choudhary, A. Murdoch, D. M. Turnbull, and M. Herbert. 2015. Concise reviews: Assisted reproductive technologies to prevent transmission of mitochondrial DNA disease. Stem Cells 33(3):639-645. License information available at: http://creativecommons.org/licenses/by/4.0/

NUCLEAR GENE BASED MITOCHONDRIAL DISEASE

NUCLEAR GENE DISORDERS COMMONLY CAUSE MITOCHONDRIAL DISEASE



- nDNA plays LARGER ROLE in mitochondrial disease
  - 1,500+ nDNA-encoded gene products in mitochondria
  - >250 nuclear genes implicated in MRC disease
    - Several 100 more novel nuclear gene causes to discover…

MITOCHONDRIAL DISEASE MOLECULAR DIAGNOSIS SUMMARY

- **Primary mitochondrial diseases commonly result from mutations in nuclear DNA genes**
  - **All inheritance patterns are seen**
  - **350+ nuclear gene disorders directly impair mitochondrial function**
    - **Genes grouped into several major functional categories**
      - **OXPHOS Subunits**
      - **OXPHOS biogenesis or regulation**
      - **mtDNA maintenance or expression**
      - **Nucleotide transport or synthesis**
      - **Membrane dynamics**
  - ***POLG* is most common, but still relatively rare (~3%), single gene cause of nuclear-based mito disease**

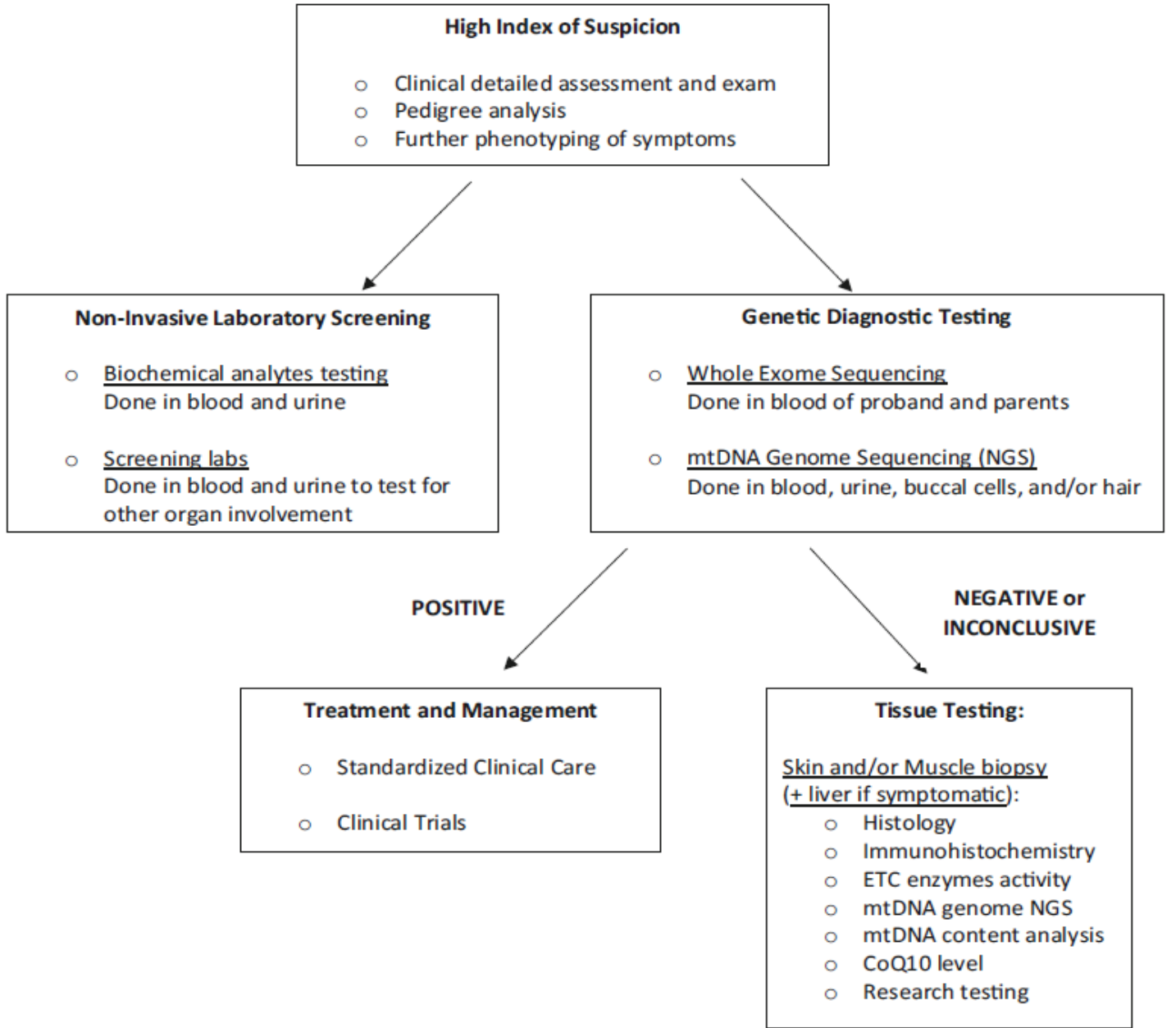
MITOCHONDRIAL DISEASE INHERITANCE

Inheritance Pattern:	Disease Example:	Recurrence Risk to Full Siblings:	Recurrence Risk to Offspring of Affected Females:	Recurrence Risk to Offspring of Affected Males:
Maternal	mtDNA point mutations; mtDNA large deletions ± duplications (rare)	1-4% if no symptoms in mother; up to 50% if symptomatic mother (EMPIRIC RISK)	Up to 50% for both sons and daughters	None
Autosomal Recessive	Mutations in nDNA-encoded respiratory chain subunits or assembly factors; mtDNA depletion (*POLG1*, *TK2*, *DGUOK*, etc.)	25%	All children will be carriers (likely asymptomatic); Affected status depends on population carrier frequency	All children will be carriers (likely asymptomatic); Affected status depends on population carrier frequency
Autosomal Dominant	Progressive external ophthalmoplegia (*POLG1*)	50% if parent is affected; <1% based on germline mosaicism if parent is asymptomatic	50% for both sons and daughters	50% for both sons and daughters
X-linked	Sideroblastic anemia (*ABC7*); Barth syndrome (*tafazzin*); Mohr-Tranebjaerg syndrome (*DDP1*)	If mother is a carrier: 50% for brothers to be affected & 50% for sisters to be carriers (likely asymptomatic); If *de novo*, <1% for brothers to be affected or sisters to be carriers	If symptomatic mother, 50% for sons to be affected and 50% for daughters to be carriers/affected (depending on her x-inactivation pattern)	None for sons; 50% for daughters to be carriers (likely asymptomatic)
Sporadic	Muscle biopsy evidence of respiratory chain dysfunction without clear genetic etiology	Uncertain	Uncertain	Uncertain

Falk MJ (2008) In: *Mito 101* CD, Eds. S. Parikh, S. DiMauro, UMDF

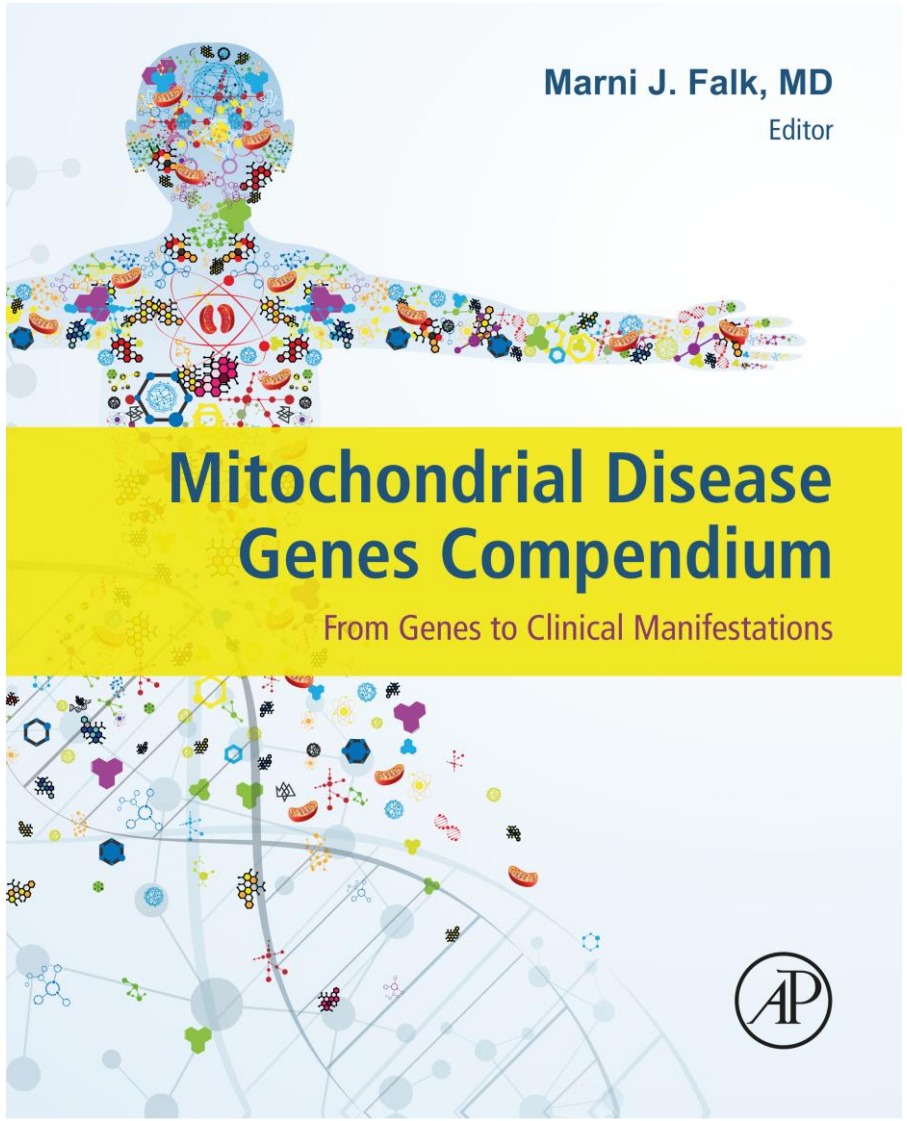
MITOCHONDRIAL DISEASE DIAGNOSTIC ALGORITHM

Curr Genet Med Rep (2018) 6:62–72



→ ddPCR to quantify heteroplasmy level if detect mtDNA deletion(s)

MITOCHONDRIAL DISEASE GENES COMPENDIUM



KEY FEATURES:

- **Provides a readily intelligible, all-in-one reference of known mitochondrial disease genes & associated conditions**
- **Features live links to MSeqDR Web pages, with regularly updated genetic variant data and bioinformatics tools**
- **Covers inheritance patterns, age spectrum affected, major clinical features, therapeutics, support groups, and research under way for over 250 mitochondrial diseases**

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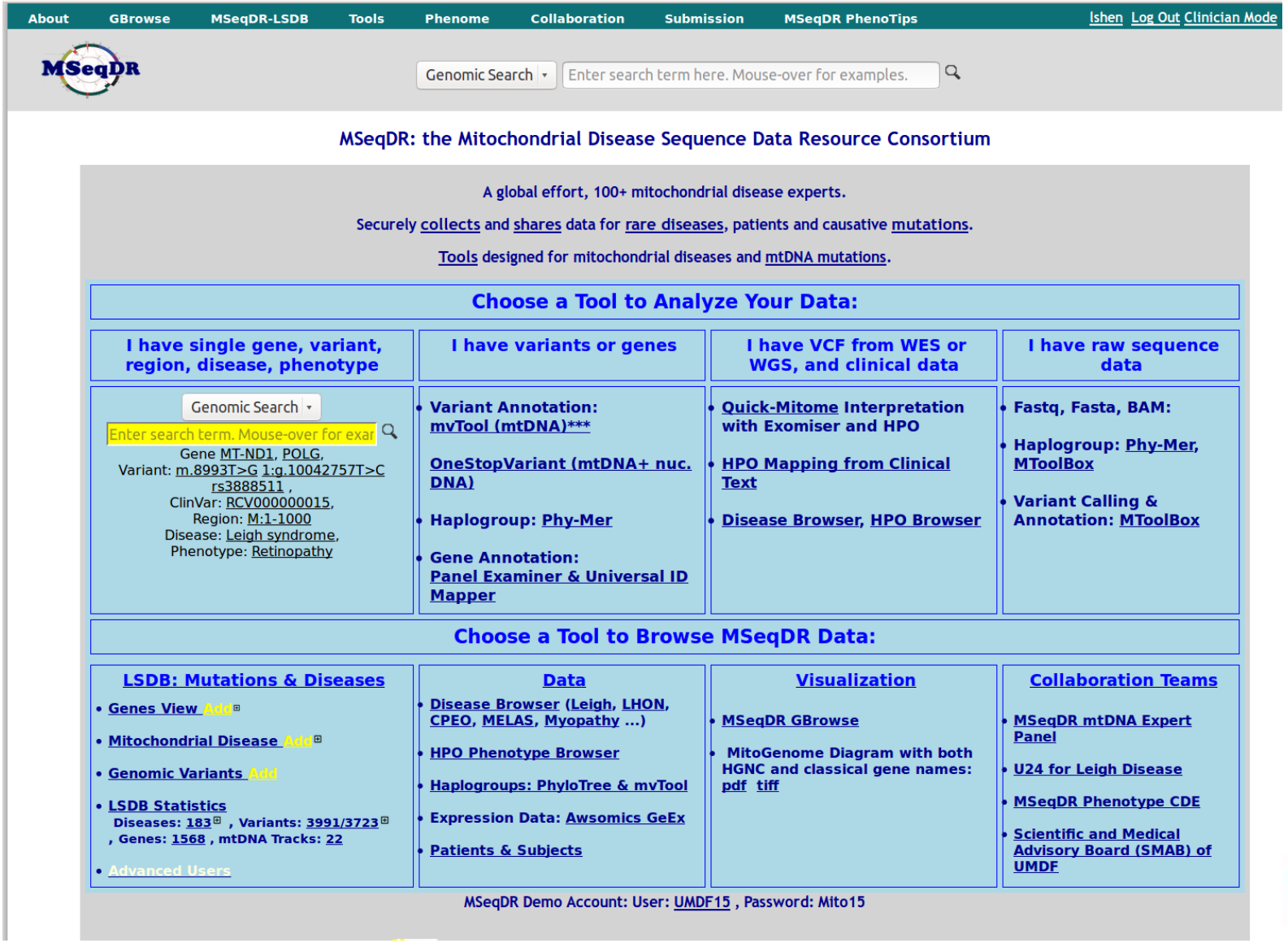
MITOCHONDRIAL DISEASE SEQUENCE DATA RESOURCE

HTTPS://MSEQDR.ORG



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Falk MJ et al, *Mol Gen Metab*, 2015; Shen L et al, *Hum Mut*, 2016; Shen L et al, *Hum Mut*, 2018

Mito-QuickExome in 5 minutes - MSeqDR Phenotype-Guided WES Quick Interpretation Toolbox

Input clinical symptoms and diagnosis description and double click "HPO Annotator" button

```
#HL_Example Phenotypes
Congenital hearing loss/deafness
Sensorineural hearing loss
Aminoglycoside-induced hearing loss
Congenital Conductive Hearing Impairment
```

HPO Annotator **HPO ID:** HPO IDs separated by comma. HP:001 * review at bottom "HPO Annotator"

Email Provide email for r send me report when finished

Upload Variant and Pedigree File (VCF format v4, the sample column is required):

Select Files

Add files | Start upload | Remove all

Drag & Drop vcf or Pedigree File Here. Using VCF format v4, the sample genotype column is required.
Optionally upload *.ped file for family-based analysis. Aftr uploading, click Refresh to see the file ...

Refresh

Variant VCF files available:

11. Demo0001.vcf - 407.786 KB - 2017-07-11 04:11:00

Annotate | Download | Delete

Pedigree files available:

1. Demo0001.ped - 0.153 KB - 2017-07-11 04:11:06 Use this *.ped file

Create PED | Download PED | Delete PED

* Do not re-submit or refresh, must leave this page to run till it is completed. The run may need about 15 minutes for input with 1500 variants. Use email function to receive result notice, or View Result Here

MSeqDR Tool Settings:

- [x] MSeqDR mtDNA annotation
- [ ] VEP annotation and filtering
- [x] ClinVar annotation
- [x] dbNSFP annotation
- [x] gnoMAD Exome AF annotation
- [x] Limit to transcribed regions and 10-bp flanking
- [x] Phy-Mer: Mitochondrial haplogroup classification
- [x] Heteroplasmy calculation
- [x] Add link to all variants, genes, HPO and OMIM entries
- [x] Create patient record

UMDF EMPOWERED COMMUNITY GENOMIC DATA ANALYSES:
Patient-directed Genomic Data File Reanalysis Through mitoSHARE in MSeqDR-OpenCGA

BACKGROUND

- Many individuals with features highly concerning for mitochondrial disease lack a confirmed genetic etiology
- Clinical genetic diagnostic testing typically only reports variants in *known* disease genes; limits opportunity for *gene discovery*
- No community-wide mechanism has existed to empower individuals and their families to choose who can access and meaningfully analyze their existing genomic data

METHODS

- UMDF-led mitoSHARE mitochondrial disease patient registry was launched in March 2022
  - Participants can be informed of research studies for which they may be eligible if interested
- MSeqDR genomic data sharing study was approved by the Institutional Review Board (IRB) at Children's Hospital of Philadelphia (CHOP, Falk PI) in January 2022

GENOMIC DATA FLOW & ANALYSIS

Subject enrolls in mitoSHARE, indicates desire to participate in MSeqDR study

Subject completes:
- Electronic ICF
- Genomic data release form
- GUID generation form

Patient/Doctor Interactions:

- MSeqDR staff informs subject of GUID
- Subject provides their doctor/researcher their GUID
- Doctor/researcher requests access to data analysis tools
- MSeqDR committee grants access to coded data

Lab/Data Interactions:

- MSeqDR staff sends release to clinical lab
- Data is transferred to CHOP cloud, then Cavatica
- MSeqDR team labels data with GUID
- Coded data transferred to analysis platform

Doctor/researcher analyzes coded data
Other cases identified and/or functional validation is performed

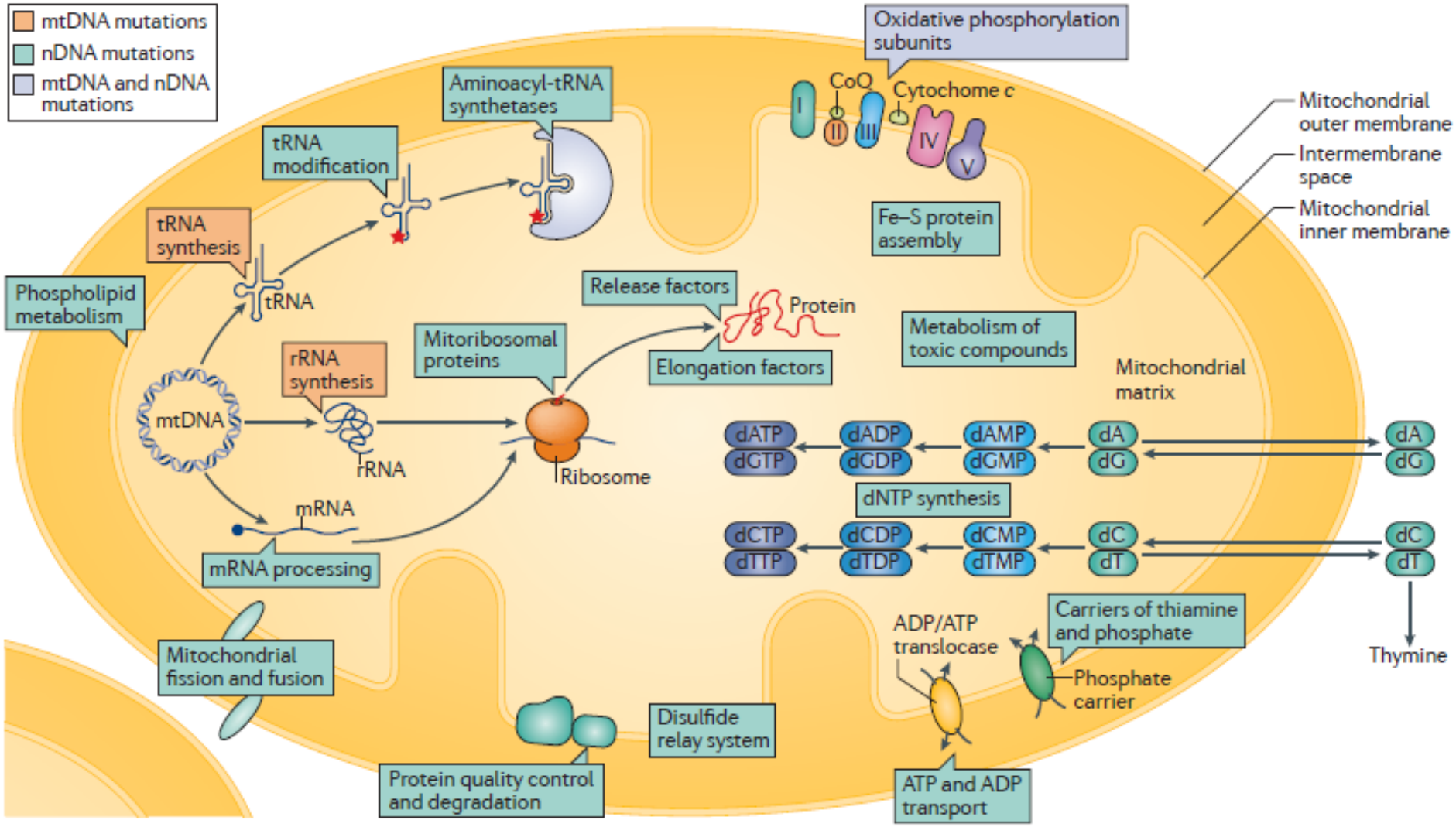
Case is SOLVED; Diagnostic odyssey ENDS

Data contributes to other genomic discoveries

Children's Hospital of Philadelphia
Mitochondrial Medicine
Elizabeth McCormick, MS, LCGC

MITOCHONDRIAL DISEASE THERAPIES

Mitochondrial disease:
Molecular pathways effected by genetic disorders



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

MITOCHONDRIAL MEDICINE SOCIETY: TREATMENT & PREVENTATIVE CARE GUIDELINES

- **Exercise guidelines**
- **Acute stroke guidelines**
- **Anesthesia guidelines**
- **Acute illness guidelines**
- **Vitamin use guidelines**

Consensus recommendations for vitamin and xenobiotic use

1. CoQ_{10} should be offered to most patients with a diagnosis of mitochondrial disease and not exclusively used for primary CoQ_{10} deficiency.
   a. Reduced CoQ_{10} (ubiquinol) is the most bioavailable form and, when used, dosing should be appropriately modified.
   b. Plasma and/or leukocyte CoQ_{10} levels are helpful in monitoring absorption and adherence to treatment. Plasma levels are more variable and less reflective of tissue levels.
2. ALA and riboflavin should be offered to mitochondrial disease patients.
3. Folinic acid should be considered in mitochondrial disease patients with central nervous system manifestations and routinely administered to those with documented CSF deficiency or with disease states known to be associated with deficiency.
4. L-Carnitine should be administered to mitochondrial disease patients when there is a documented deficiency and levels should be monitored during therapy.
5. When beginning supplement therapy, one should begin one at a time when possible, taking into account a patient's clinical status.
6. There is no evidence to suggest that one can adjust a person's diet on the basis of ETC results.
7. Goal levels for most vitamin therapy used are not yet known; it is prudent to replace deficiency states.

***Parikh S et al, *Genetics in Medicine,* 2014; Parikh et al, *Genetics in Medicine* 2017**

MITOCHONDRIAL DISEASE HAS NO FDA APPROVED THERAPIES OR CURES

- **Individually rare disorders**
  - High genetic heterogeneity: >350 genes
  - High phenotypic heterogeneity: 16 symptoms/patient average*
  - No universal biomarker
- **Therapeutic options are limited**
  - Exercise: aerobic and isotonic
  - Nutritional therapy underexplored
  - One-size-fits-all empiric "*supplement cocktails*" **
    - Enzyme co-factors (vitamin B1 or B2)
    - Metabolite therapies (arginine, folinic acid, creatine)
    - Enzyme activators (dichloroacetate)
    - Antioxidants (vitamin C or E, lipoic acid, coenzyme Q)

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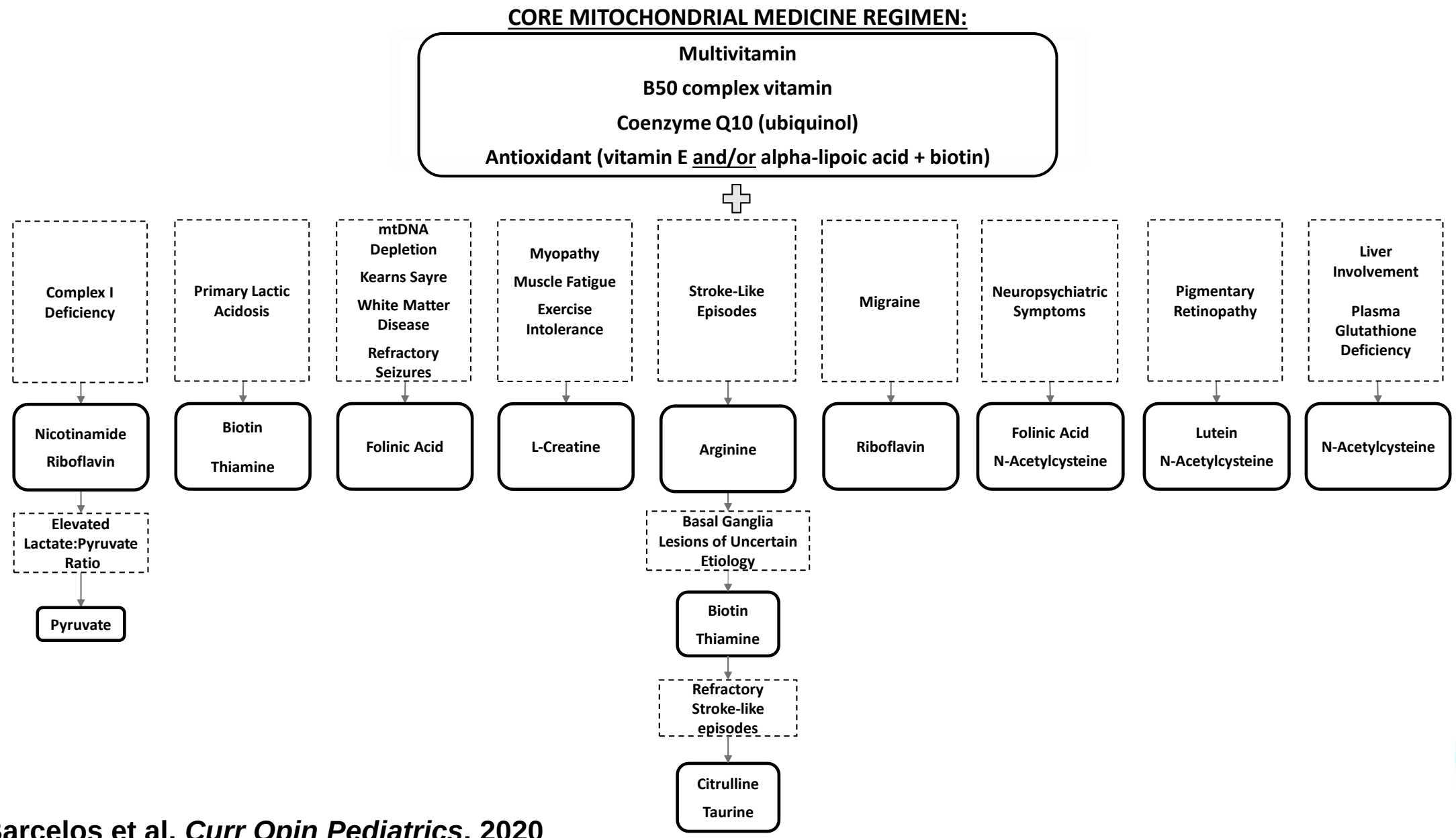
*Zolkipli-Cunningham et al, *PLOS ONE*, 2018; **Parikh S et al, *Curr Treat Options in Neurol*, 2009

Mitochondrial Medicine Therapies



Barcelos et al, *Curr Opin Pediatrics*, 2020

Treatable Gene-Specific Mito Disorders

Affected pathway	Clinical syndrome	Affected gene(s)	Clinical phenotype	Therapeutic substance	Treatment response
Primary disorders of mitochondrial vitamin cofactor metabolism	Brown-Vialetto-Van Laere syndrome / Fazio-Londe disease	*SLC52A2, SLC52A3, (SLC52A1)*[a]	Sensorineural hearing loss, cranial nerve palsies	Riboflavin (oral: 10–50 mg/kg/day)[b]	Generally good
	Biotin-thiamine-responsive basal ganglia disease	*SLC19A3*	Episodic encephalopathy, dystonia, seizures	Thiamine (oral: 10–20 mg/kg/day), biotin (oral: 10–15 mg/kg/day)[c]	Generally good
	Biotinidase deficiency	*BTD*	Dermatitis, muscular hypotonia, developmental regression	Biotin (oral: 5–10 mg/kg/day)[d]	Generally good
	Holocarboxylase synthetase deficiency	*HLCS*	Skin lesions, metabolic acidosis, seizures, developmental delay	Biotin (oral: 10–20 mg/kg/day)[e]	Variable but generally good
	Thiamine pyrophosphokinase deficiency	*TPK1*	Episodic encephalopathy, dystonia, spasticity	Thiamine (oral: ~20 mg/kg/day)[f]	Variable (< 10 patients treated so far)
Disorders with indirect response to mitochondrial vitamin cofactor supplementation	ACAD9 deficiency	*ACAD9*	Encephalopathy, myopathy, hypertrophic cardiomyopathy	Riboflavin (oral: 10–20 mg/kg/day)[g]	Variable
	Multiple acyl-CoA dehydrogenase deficiency	*ETFA, ETFB, ETFDH, SLC25A32, FLAD1*	Early childhood multisystem disease or late-onset form with muscle weakness, hepatopathy, etc.	Riboflavin (oral: ~10 mg/kg/day)[h]	Generally good
	Thiamine-responsive pyruvate dehydrogenase deficiency	*PDHA1*	Neonatal lactic acidosis, seizures, developmental regression, spasticity	Thiamine (oral: 30–40 mg/kg/day)[i]	Variable
Disorders of mitochondrial non-vitamin cofactor metabolism	Coenzyme Q_{10} deficiency	*PDSS1, PDSS2, COQ2, COQ4, COQ6, COQ7, ADCK3, ADCK4, COQ9*	Variable phenotypes, ranging from adult-onset myopathy to fatal neonatal presentations	Coenzyme Q_{10} (oral: 10–30 mg/kg/day)[j]	Highly variable depending on the underlying defect
Disorders of mitochondrial inorganic cofactor metabolism	Cytochrome c oxidase deficiency	*SCO2, COA6*	Infantile encephalocardiomyopathy	Copper–histidine (dose unclear; subcutaneous injections of up to 500 μg daily were suggested)[k]	Unclear, only one *SCO2* patient treated; only *in vitro* evidence for *COA6*
	Molybdenum cofactor deficiency	*MOCS1, MOCS2, GPHN*	Infantile-onset epileptic encephalopathy, progressive brain damage	Cyclic pyranopterin monophosphate (intravenous: 80–320 μg/kg/day)[l]	Generally good in MoCD type A patients
'Inhibitors' of mitochondrial metabolism	3-Hydroxyisobutyryl-CoA hydrolase deficiency	*HIBCH*	Infantile Leigh-like phenotype	Valine-restricted diet[m]	Unclear, only few patients treated
	Enoyl-CoA hydratase deficiency	*ECHS1*	Infantile Leigh-like phenotype	Valine-restricted diet[n]	Unclear, only few patients treated so far
	Thioredoxin 2 deficiency	*TXN2*	Cerebellar atrophy, dystonia, seizures, peripheral neuropathy	Antioxidant treatment (e.g. Idebenone up to 20 mg/kg/day)[o]	Apparently good (only one patient reported)
	Ethylmalonic encephalopathy	*ETHE1*	Severe, multisystem infantile disorder	Metronidazole, *N*-acetyl cysteine as glutathione precursor, liver transplantation[p]	Variable

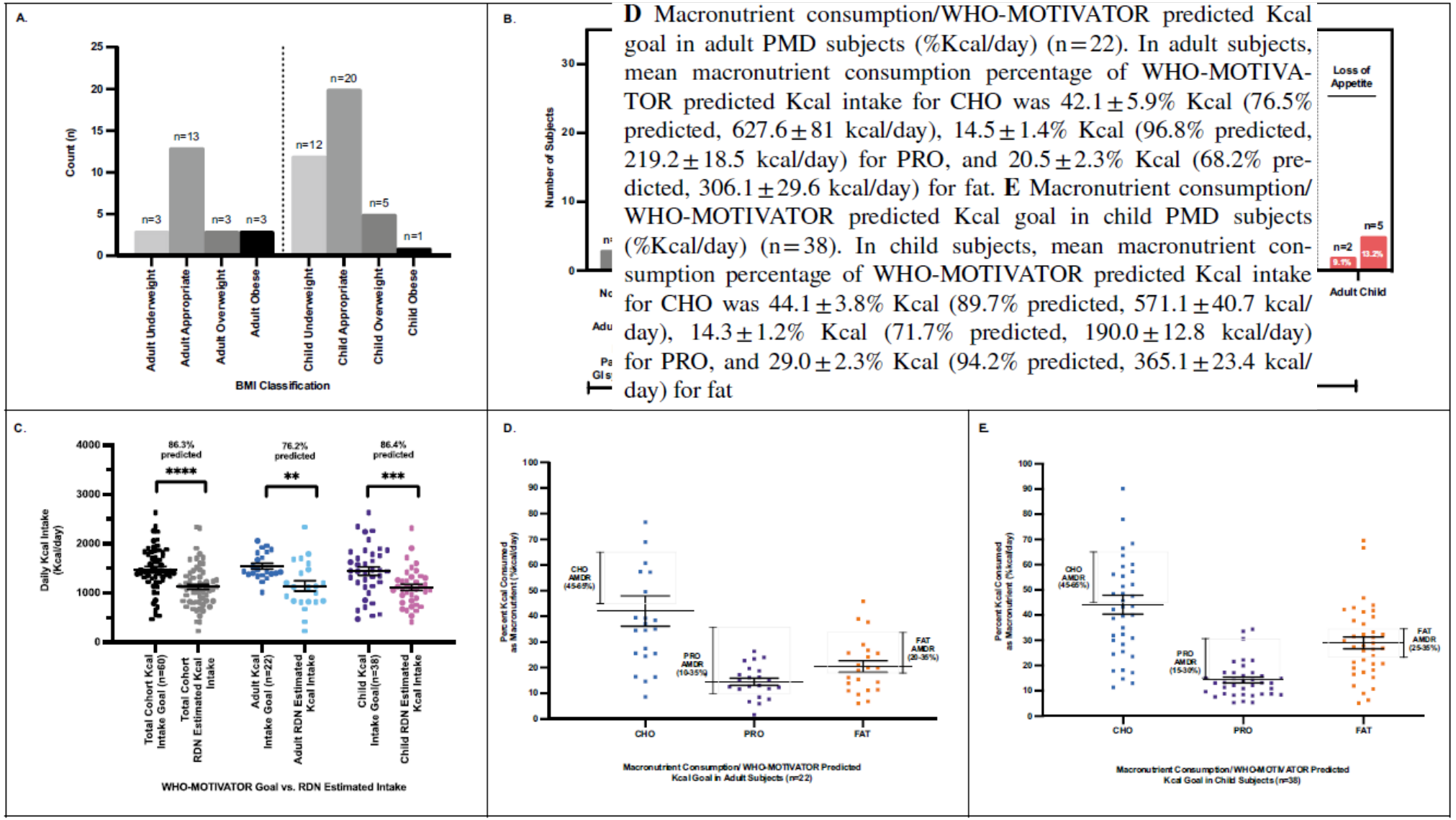
*Distelmaier F. et al, *Brain*, 2017

Nutritional Guidance is Limited in Mitochondrial Disease

- Evaluate energy, protein & micronutrient intake
  - Assess for relative under-nutrition
    - Consider energy expenditure, intake, and absorption*
    - May require gastrostomy tube or parental nutrition
    - Treat swallowing dysfunction, abnormal gut motility, behavioral feeding issues, and gastroesophageal reflux to optimize nutritional intake**
  - Monitor for essential micronutrient deficiency
    - B12 (13%), D (>80%), folate, zinc, selenium, carnitine, vit A & E*
    - Multivitamin supplement is safe and may alleviate deficiencies
      - If LHON, NARP, or retina involved: multivitamin w/ lutein
- Avoid fasting and encourage frequent small meals
- Increase fluid intake with heat and metabolic stress

*Mark Tarnopolsky, *Mito 101: Nutrition*, 2010; **Parikh S et al, *Curr Treat Options in Neurol*, 2009

Mitochondrial Disease Patients have Nutritional Deficiencies



D Macronutrient consumption/WHO-MOTIVATOR predicted Kcal goal in adult PMD subjects (%Kcal/day) (n = 22). In adult subjects, mean macronutrient consumption percentage of WHO-MOTIVATOR predicted Kcal intake for CHO was 42.1 ± 5.9% Kcal (76.5% predicted, 627.6 ± 81 kcal/day), 14.5 ± 1.4% Kcal (96.8% predicted, 219.2 ± 18.5 kcal/day) for PRO, and 20.5 ± 2.3% Kcal (68.2% predicted, 306.1 ± 29.6 kcal/day) for fat. **E** Macronutrient consumption/WHO-MOTIVATOR predicted Kcal goal in child PMD subjects (%Kcal/day) (n = 38). In child subjects, mean macronutrient consumption percentage of WHO-MOTIVATOR predicted Kcal intake for CHO was 44.1 ± 3.8% Kcal (89.7% predicted, 571.1 ± 40.7 kcal/day), 14.3 ± 1.2% Kcal (71.7% predicted, 190.0 ± 12.8 kcal/day) for PRO, and 29.0 ± 2.3% Kcal (94.2% predicted, 365.1 ± 23.4 kcal/day) for fat

Divito D et al, *Neurotherapeutics*, 2023

PMD patients with inadequate calories had strong correlation between protein intake & outcome measures

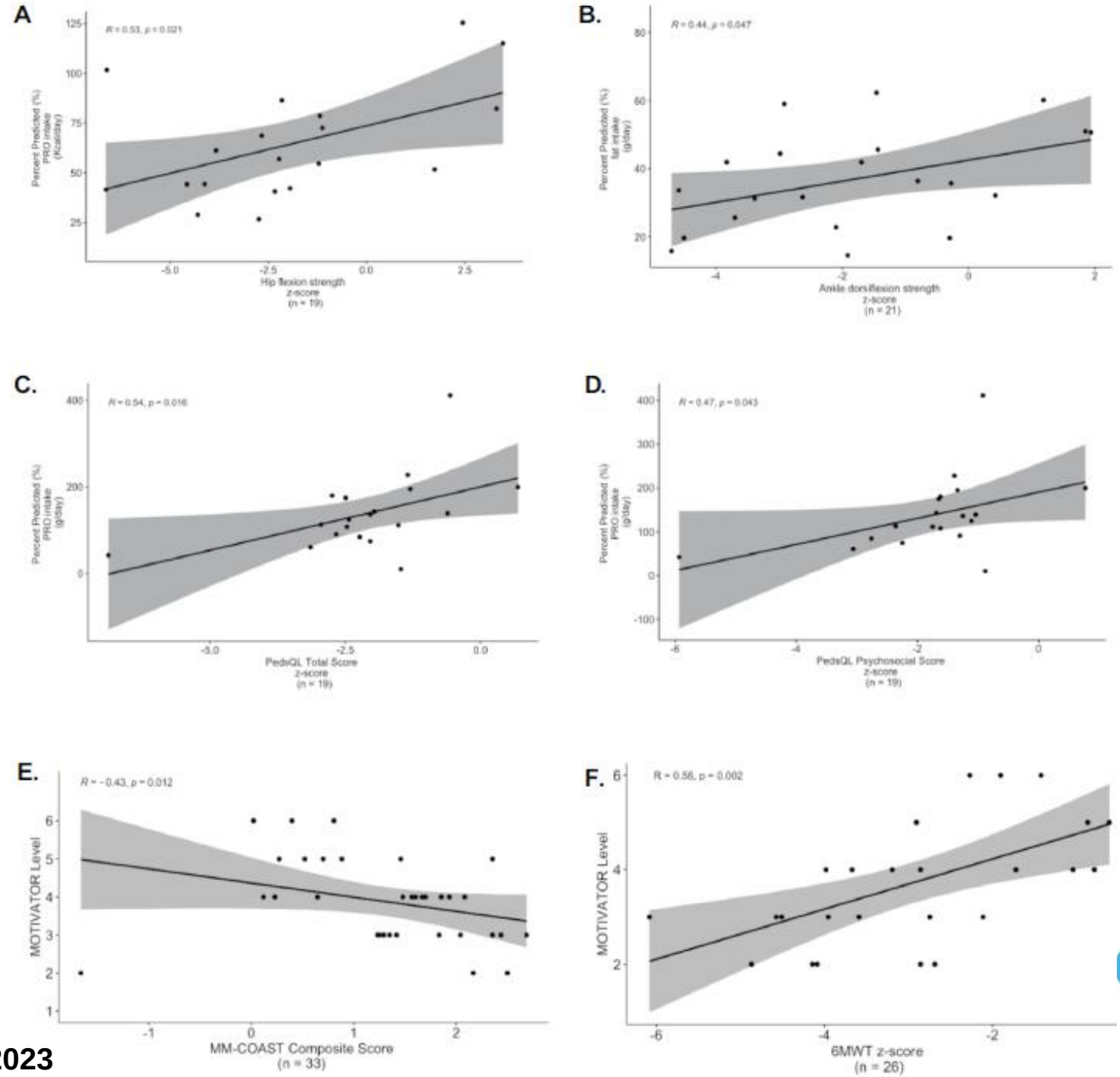
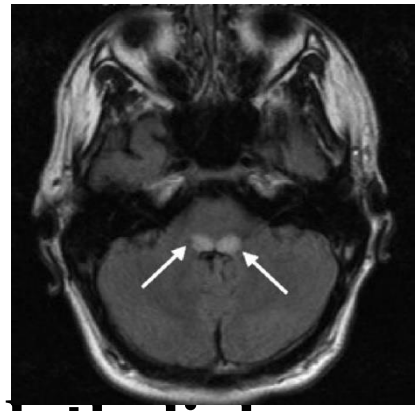


Fig. 7 Correlations of objective assessments and surveys to macronutrient consumption (g/day and Kcal/day) in subjects with inadequate Kcal intake (≤75% predicted) (n=29). A Hip flexion strength

Divito D et al, *Neurotherapeutics*, 2023

Amino Acid Therapies for Mitochondrial Disease



- **Arginine or Citrulline**
  - **Nitric oxide donors target microvascular endothelial ischemia that occurs in metabolic stroke**
  - **Intravenous use**
    - **Acute stroke treatment in MELAS (Koga et al, 2005)**
      - **Expert panel consensus to consider use (Parikh S et al, 2018)**
      - **Well-tolerated (monitor for hypotension & hypoglycemia)**
    - **Acute stroke treatment in diverse pediatric mito disease strokes beyond MELAS (Ganetzky and Falk, 2018)**
      - **Hemiplegic strokes: >50% clinical response by discharge**
  - **Enteral use**
    - **Prophylaxis for metabolic stroke occurrence/recurrence**
    - **Comparative arginine vs citrulline study underway**
      - **Fernando Scaglia, Baylor (NAMDC U54, NIH)**

No Clear Macronutrient Profile for Mitochondrial Disease

- **No scientific data supports specific macronutrient profiles (ratios of protein, carbohydrate and fat) in mito disease[1]**
- **KETOGENIC DIET is controversial**
  - **Increase ketones & succinate, starvation response, mitochondrial biogenesis, glutathione**
    - **KD slowed mitochondrial myopathy progression in *C10ORF2* mice[2]**
    - **High-fat diet slowed neurologic progression in CI deficient *AIFM1* mice[3]**
    - **KD exacerbated disease in *MTRF2 & MPV17* mice[4]**
  - **KD is often not tolerated in patients[5]**
    - **Mito disease patients often have hypertriglyceridemia[6]**
    - **Mito disease patients often have decreased FAO & PPAR activity[7]**
  - **Long-term health risks may preclude KD use (? refractory epilepsy[1])**

[1]Tarnopolsky M, *Mito 101*, 2010; [2]Ahola S, *Hum Mol Gen,* 2010;
[3]Schiff M et al, *PLOS ONE*, 2011; [4]Viscomi et al, *BBA*, 2015; [5]Ahola S, *EMBO MM*, 2016;
[6]Clarke C et al, *Mol Gen Metab*, 2013; [7]Zhang Z et al, *PLOS ONE*, 2013

Modified Atkins Diet is Not Tolerated in mtDNA Deletion Myopathy Patients

- **Modified Atkins Diet (mAD, 10 mos) well-tolerated & rescued myopathy in mtDNA 'deletor' myopathy *mice***
- **Small *human* myopathy subject mAD clinical trial***
  - **5 adult subjects with mtDNA deletions (2 single, 3 multiple) and 10 matched healthy controls**
  - **Switched from normal diet (ND) to planned 4 weeks on mAD**
    - **ND: 41-48% carb, 14-20% protein, 27-38% fat**
    - **mAD: 3-9% carb**
  - **mAD diet tolerability and effects:**
    - **Healthy controls: no problems completing 4 week trial**
    - **mtDNA deletion myopathy subjects: all 5 stopped diet after 4-11 days due to severe muscle pain/burning progressive from legs>back>arms>neck, headaches, and increased tiredness**
      - **Increased muscle fiber necrosis, increased CK, increased lactate with exercise, muscle fibers highly glycolytic**

*Ahola S, *EMBO Mol Med*, 2016

KETOGENIC DIET COMPONENTS MAY HAVE BENEFIT IN MITO DISEASE

- **Ketogenic diet *components* may hold potential therapeutic value**
  - **Triacylglyerol infusions improved exercise endurance in complex I deficiency mito myopathy patients[1]**
  - **Triheptanoin is anaplerotic and succinate precursor to bypass CI deficiency – showed benefit on cardiomyopathy in LCFAO disorder[2]**
  - **Decanoic acid (C10) improved mitochondrial mass, complex I activity, & PPARγ activity over 6 days in neuronal culture[3]**

[1]Roef MJ, *Am J Clin Nut,* 2002; [2]Roe CR et al, JCI, 2002; [3]Hughes SD, *JNC*, 2014

Low-Glycemic Carbohydrate Diet May Have Therapeutic Role in Mito Disease

SUPPORTING EVIDENCE:

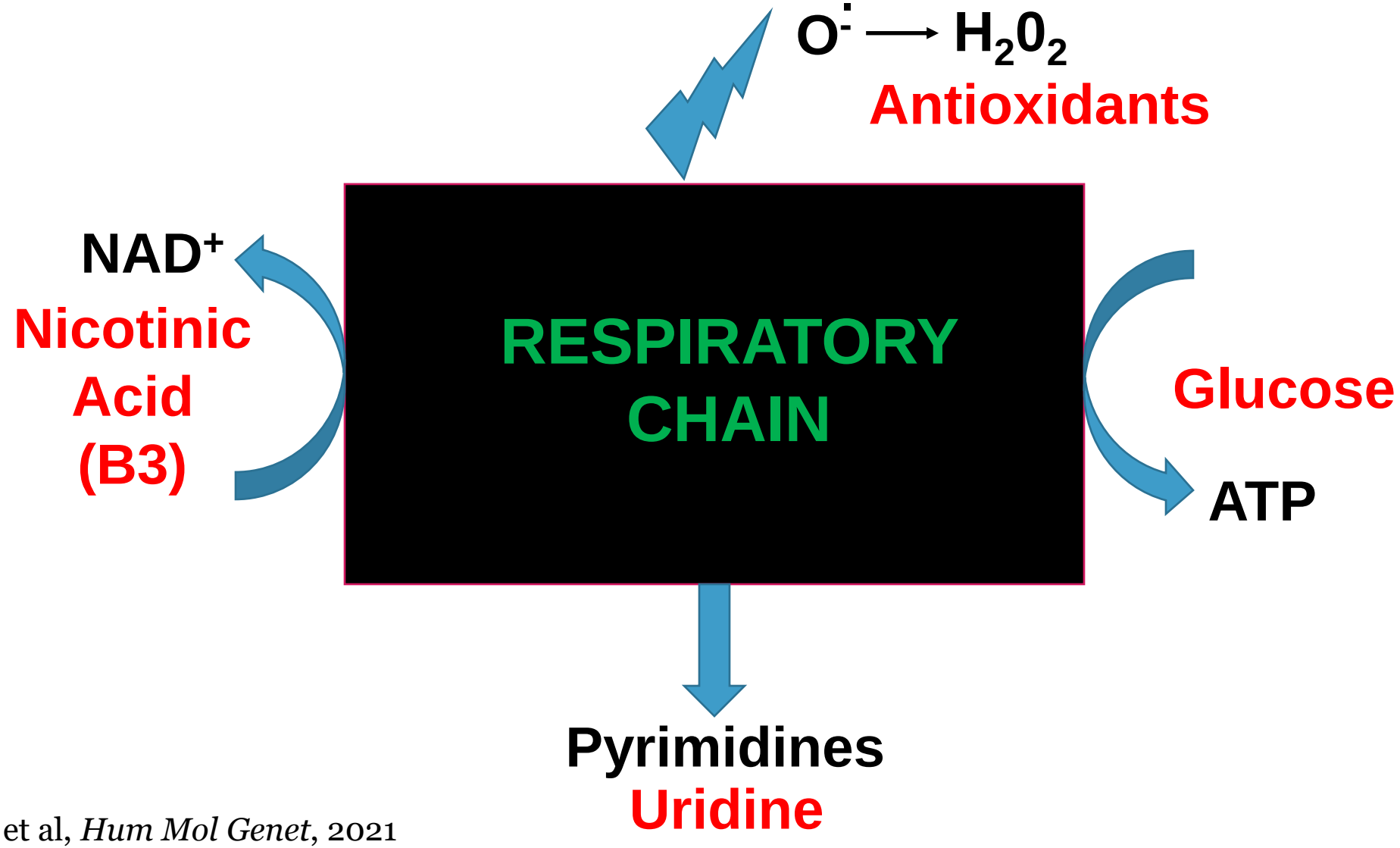
- Glycolytic rate is increased in primary mitochondrial disease[1, 2]
- Dextrose-containing IV fluids often used to prevent catabolism in metabolic crisis
- Anecdotal patient reports of carb-craving (including within minutes of awakening), with improved cognition and feeling of wellness after eating
- *In vitro* dysfunction in mito disease models & cells resolves with glucose treatment[3]
- Low glycemic carbohydrates may improve health outcomes (Shana McCormack)

CONCERNS:

- Glucose infusion may precipitate metabolic crisis by altered NADH/NAD^+ balance
- Drosophila model of mito translation defect had reduce growth in high glucose[2]
- Glucose dysregulation is common in mitochondrial diseases
  - Diabetes mellitus in some patients (adults>kids)
  - Hypoglycemia in some patients (kids>adults)

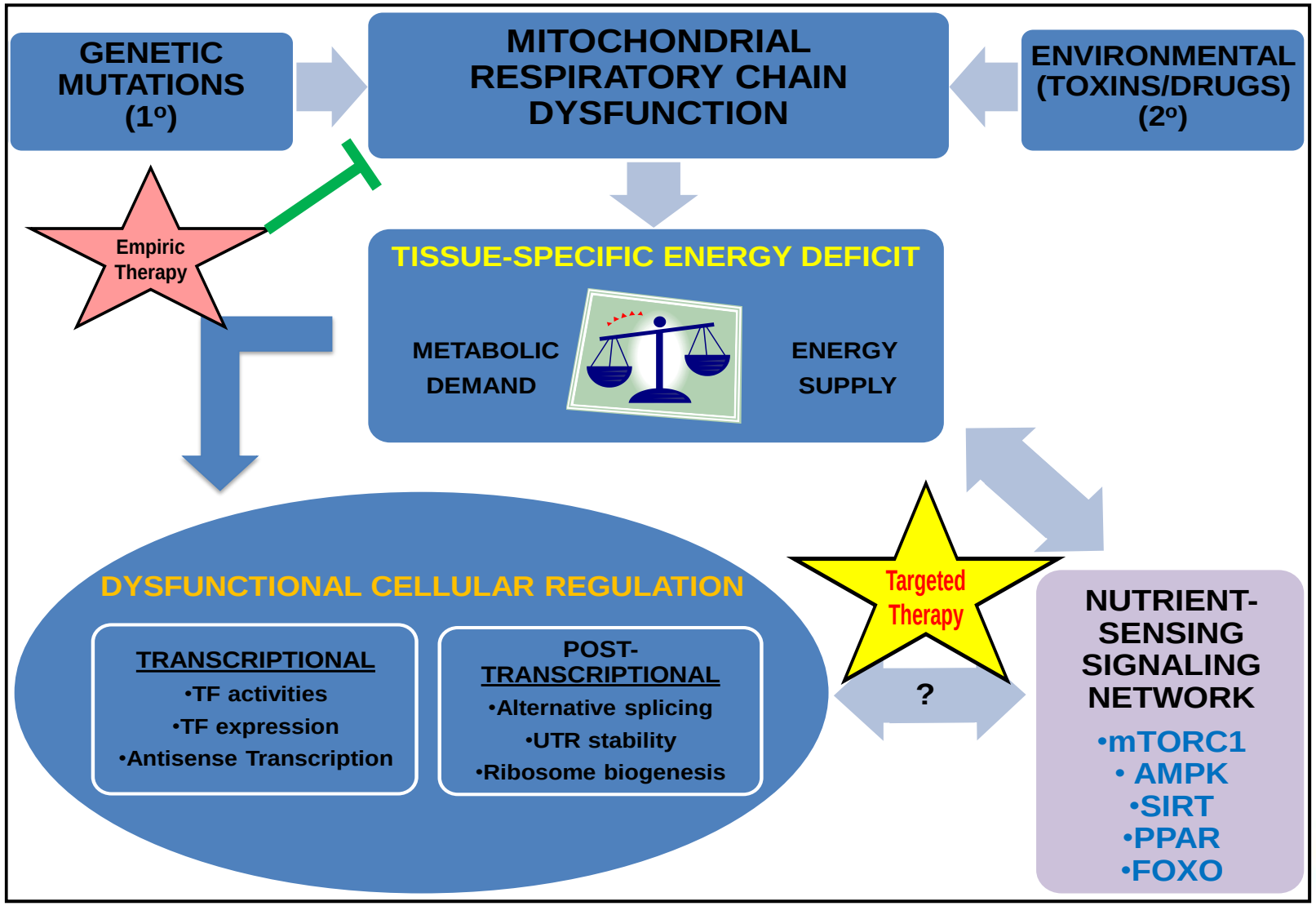
[1]Schrier-Vergano S et al, *Mol Gen Metab* 2014; [2]Kemppainen E et al, *PLOS ONE*, 2016;
[3]Peng M et al, *Hum Mol Genet*, 2015; Kwon YJ et al, *Mitochondrion*, 2017

"Black Box Product Therapies" Framework for Mitochondrial Disease



Guha M et al, *Hum Mol Genet*, 2021

TARGETING MITOCHONDRIAL DISEASE THERAPIES TO DYSREGULATED CELLULAR PATHWAYS



Zhang and Falk, *IJBCB*, 2014

EMERGING THERAPEUTIC ARSENAL FOR MITOCHONDRIAL DISEASE

Therapeutically targeting central alterations in the nutrient-sensing signaling network & basic cell processes that regulate proteotoxic stress may offer a personalized way to modify effects 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

VISION:

DEVELOP LAB TESTS TO DESIGN OPTIMAL DRUG TREATMENT(s) FOR EACH PATIENT USING THEIR OWN CELLS & ANIMAL MODELS

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Mitochondrial Medicine

New Model to Develop Precision 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

Lab testing of drugs in mito disease models

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

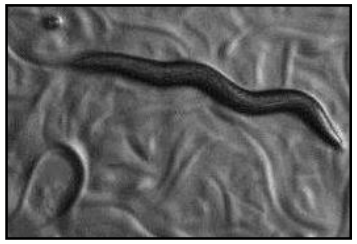
Clinical Trials

Standard of Care

*Falk MJ et al, Natl Acad Med Workshop: 'Enabling Precision Medicine', 2017

Precision Mitochondrial Medicine

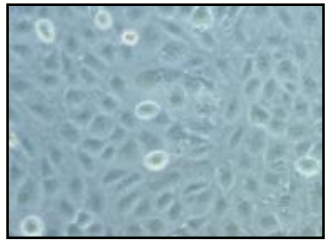
Mitochondrial Disease Model System Selection:



Invertebrates

Vertebrates

Mammals



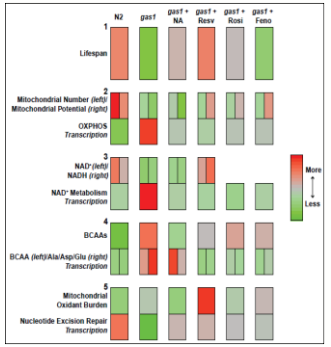
Human Cells

Pre-Clinical Model Treatment Efficacy & Safety Profiling:

- Empiric
- Drug Libraries
- Genetic Libraries

Candidate Therapies

+



1º & 2º Endpoints

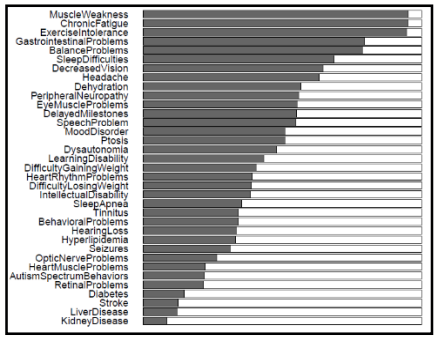
Precision Clinical Trial Design:

Therapeutic Lead

+

Rare Disease Patient(s)

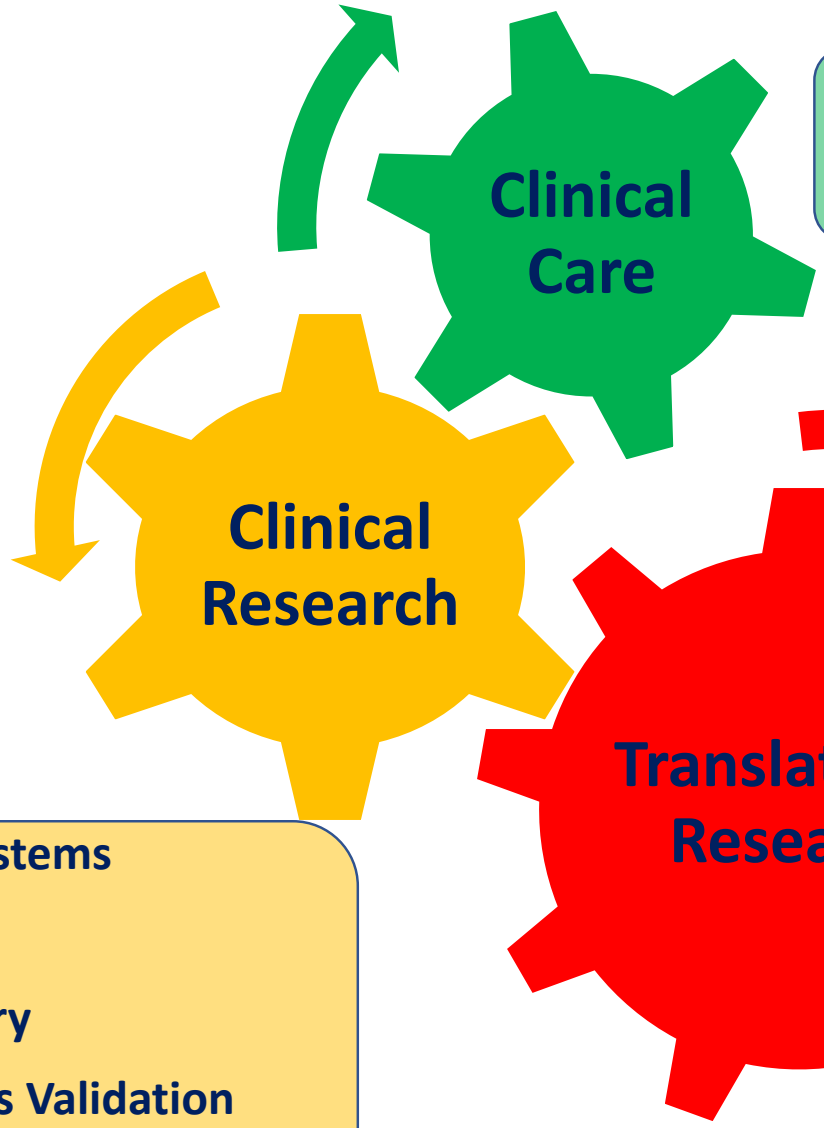
+



Patient-Important Outcome

Falk MJ, *J Inherited Met Disease*, 2020

MITOCHONDRIAL MEDICINE FRONTIER PROGRAM



- Clinical Diagnostics
- Clinical Management

- Disease Gene Discovery/Validation
- Patient Cell & Animal Model Studies
- Optimize/Derisk Precision Therapies
- Develop Companion Diagnostics

- Integrated Data Systems
- Patient Registry
- Tissue Biorepository
- Outcome Measures Validation
- Clinical Trials Design/Performance

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Mitochondrial Medicine

CONCLUSIONS

1. **MITOCHONDRIAL DISEASE IS HIGHLY HETEROGENEOUS BUT RECOGNIZABLE**
   - **Phenotypic and genetic variability**

2. **MOLECULAR DIAGNOSTIC TESTING IS ESSENTIAL IN SUSPECTED MITOCHONDRIAL DISEASE**
   - **Nuclear (>350) and mtDNA (37) gene disorders**

3. **STANDARD OF CARE GUIDELINES NOW EXIST FOR MITOCHONDRIAL DISEASE MANAGEMENT**
   - **Precision mitochondrial medicine is increasing possible**

THANK YOU!
ANY QUESTIONS?

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