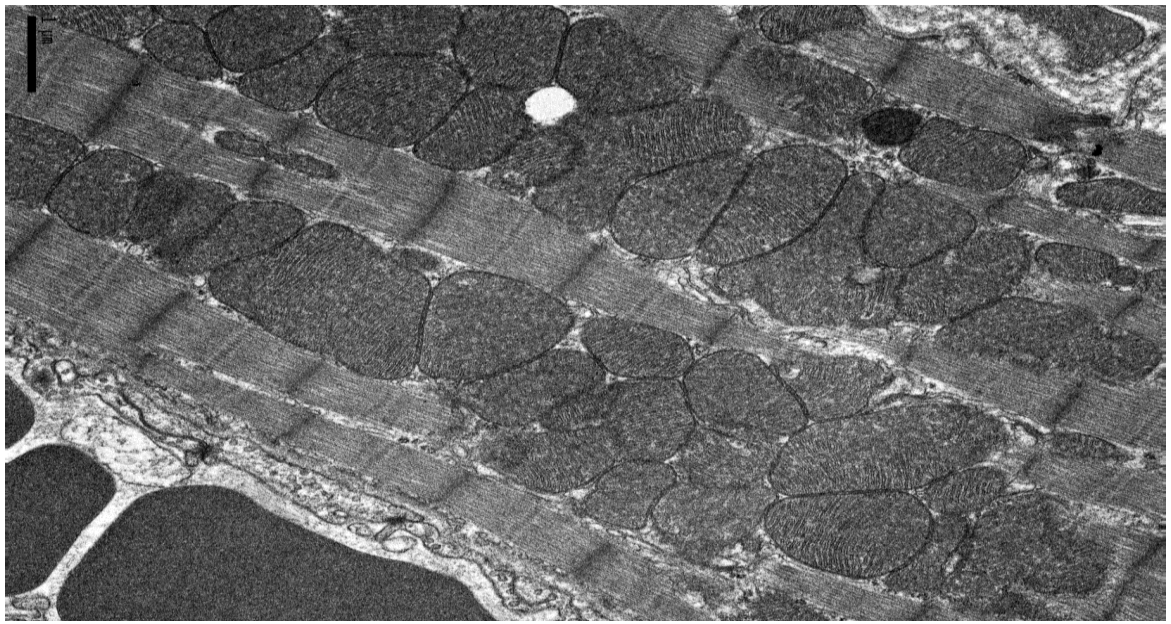
Noi abordări ale diagnosticului şi terapiei în bolile rare. Afecţiuni ale metabolismului bioenergetic

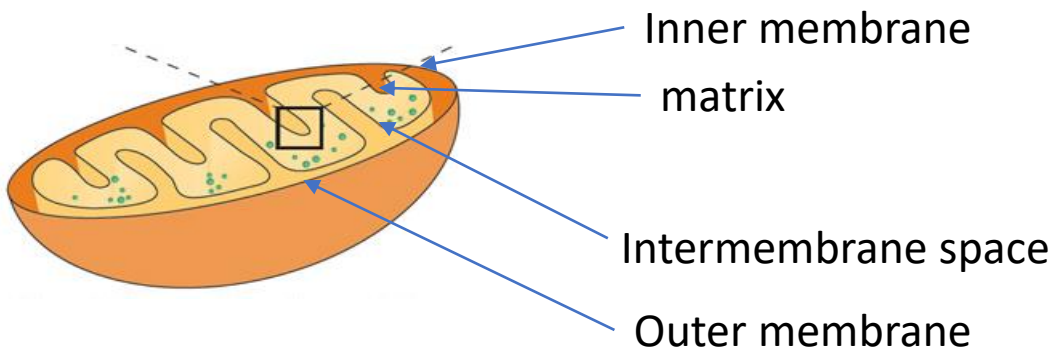
Smart Diaspora: Diaspora în Învățământul Superior, Știință, Inovare și Antreprenoriat, 10-13 aprilie 2023, Timișoara.

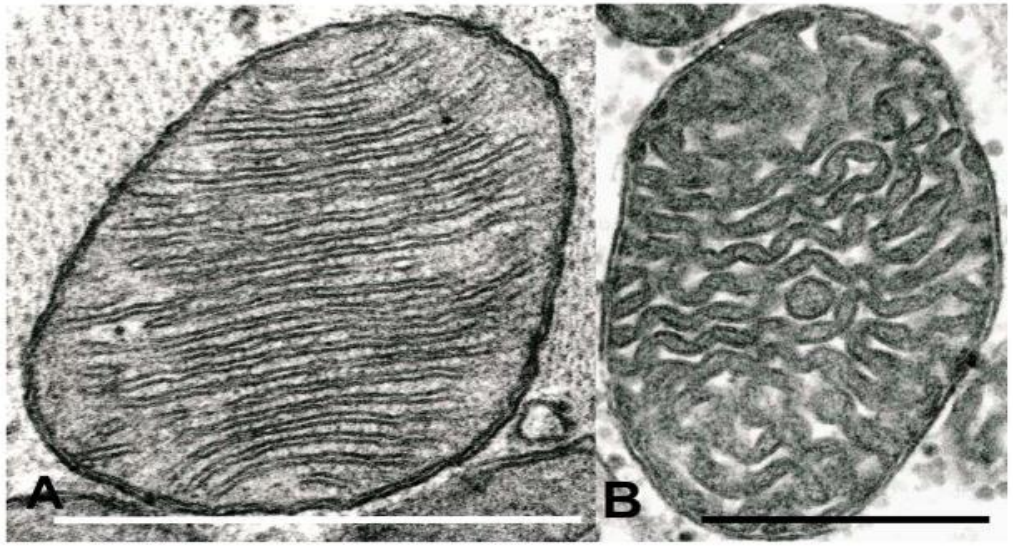
„VICTOR BABES“ UNIVERSITY OF MEDICINE AND PHARMACY FROM TIMISOARA

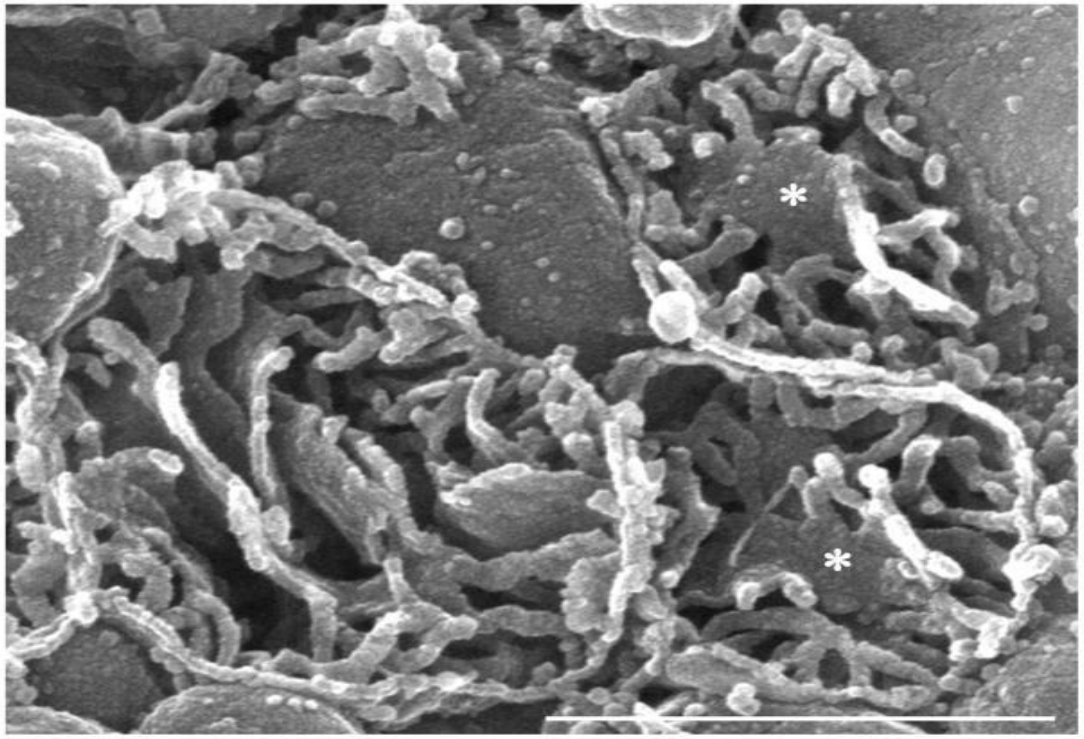
Mariana Rosca, MD

COLLEGE OF MEDICINE
CENTRAL MICHIGAN UNIVERSITY







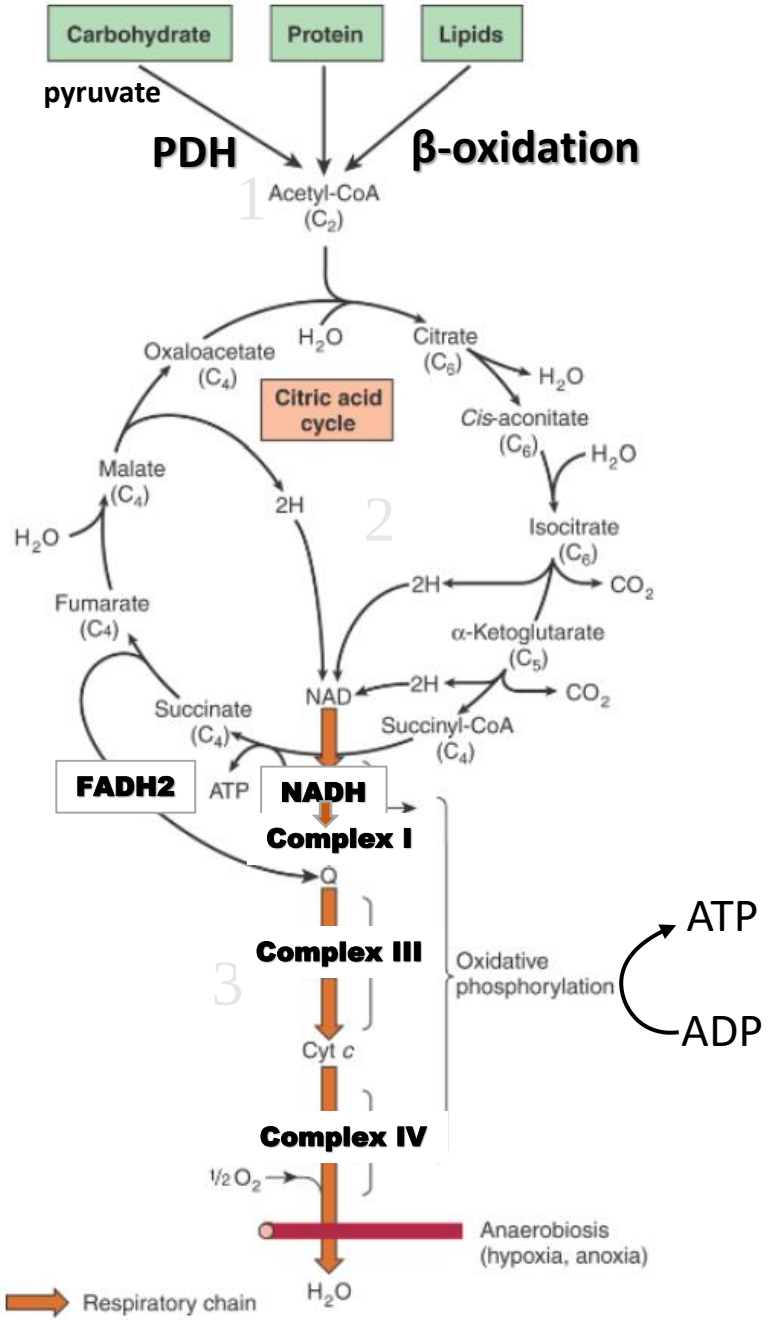


Cardiovasc Res. 2010 Oct 1;88(1):40-50. doi: 10.1093/cvr/cvq240

Int J Biochem Cell Biol. 2009 Oct; 41(10): 1949–1956. doi: 10.1016/j.biocel.2009.05.004

Mitochondrial metabolism

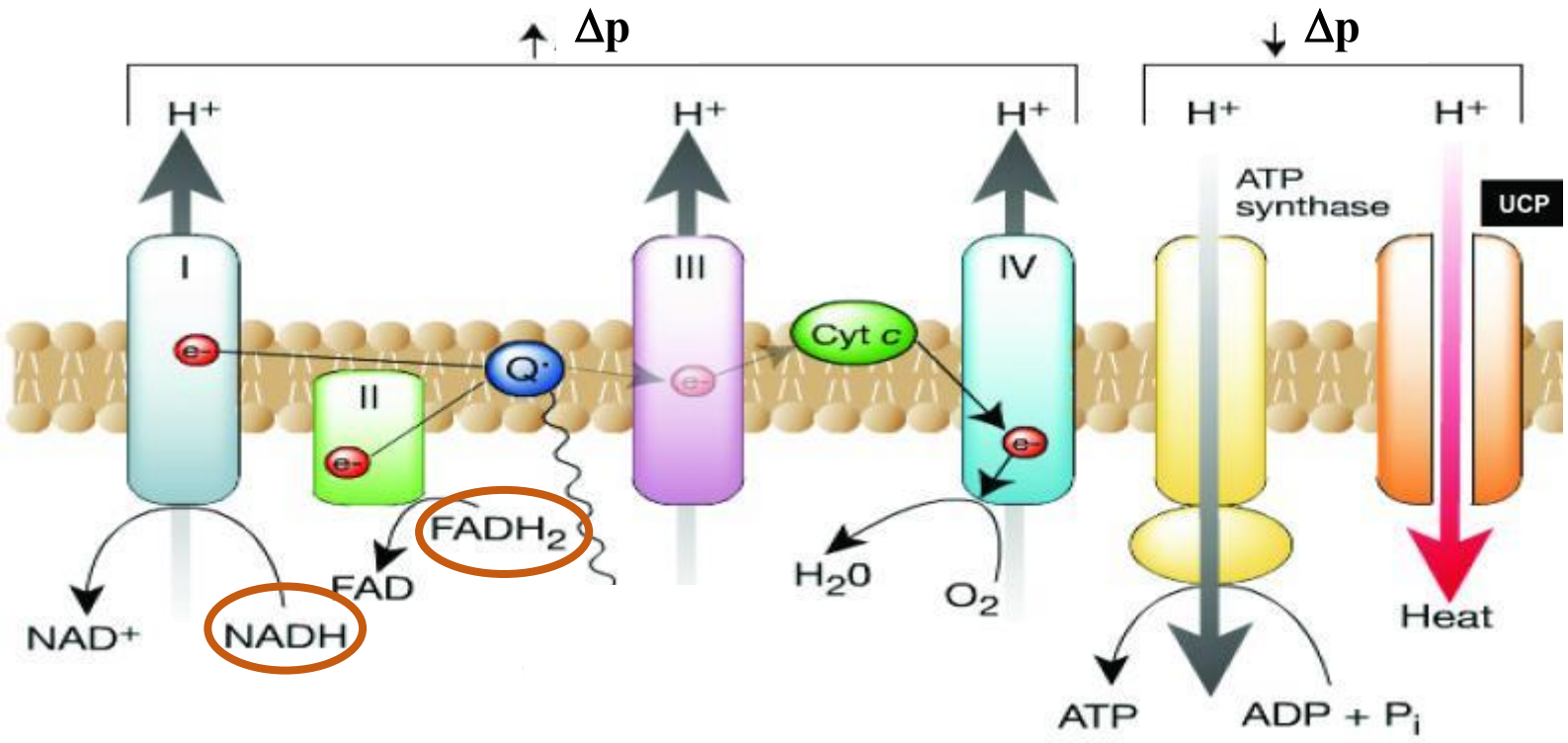
$$Acetyl\text{-}CoA + O_2 = CO_2 + H_2O + ATP$$



Regulated by the **<u>ATP demand</u>** and **oxygen availability.**

From Harper's Biochemistry, VitalSources

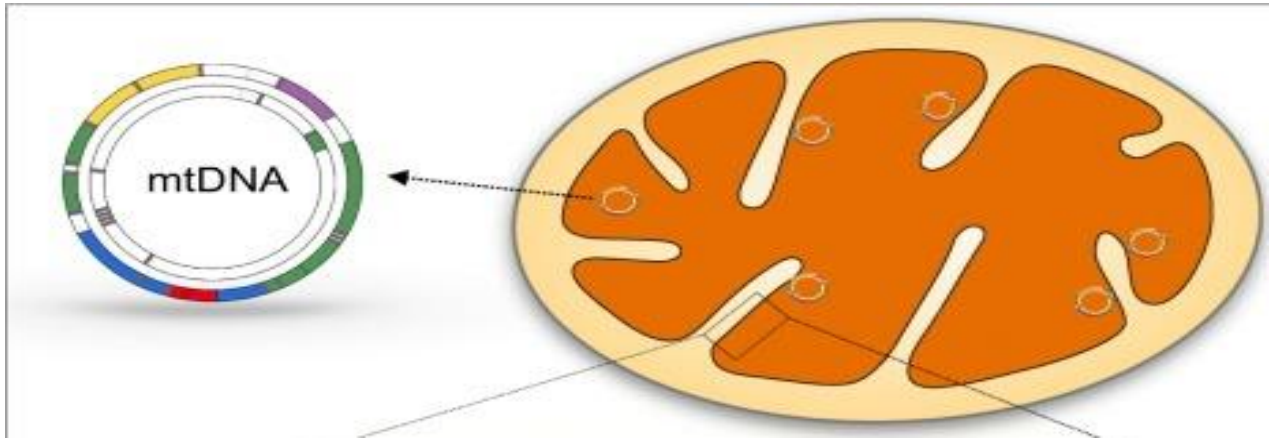
Oxidative phosphorylation



Proton-motive force (Δp): ΔpH and ΔΨ

Circ Res. 2016 27;118(11):1808-29. doi: 10.1161/CIRCRESAHA.116.306923.

Mitochondrial DNA



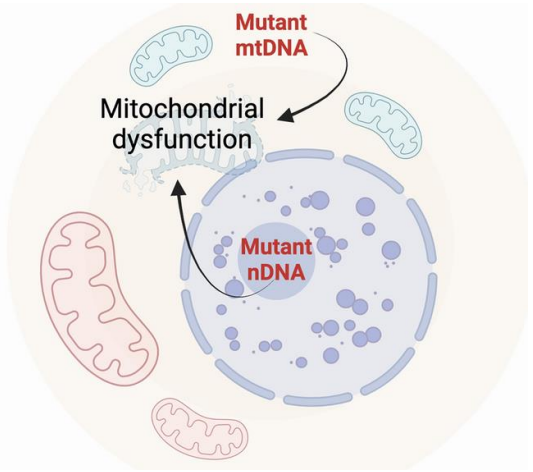
Proc Natl Acad Sci U S A. 2015 Dec 1;112(48):E6614-23. doi: 10.1073/pnas.1515733112.

- 5-10 copies per mitochondrion
- circular
- only 16,500 base pairs
- encodes 22 tRNAs, 2 rRNAs, and 13 mRNAs that are translated into **13 peptide subunits of four mitochondrial complexes**

1500 mitochondrial proteins

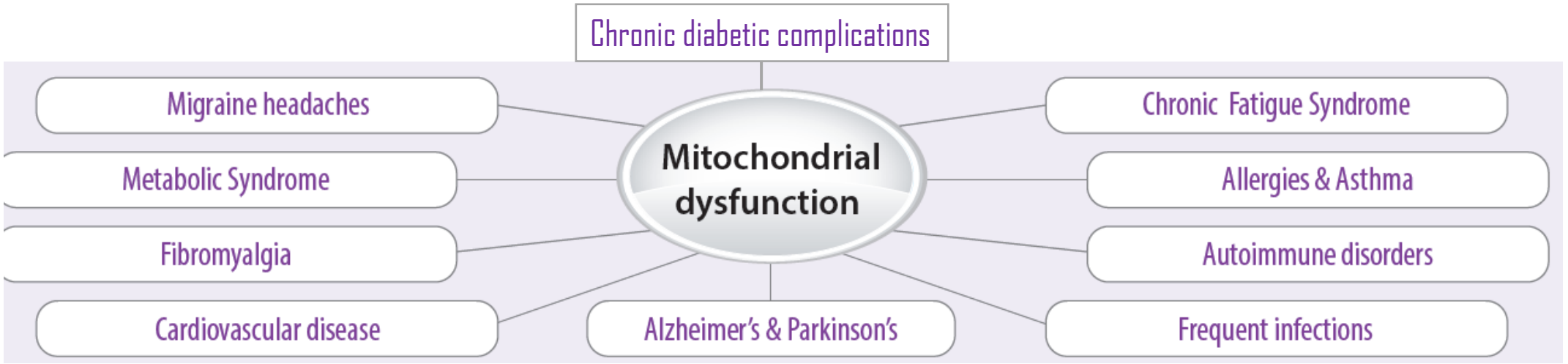
- **13 encoded by the mtDNA**
- **the rest encoded by the nuclear DNA**

Primary mitochondrial diseases (MD).
General characteristics



- affect 1:6000-1:8000 live birth (≈ childhood cancer)
- induced by mutations in either nuclear (childhood) or mtDNA (adult onset)
- 1:200 healthy humans have a pathogenic mtDNA mutation that can result in an MD
- USA: estimation that 1.4 mil people harbor a pathogenic mtDNA mutation
- 700,000 are female, 1/3 at reproductive age with the potential to transmit the disease

SECONDARY (acquired) mitochondrial defects

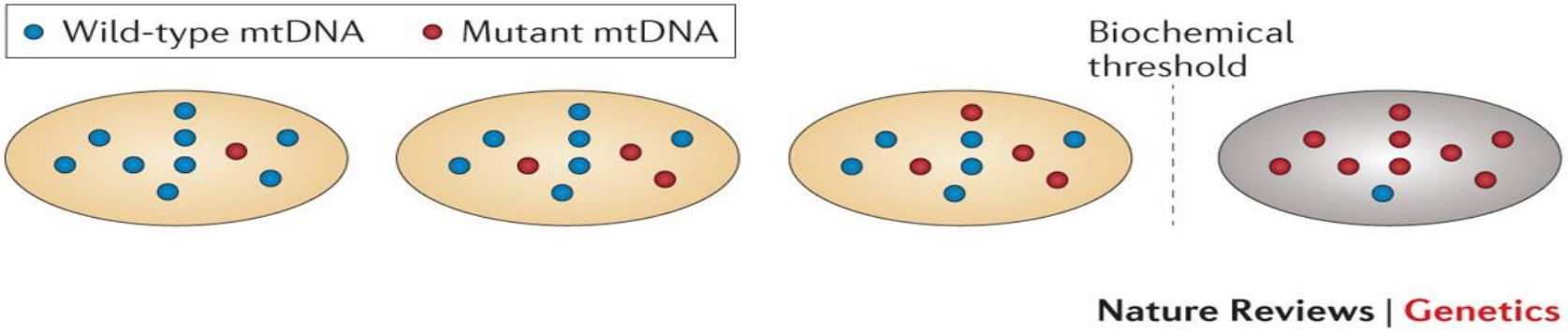


Primary MD: transmission

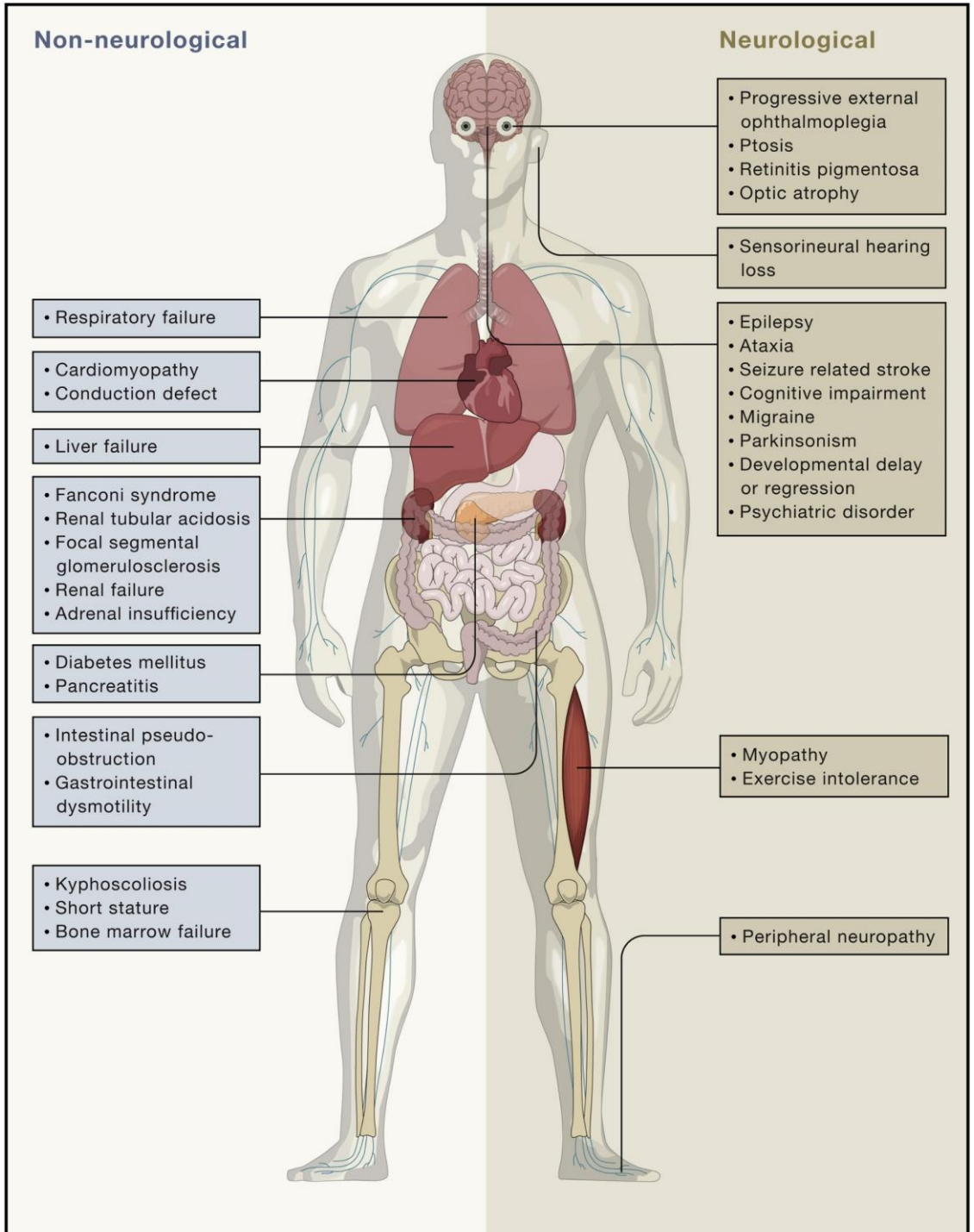
- Defects in the nDNA (~ 90%): **Mendelian inheritance**
- Defects in the mtDNA: **maternal inheritance**

  Heteroplasmy: co-existence of wild-type mtDNA with mutant mtDNA.

  Clinical expression depends on the load of mutated mtDNA.



Primary MD: clinical features



Cell. 2020 181 (1):168-88. doi: 10.1016/j.cell.2020.02.051

Primary MD: Clinical syndromes induced by mutations in nDNA

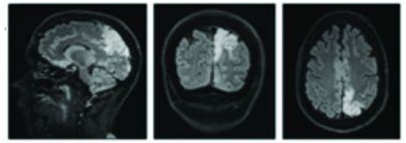
Mechanism	Gene	Inheritance	Phenotype	
Multiple mtDNA deletions	TP (Thymidine phosphorylase)	AR	MNGIE (mitochondrial neurogastrointestinal encephalopathy)	N, GI,
	ANT1	AD	adPEO: Progressive External Ophthalmoplegia	O
	TWINKLE mtDNA helicase	AD, AR	adPEO, IOSCA: Infantile Onset Spinal Cerebellar Atrophy	O, C
	POLG	AD, AR	ad/arPEO, SANDO (Sensory Ataxia, Neuropathy, Dysarthria, Ophthalmoplegia), parkinsonism	O, C, P
mtDNA depletion	POLG	AR	Alpers syndrome (neurologic: seizures, dementia, and liver)	N, L
	TK2	AR	MM (mitochondrial myopathy), SMA (Spinal Muscular Atrophy)	MM
	SUCLA2	AR	LS (**Leigh Syndrome**: subacute necrotizing encephalomyelopathy)	N
	DGUOK	AR	Alpers syndrome (Diffuse Degeneration of Cerebral Gray Matter with Hepatic Cirrhosis)	N, L
	MPV17	AR	Alpers syndrome	N, L
ETC subunit defect	NDUSFx	AR	LS, GRACILE (Growth Retardation, Aminoaciduria, Cholestasis, Iron Overload, Early Death)	N, K
	NDFVx	AR	LS	N
	SDHA	AR	LS	N
Ancillary protein defect	BCS1L	AR	LS	N
	SURF1	AR	LS	N
	SCO2	AR	LS, hypertrophic cardiomyopathy, neuropathy	N, H
	COX15	AR		
	ATP12	AR	Hypertrophic cardiomyopathy, LS	H, N
CoQ synthesis defect	COQ2	AR	Encephalomyopathy, tubulopathy, ataxia	N, C, K
	PDSS2	AR	Encephalomyopathy, tubulopathy, ataxia	N, C, K
Iron metabolism defect	ALAS2	X-linked	Sideroblastic anaemia	A
	ABCB7	X-linked	Sideroblastic anaemia and ataxia	A
	FRDA (*Fxn*)	AR	Friedreich's ataxia	C
Motility defect	KIF5A	AD	Spastic paraplegia	N
Fusion defect	MFN2	AD	CMT2A (Charcot-Marie-Tooth disease type 2A): nervous system degeneration	N
	OPA1	AD	Optic nerve atrophy	E
Fission defect	DLP1	AD	Microcephaly, optic atrophy, lactic acidosis	N, E, LA

Acta Myol. 2009 Jul; 28(1): 16–23. PMCID: PMC2859630PMID: 19772191, Mitochondrial disorders of the nuclear genome, C Angelini, L Bello, M Spinazzi, and C Ferrati

Primary MD: Clinical syndromes induced by mutations in mtDNA

Mitochondrial Encephalopathy, Lactic Acidosis, Stroke-like syndrome (MELAS)

- Point mutations in the $tRNA^{Leu}$ gene → generalized defect in translation of all 13 mtDNA encoded proteins → multiple OXPHOS defects
- Brain MRI: stroke-like lesions mimic an ischemic stroke but no vascular territory → migraine, seizures
- Large variability of clinical phenotypes:
  - Maternally-inherited diabetes
  - Sensory-neural deafness
  - Cardiomyopathy
  - Ileus paraliticus
  - CPEO (Ophthalmoplegia)
  - Encephalomyopathy without stroke
  - Psychiatric disorders
  - Skeletal myopathy

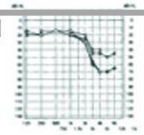


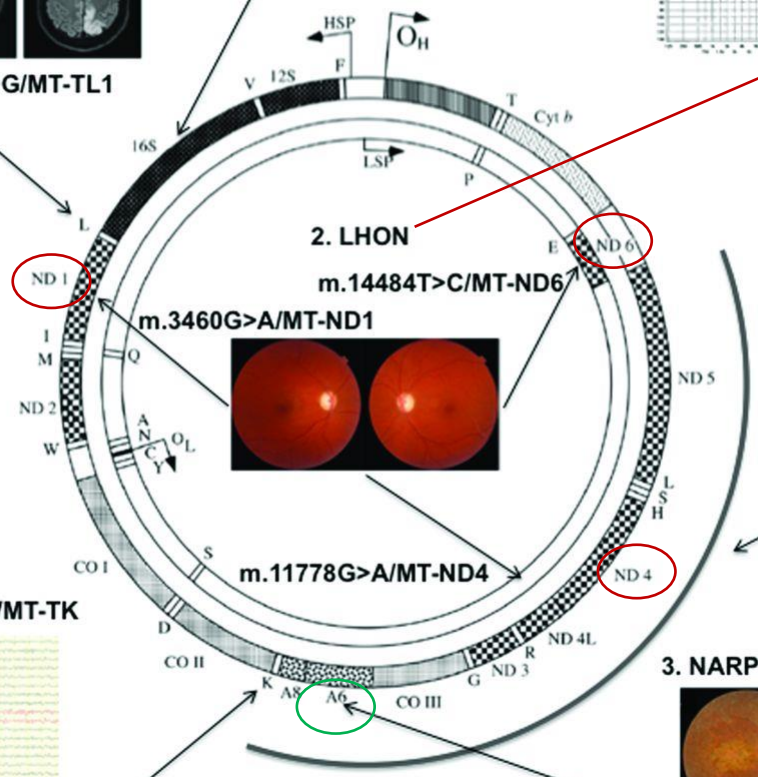
4. MELAS m.3243A>G/MT-TL1

Maternally inherited and aminoglycosides-induced deafness (Sensorineural Hearing Loss, SNHL)

Point mutations affecting mitochondrial RNA genes

6. SNHL m.1555A>G/MT-RNR1





Leber's hereditary optic neuropathy (LHON)

- Three known mtDNA mutations, all affecting complex I subunit genes
- Higher homoplasmy (100%)
- Variable penetrance, higher in males than females
- Although the mutation is homoplastic in all cells in the body, ganglion retinal cells are the most sensitive
- Causes optic atrophy and blindness

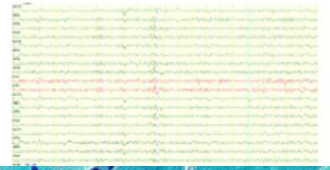
1. CPEO-single mtDNA deletion

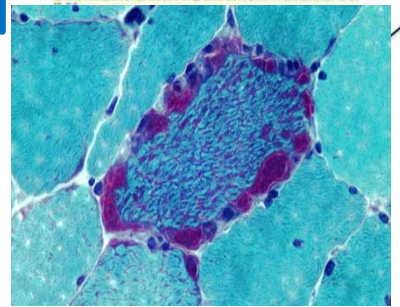
1. **Chronic External Progressive Ophthalmoplegia**
2. **Kearn-Sayre syndrome**: CEPO with systemic signs (retinopathy, AV block)
3. **Pearsone syndrome**: pancytopenia evolving to KSS later in life

Myoclonic Epilepsy with Ragged Red Fibers (MERRF)

Point mutation in the $tRNA^{Lys}$ gene → generalized defect in translation of all 13 mtDNA encoded proteins → multiple OXPHOS defects

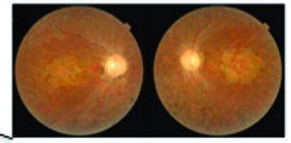
5. MERRF m.8344A>G/MT-TK





http://epilepsyu.com/2015/05/05/myoclonus-epilepsy-associated-with-ragged-red-fibers-merrf-diagnosis-discussed-by-researchers/

3. NARP m.8993T>G/*MT-ATP6*

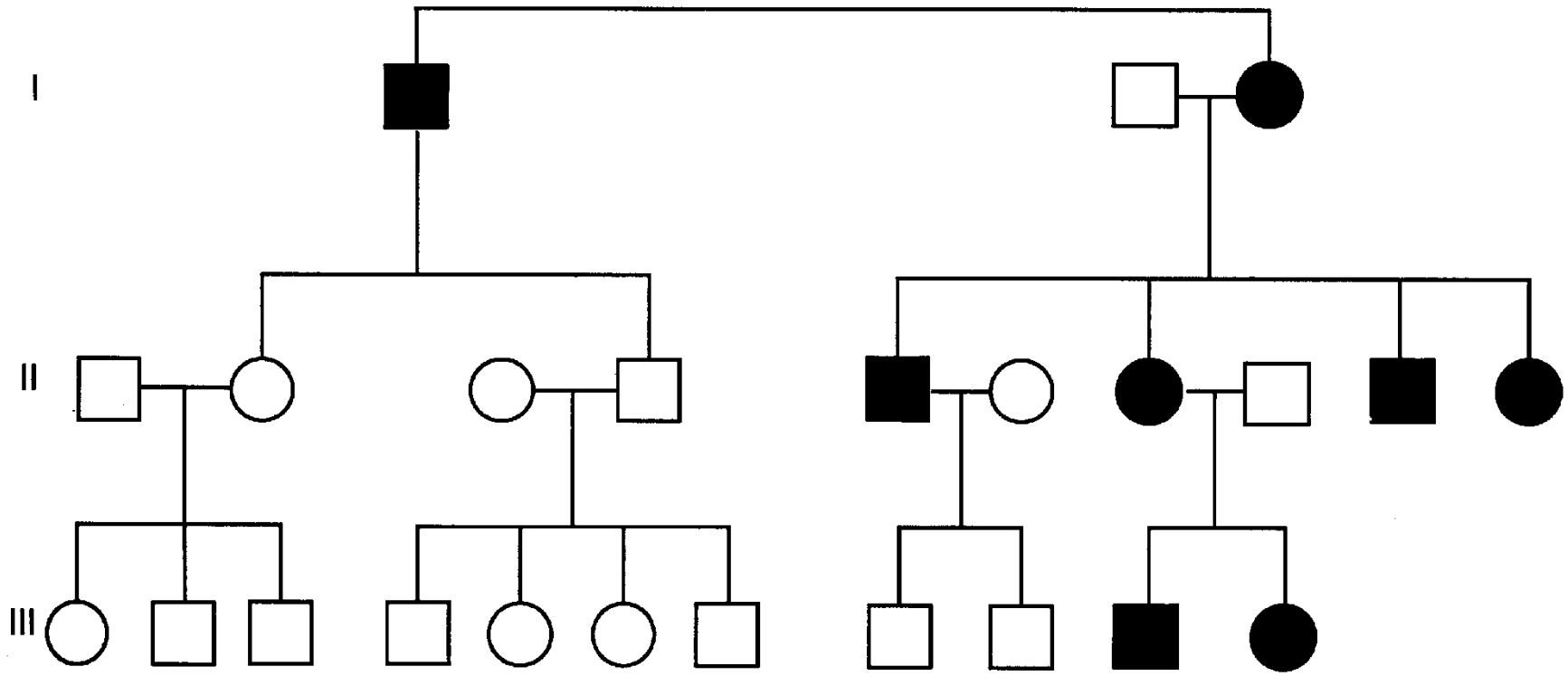


Neuropathy, Ataxia, Retinitis Pigmentosa and maternally inherited Leigh syndrome

- Induced by missense mutations in the ATP synthase subunit genes
- ↓ ATP synthesis, ↑ oxidative stress → cell death
- Complete homoplasmy is not compatible with life
- Predictable genotype/phenotype correlation

Adapted from Carelli V and al, Essays in Biochemistry 2018, 62:235-54

Leber's Hereditary Optic Neuropathy (LHON)



First described by **Von Graefe in 1858**, then characterized formally into a distinct clinical entity by Leber in 1871.

First published pedigree showing mitochondrial maternal inheritance.

Originally believed to be X-linked and inherited with partial penetrance

MD: Diagnostic challenge

❑ **History for:**
- unusual childhood diseases (neonatal death, seizure disorders, progressive neurologic deficits)
- deafness or diabetes in family members
- pattern suggesting maternal inheritance

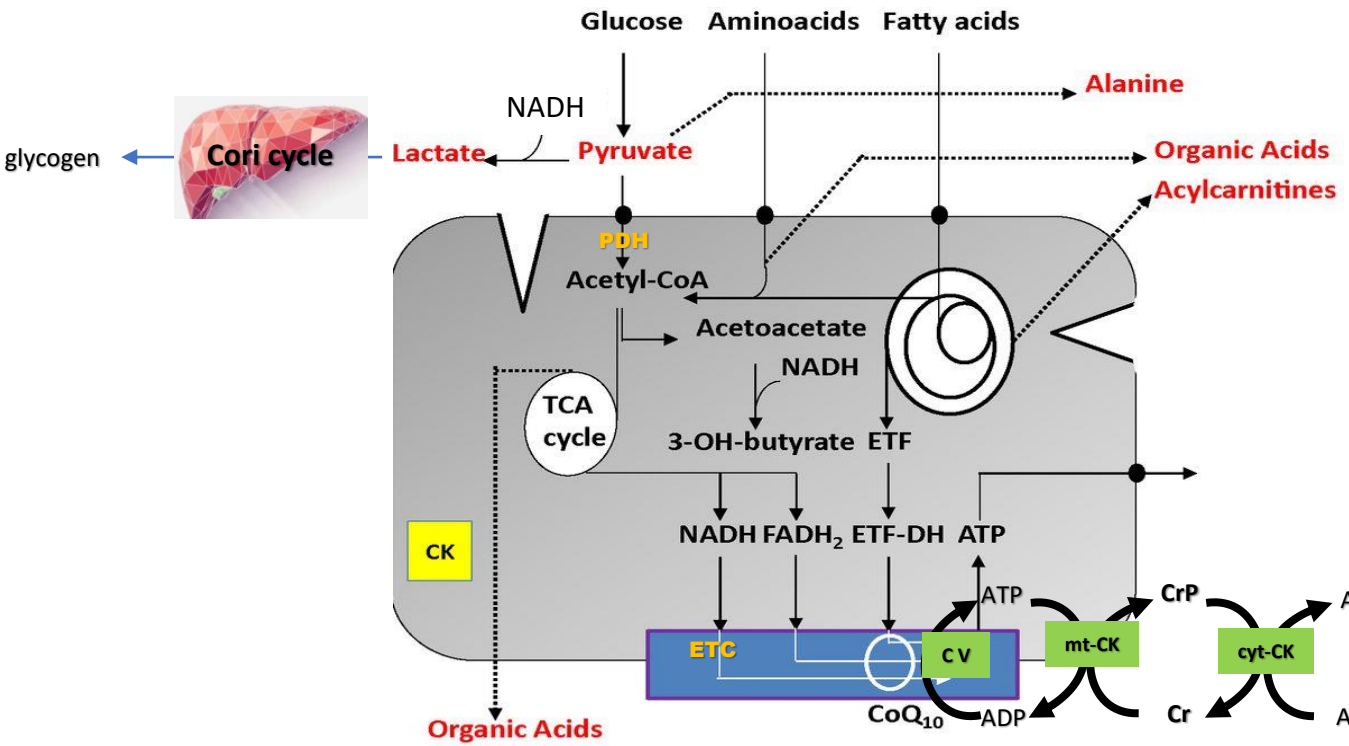
❑ **Biochemical markers** (serum, urine, CSF)

❑ **Muscle biopsy**
- ragged red fibers (modified Gomori stain)
- absence of ETC complexes by histochemical staining

❑ **Others**
- In Leigh syndrome or MELAS - CT or MRI may show some of the characteristic cerebral lesions

❑ **Novel techniques**

MD: biochemical markers



Pyruvate dehydrogenase deficiency →
↑ Pyruvate
↑ Lactate
Normal Lactate/pyruvate ratio
↑ Alanine

Electron transport chain (ETC) defect →
Little change in Pyruvate
↑ Lactate
↑ Lactate/pyruvate ratio

Creatine (Cr), creatine kinase (CK), creatine phosphate (CrP)
↑ serum CK and lactate: suggestive for mitochondrial myopathy.

Acylcarnitines are the result of incomplete fatty acid oxidation.
- usually normal in mitochondrial ETC defects
- ↑ in fatty acid oxidation defects

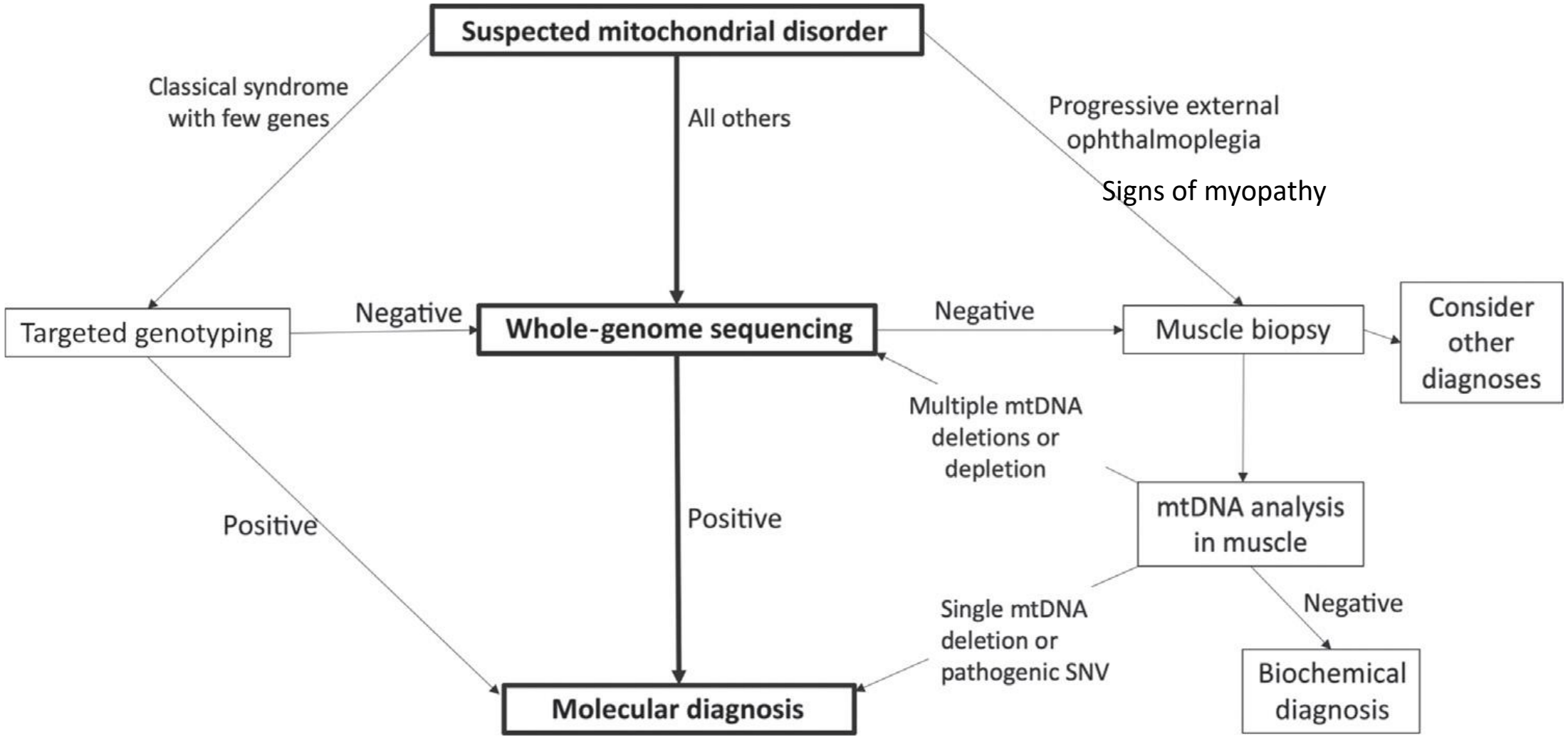
MD: Integrated “OMICS” approaches

High-throughput technologies for molecular diagnosis:

1. **Genomics:** genetic testing using Next Generation Sequencing (NGS) technologies
    - whole exome sequencing (WES)
    - whole genome sequencing (WGS)

    Challenges:
    - difficult to identify heteroplastic mtDNA mutations with low mutant load
    - determine the causal link between the mutation and phenotype
2. **Transcriptomics:** RNA sequencing
3. **Quantitative proteomics**
4. **Metabolomics**

Proposed Diagnostic Algorithm for Investigation of Patients with Suspected Mitochondrial Disease



CAZ CLINIC (Ultrastructural Pathology, 2014; 38(1): 13–25; DOI: 10.3109/01913123.2013.831158)

Barbat de 45 ani se prezinta pentru oboseala musculara progresiva, cefalee, ameteli si sincopa.

Fara antecedente patologice pina la 41 de ani. La 45 de ani a prezentat un episod the grand-mal epileptic.

Examen fizic: tremor, modificari de mers, scaderea contractilitatii musculare la membrele superioare si inferioare

Laborator: hematologic normal

Metabolic in sange:

- Acid lactic crescut
- Carnitina libera si totala scazute
- Profil acylcarnitine in plasma normal
- Pyruvate dehydrogenaza (limfocite): inconclusiv

Ecocardiografie: usoara depresie a functiei sistolice a ventricolului stang

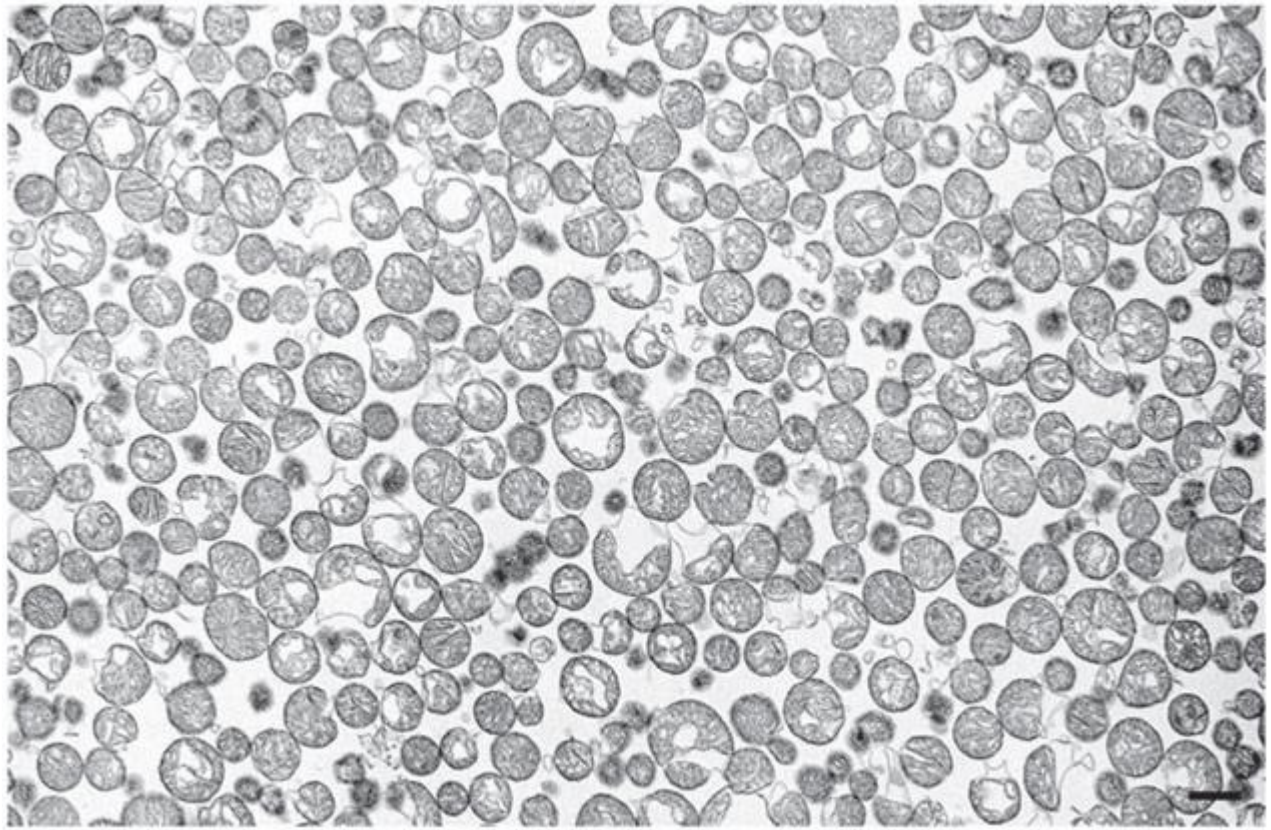
Electromyography: traseu miopatic

PET scan, alte investigatii exclud un sindrom paraneoplazic care sa explice miopatia

Biopsie musculara din cvadriceps:

- preparate pentru microscopie
- izolare de mitocondrii
- fosforilare oxidativa
- activitatile complexelor lantului respirator (spectrophotometric) in muschi homogenizat si mitocondrii
- analiza DNA mitocondrial

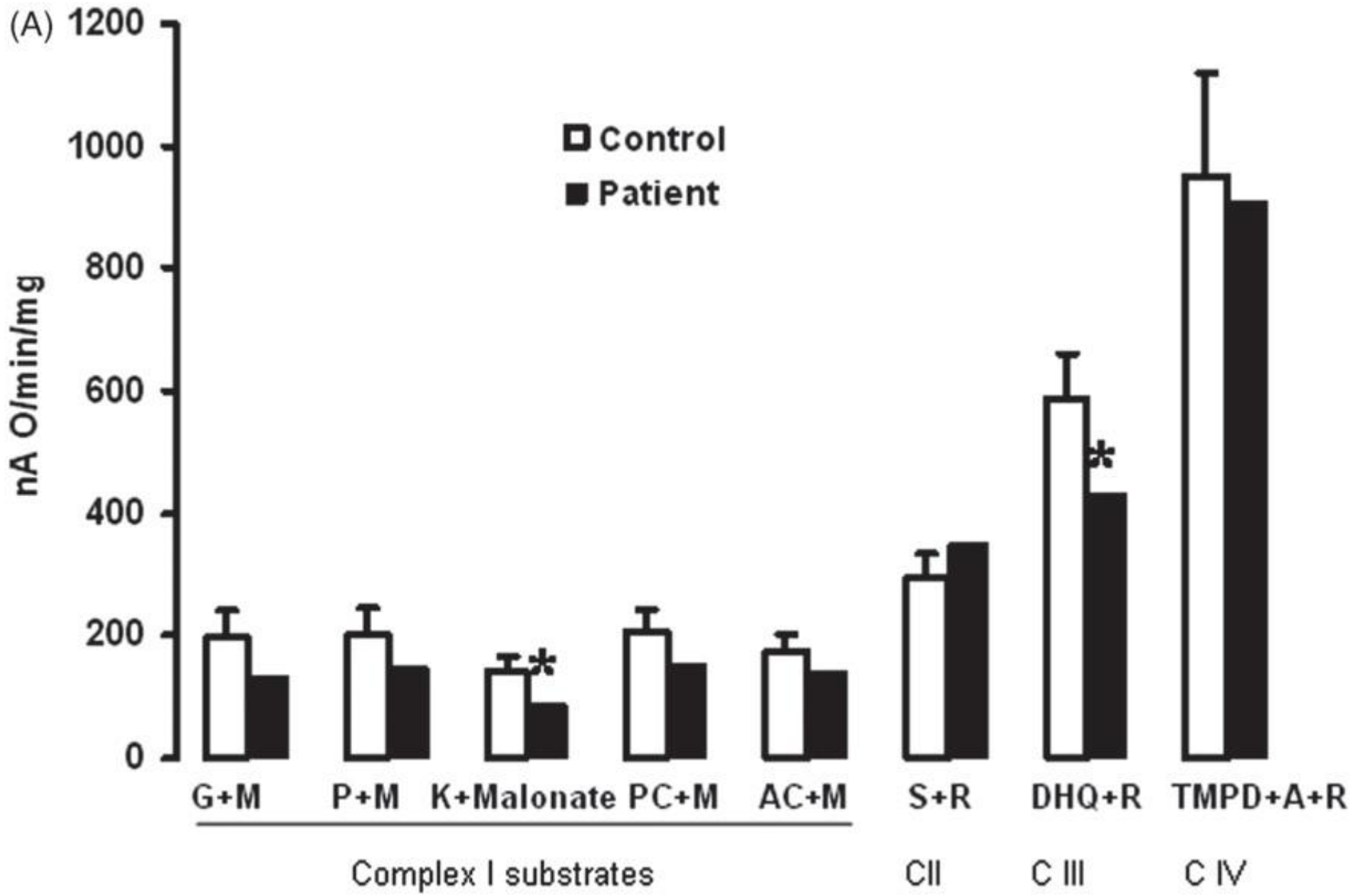
Quality control. Preparat the mitocondrii izolate.
Microscopie electronica



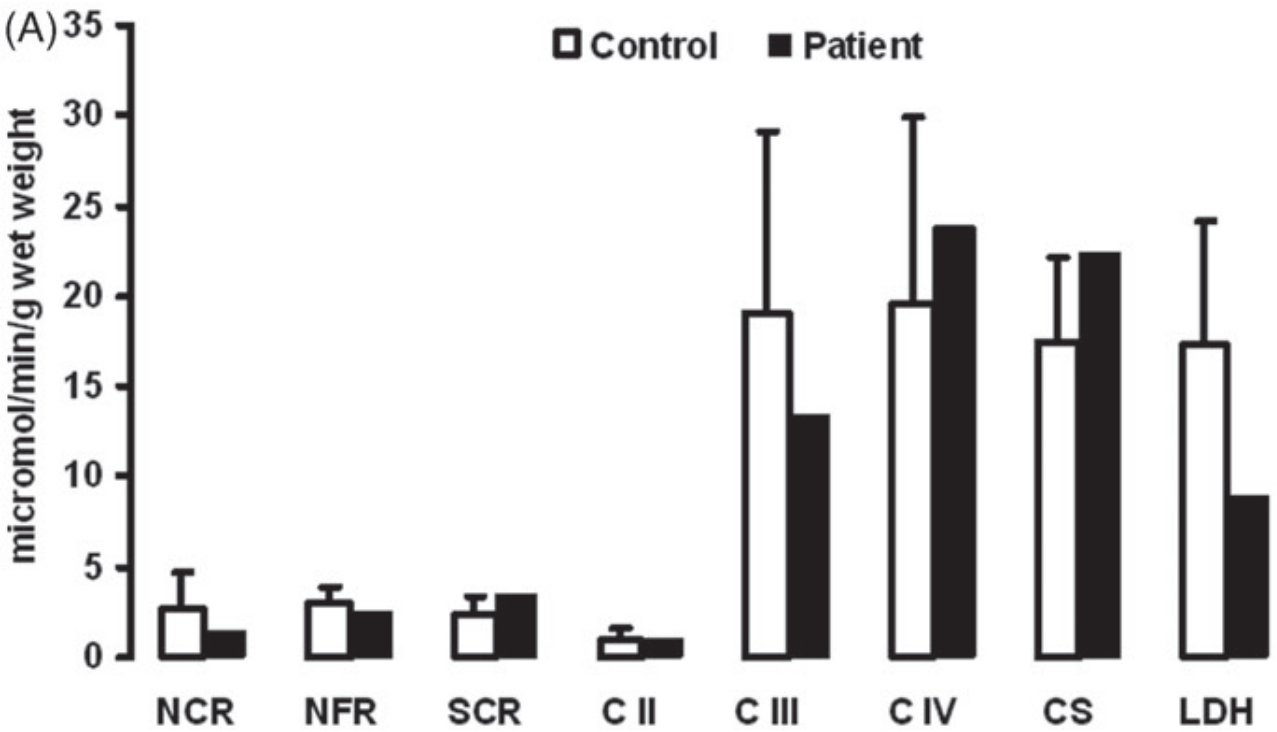
Fosforilare oxidativa: consum de O2 in prezenta unui substrat energetic, glutamat

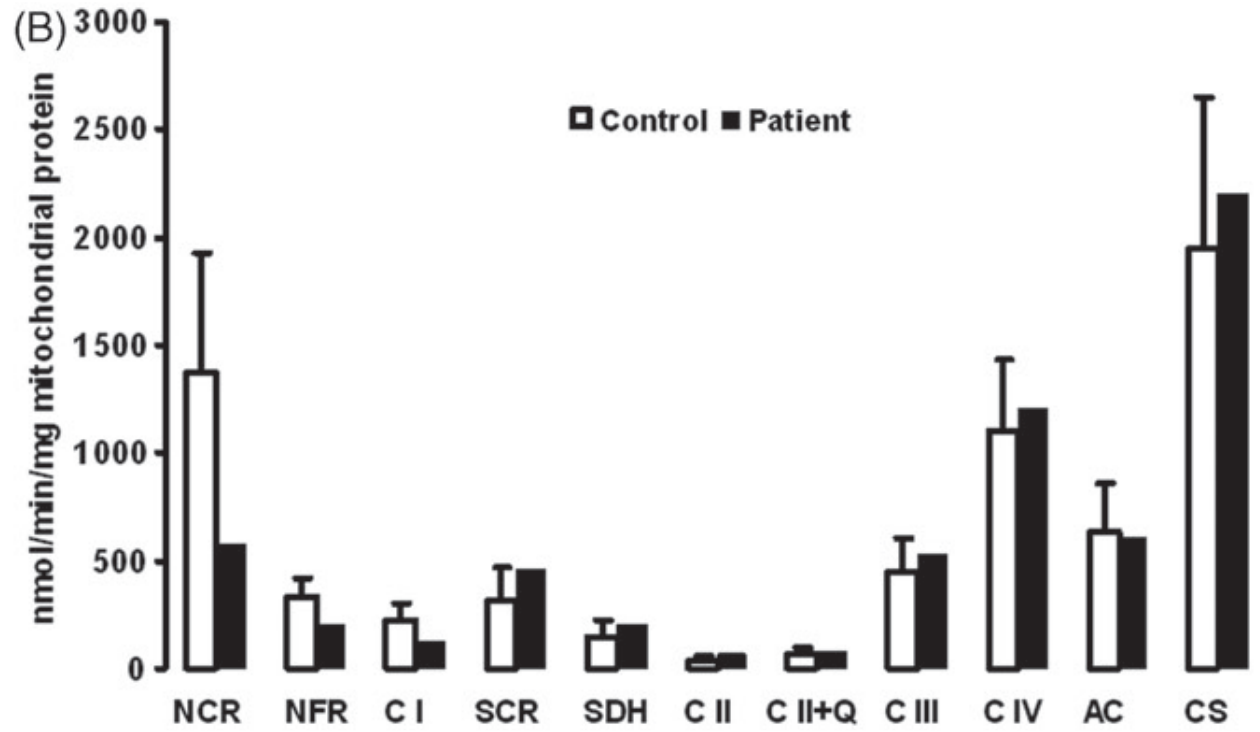
	Pacient	Control (N=68)
State 3 (ADP-dependent)	116.3	164 ± 44.1
State 4	14.5	16.1 ± 6.6
Respiratory Control Ratio (S3/S4)	8.0	13.2 ± 8.5
ADP/O	2.7	2.8 ± 0.2
Respiratie maxima	134.2	175.6 ± 46.6
Respiratie maxima uncoupled	129.7	201.1 ± 70.2

Fosforilare oxidativa cu 19 substrate energetice care folosesc cai alternative pentru a suplea lantul respirator



Activitatea specifica a complexelor lantului respirator in homogenat de tesult muscular scheletic (A.) si mitocondrii isolate (B.)



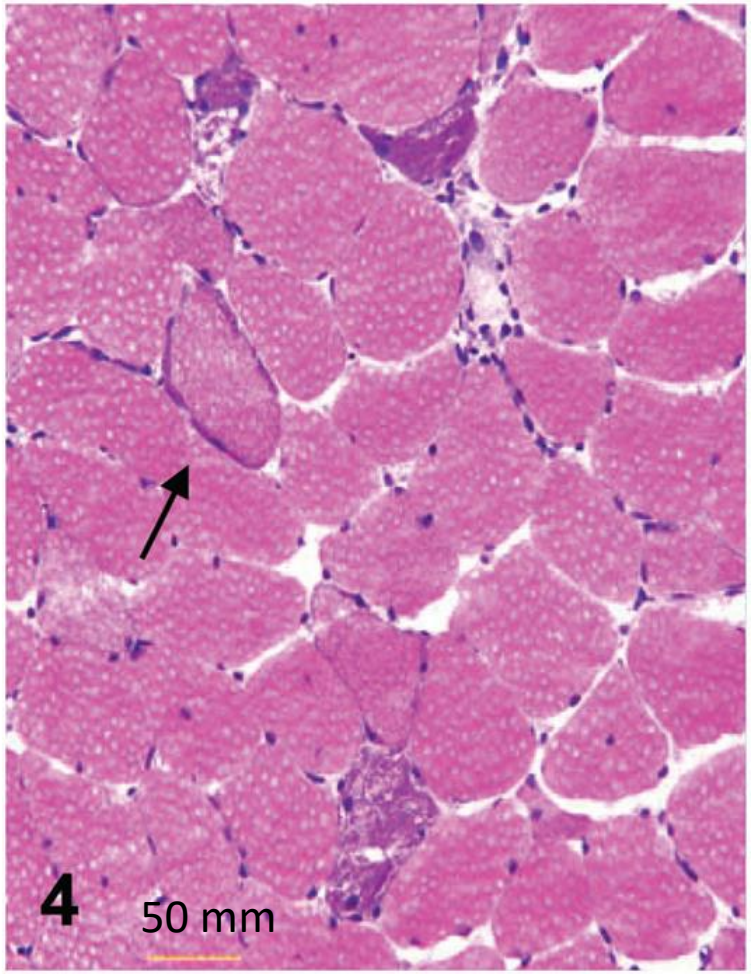


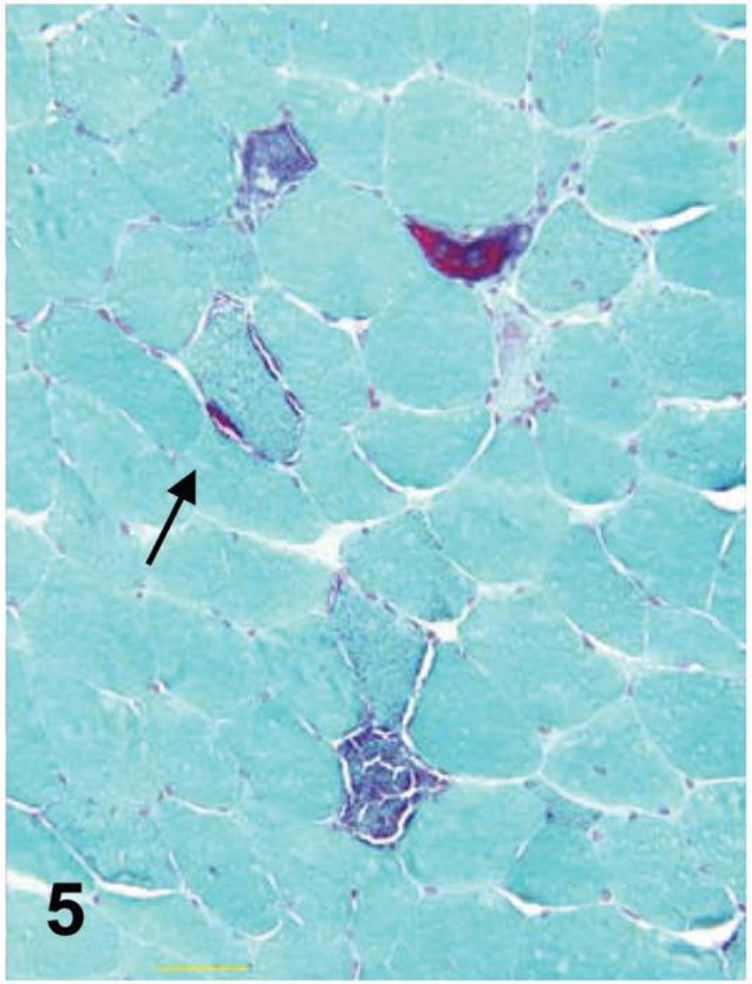
Mitochondrial Genome Sequence Evaluation:

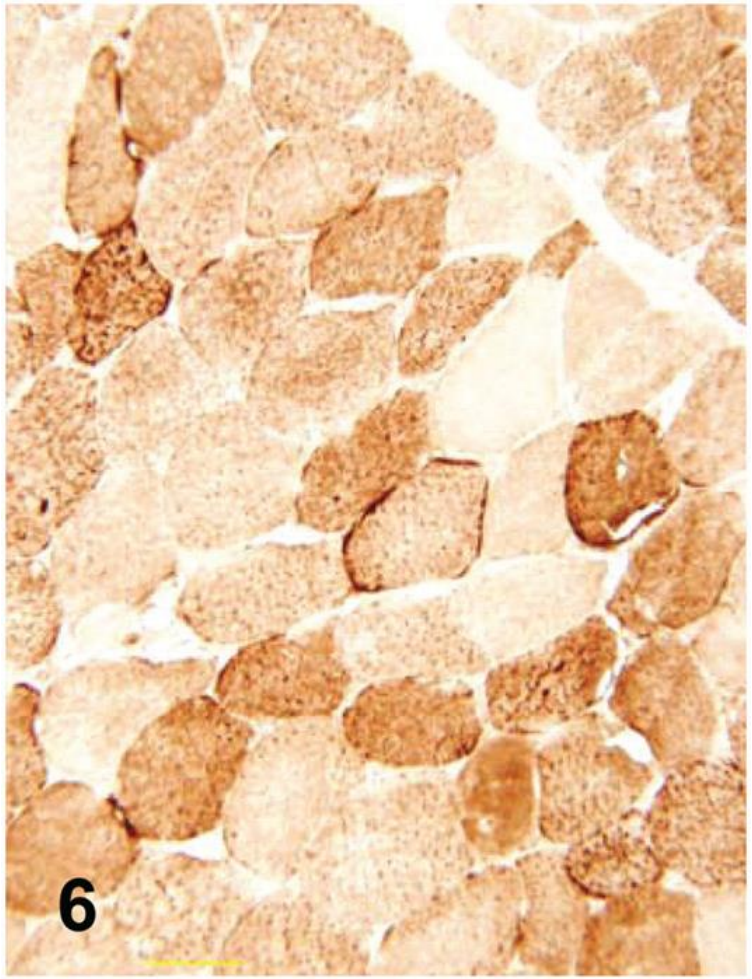
Heteroduplex scanning revealed **3255G>A** in the mtDNA

75% in the skeletal muscle biopsy sample, confirmed by

Sanger sequencing





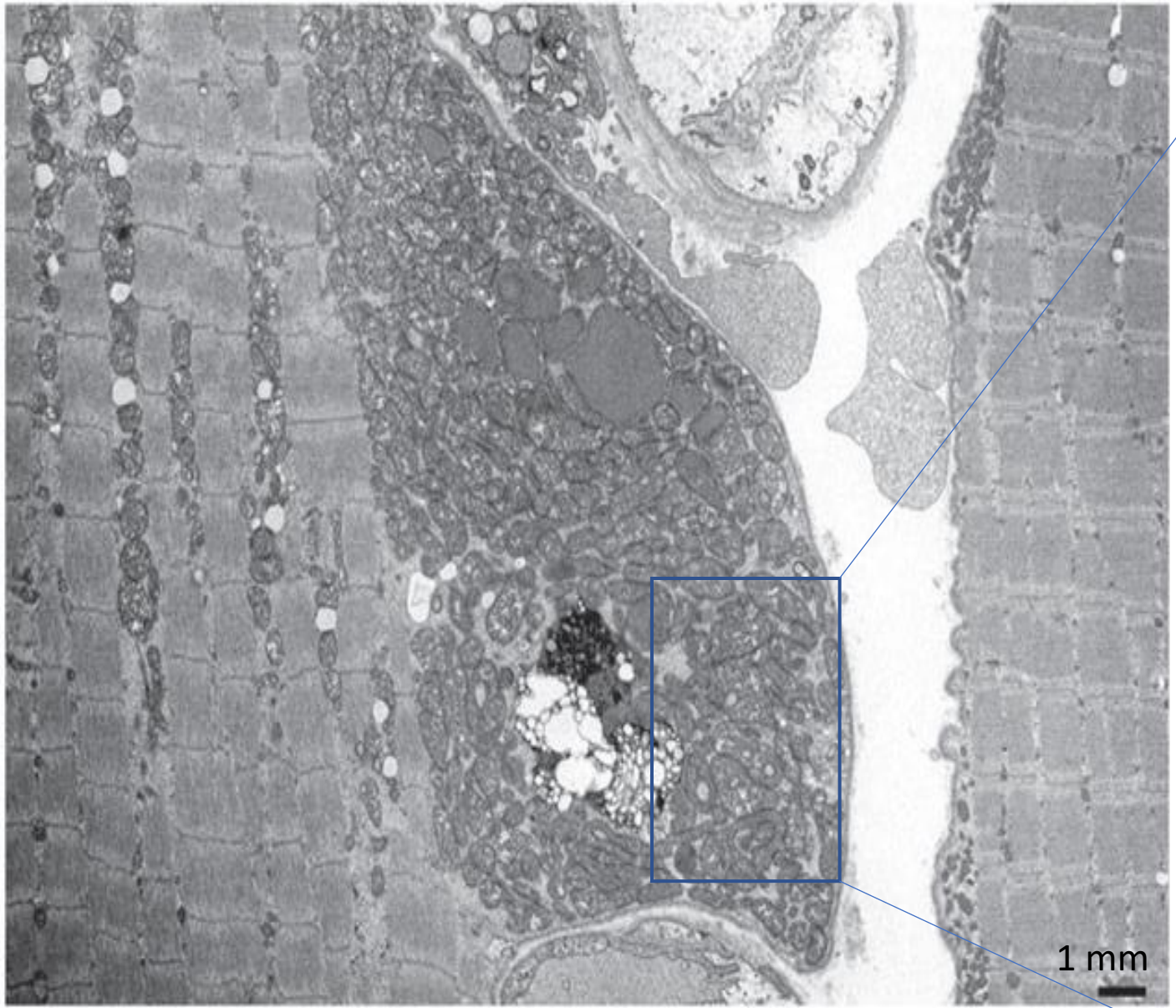


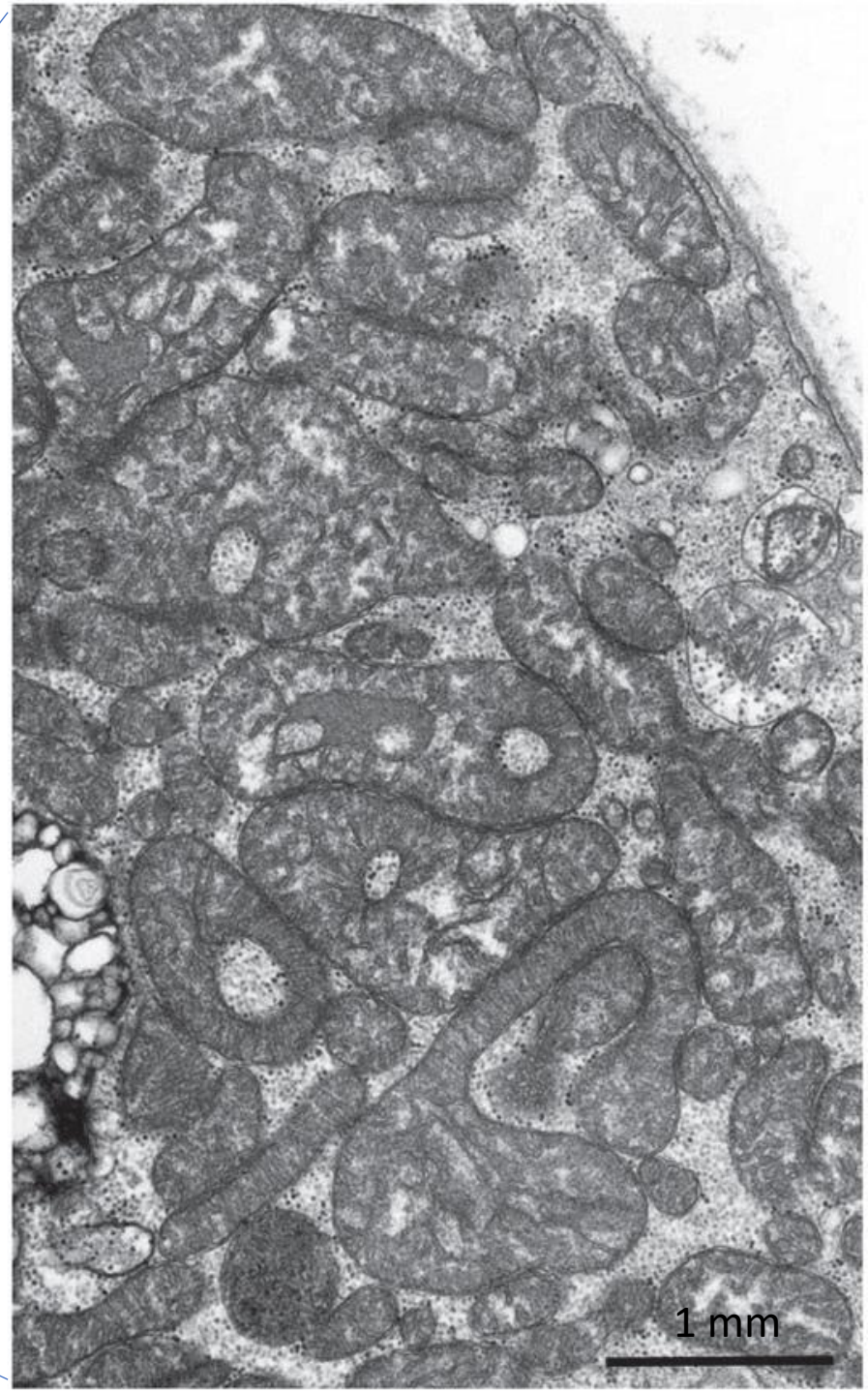
Skeletal muscle (HE stain): some myocytes show subsarcolemmal basophilia

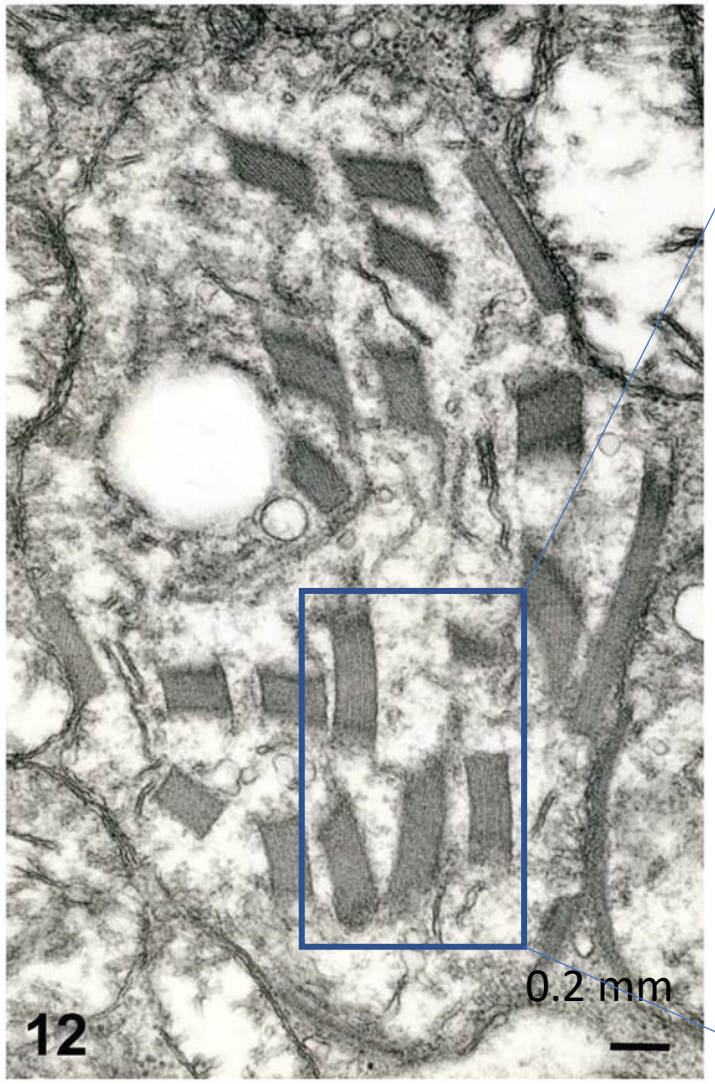
Ragged fibers (Gomori trichrome) correspond to the hyperbasophilic fibers

Cytochrome oxidase staining highly variable among the myofibers

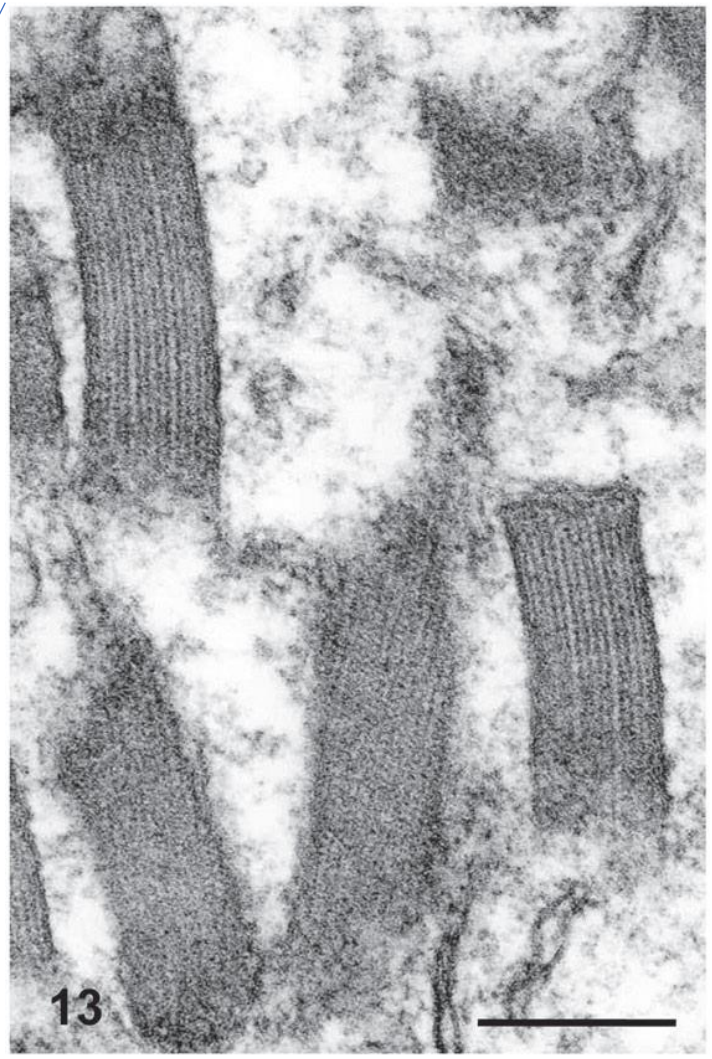
Electron micrograph of a mitochondria-rich myocyte



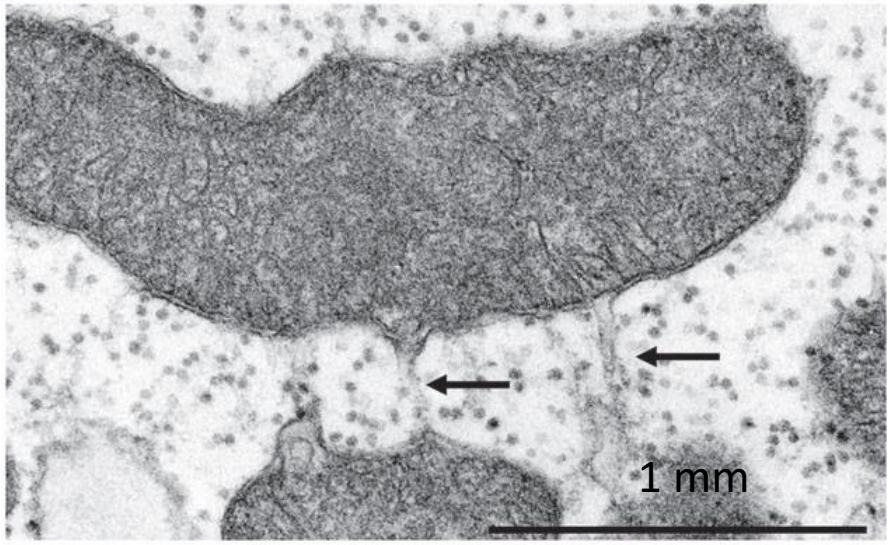




Interfibrillar mitochondria containing numerous crystalloids



Mitochondrial crystalloids at high magnification



Mitochondrial outer membrane inter-mitochondrial contact bridges

Conclusions

1. Heteroplasmic (>75%) mtDNA 3255G>A mutation in tRNA (G is evolutionary highly conserved, region intolerant to mutations)
2. Mitochondrial abnormalities
3. Myopathy
4. mtDNA heteroplasmy associated with heterogeneity in mitochondrial myocyte morphology

Prevention of Transmission of Mitochondrial Diseases

nDNA defects:

- Counselling
- Prenatal diagnosis (chorionic villus biopsy or amniocentesis)
- Preimplantation genetic diagnosis (PGD)
- Correction of the genetic defect at the germ cell or embryo stage (safety, efficacy, and ethics of using these techniques at such stages needs to be established)

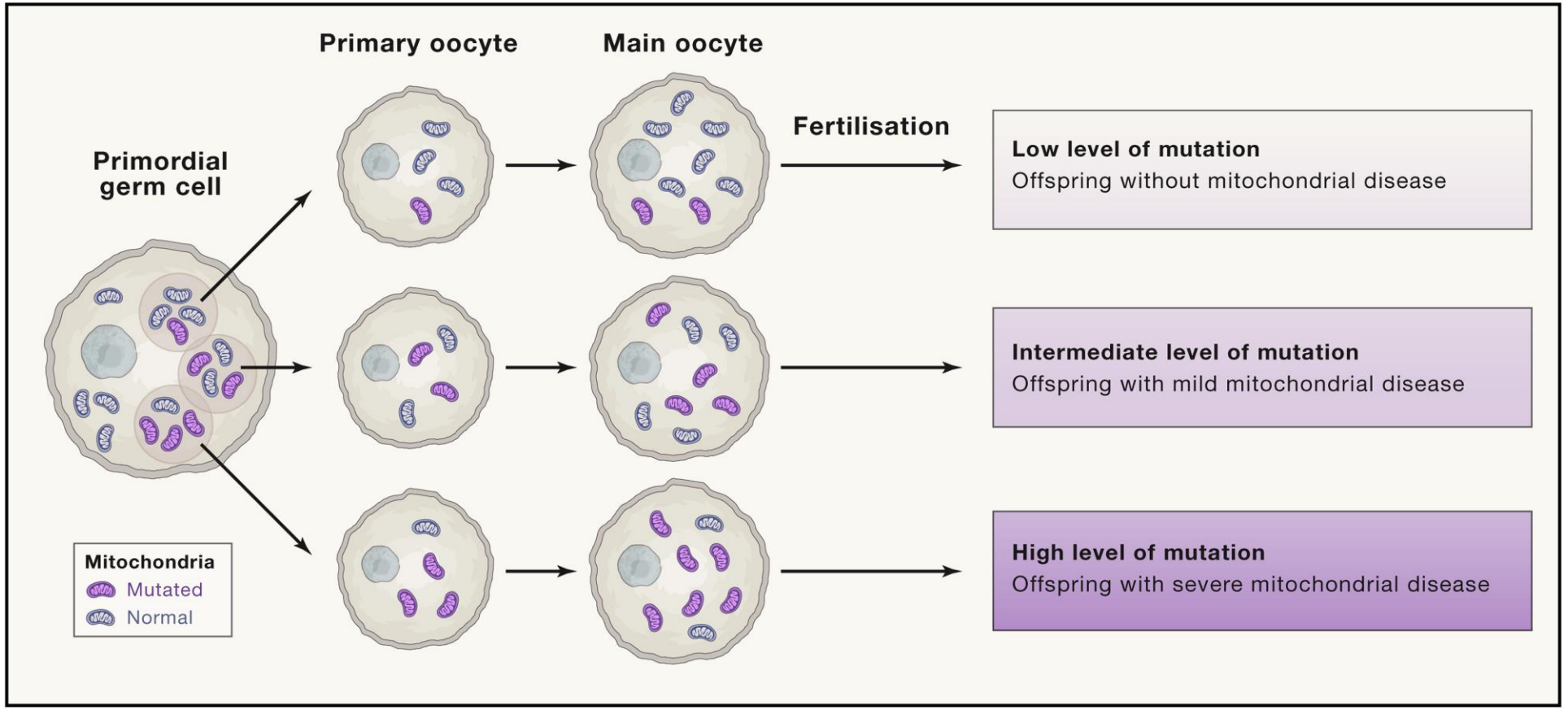
mtDNA defects: Challenges

- mtDNA is almost invariably maternally inherited
- genetic bottleneck during development → heteroplasmy

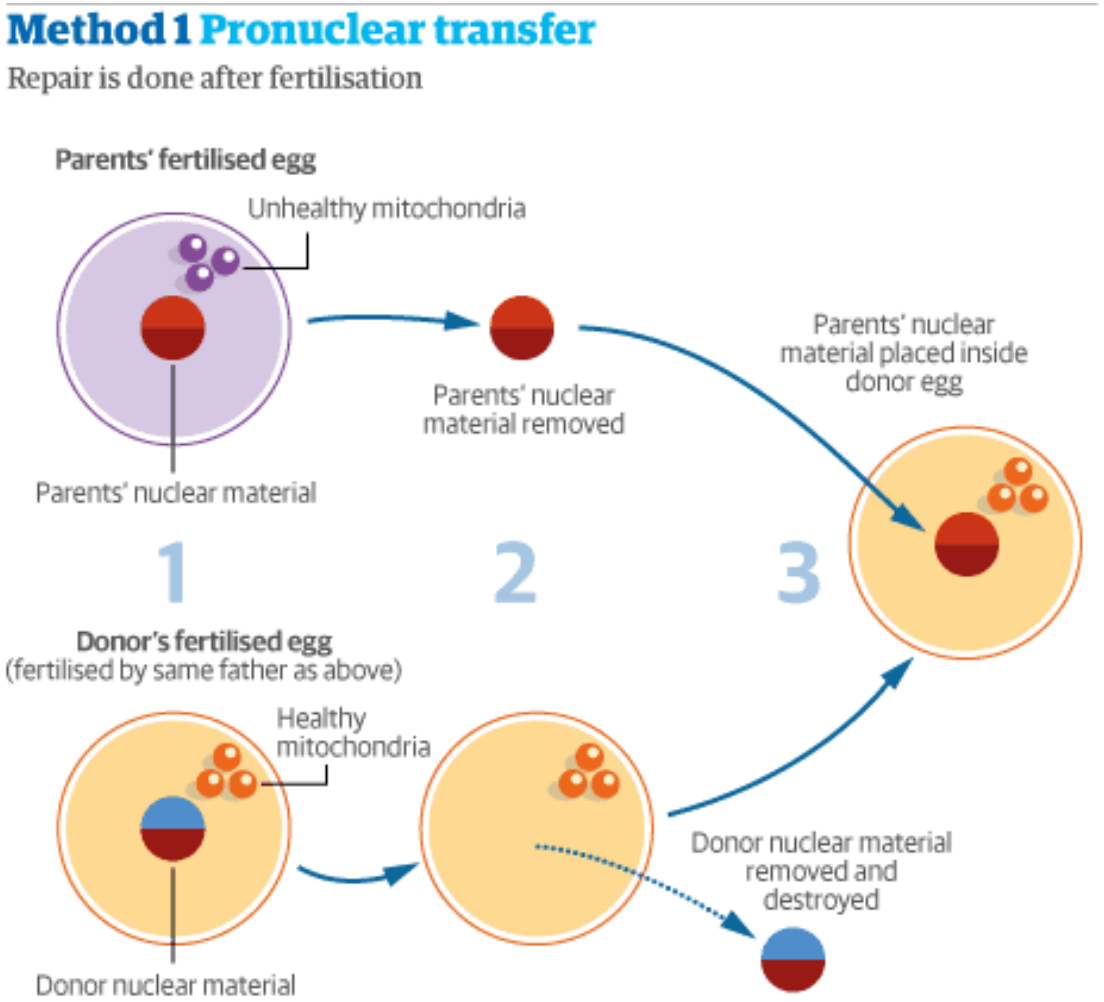
Strategies:

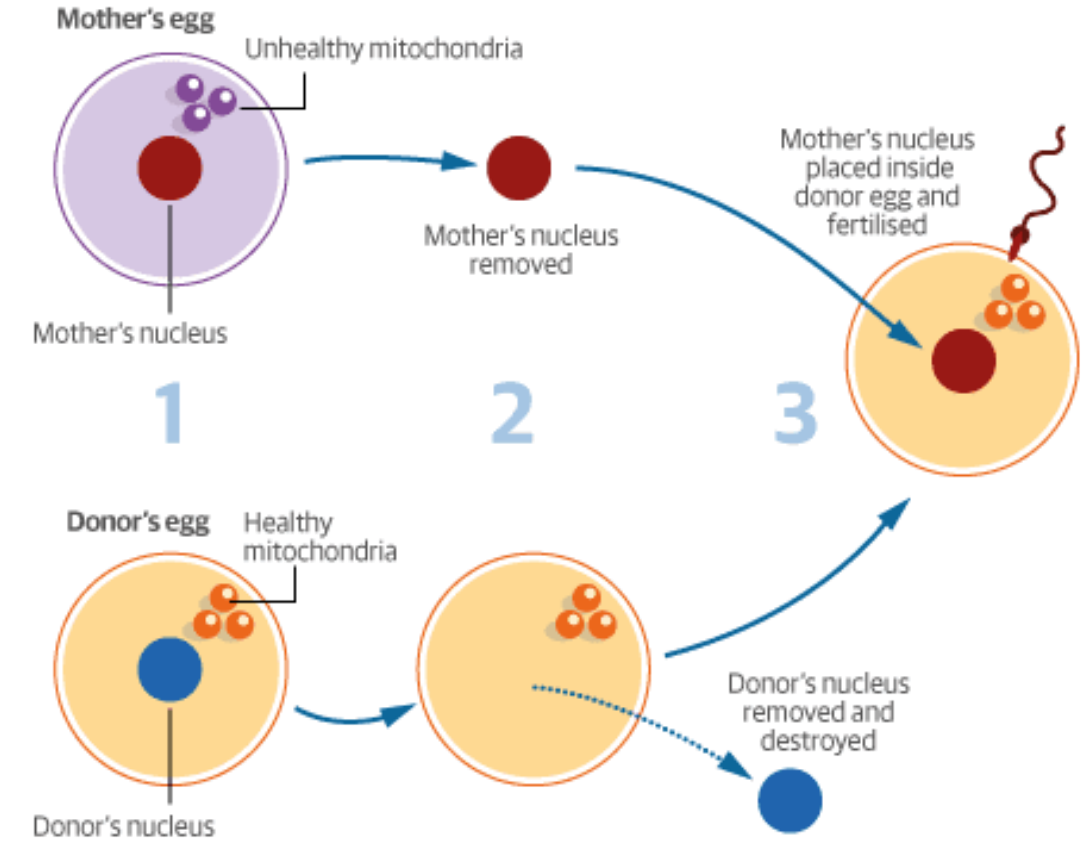
- Voluntary childlessness, adoption
- Prenatal testing
- Preimplantation genetic diagnosis (PGD)
- Oocyte donation
- Mitochondrial replacement (MRT) or mitochondrial donation

The mitochondrial genetic bottleneck → the result of any pregnancy is uncertain

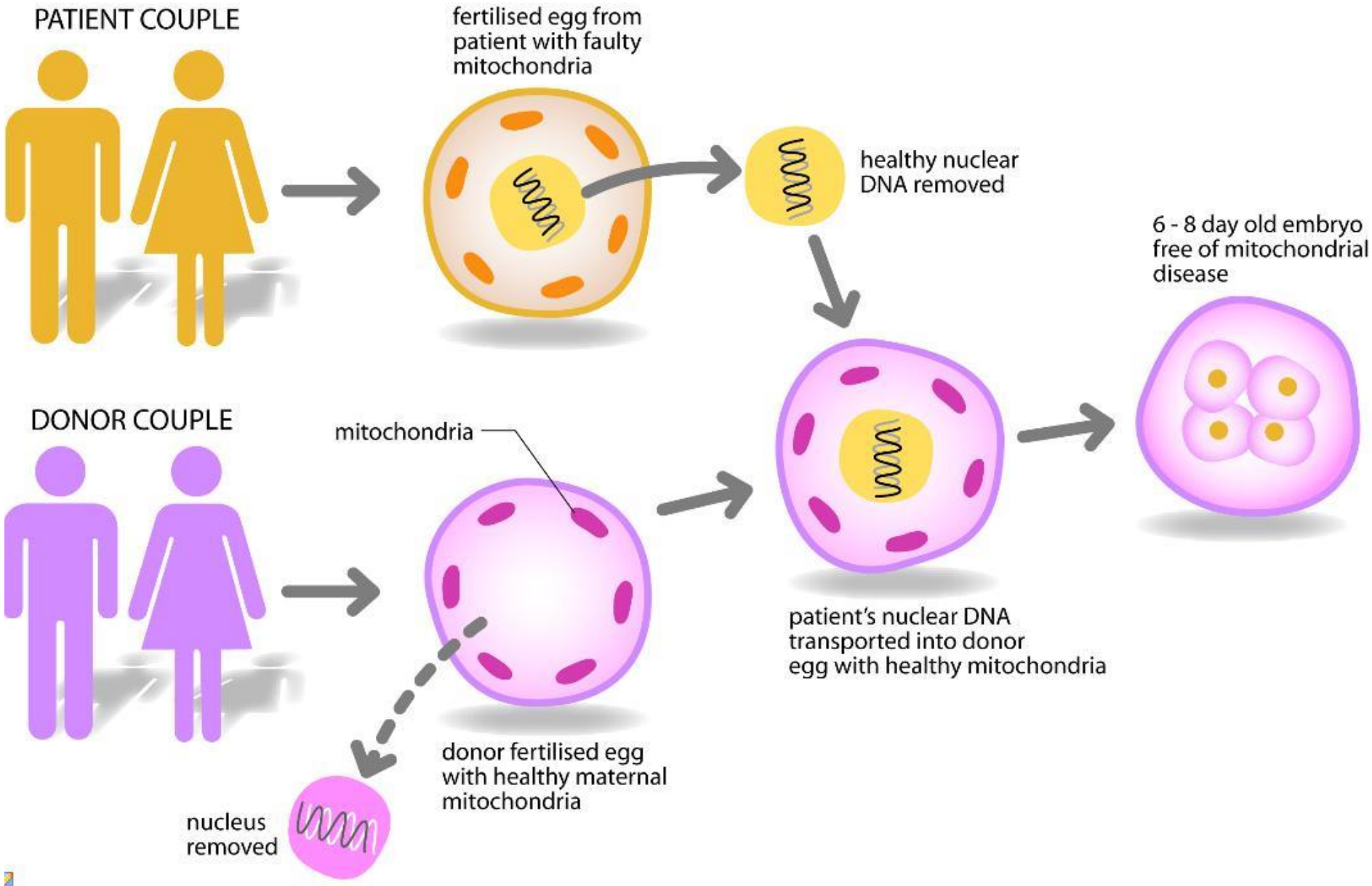


Mitochondrial replacement therapy



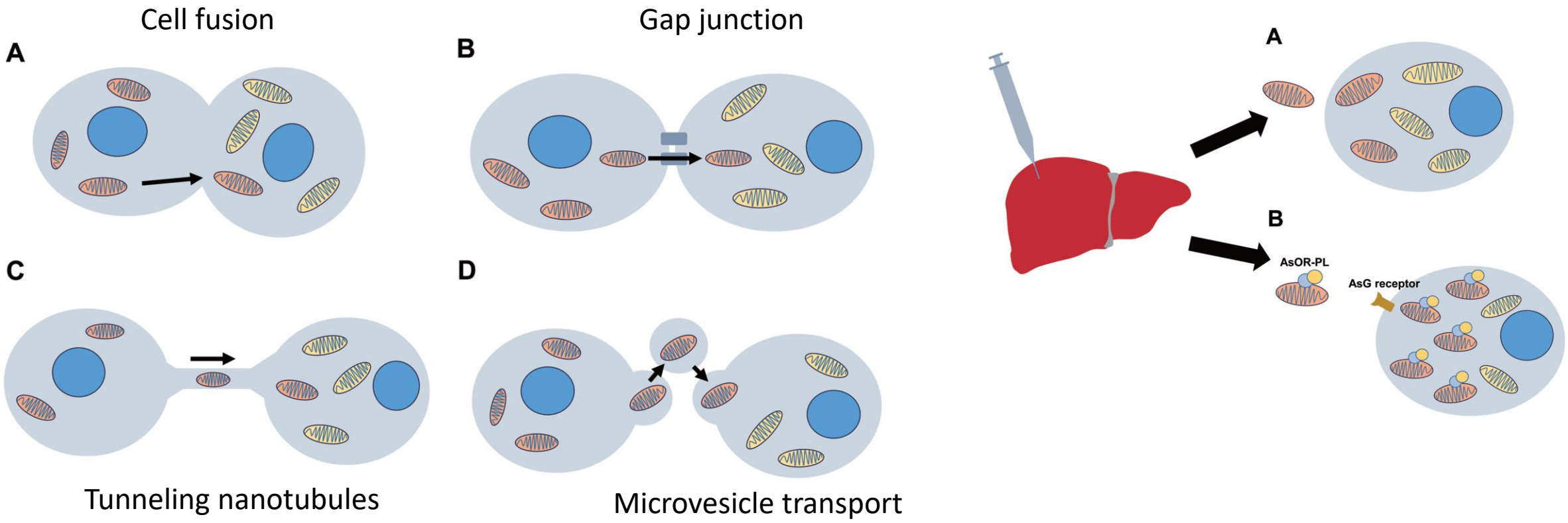


Pronuclear transfer in human embryos



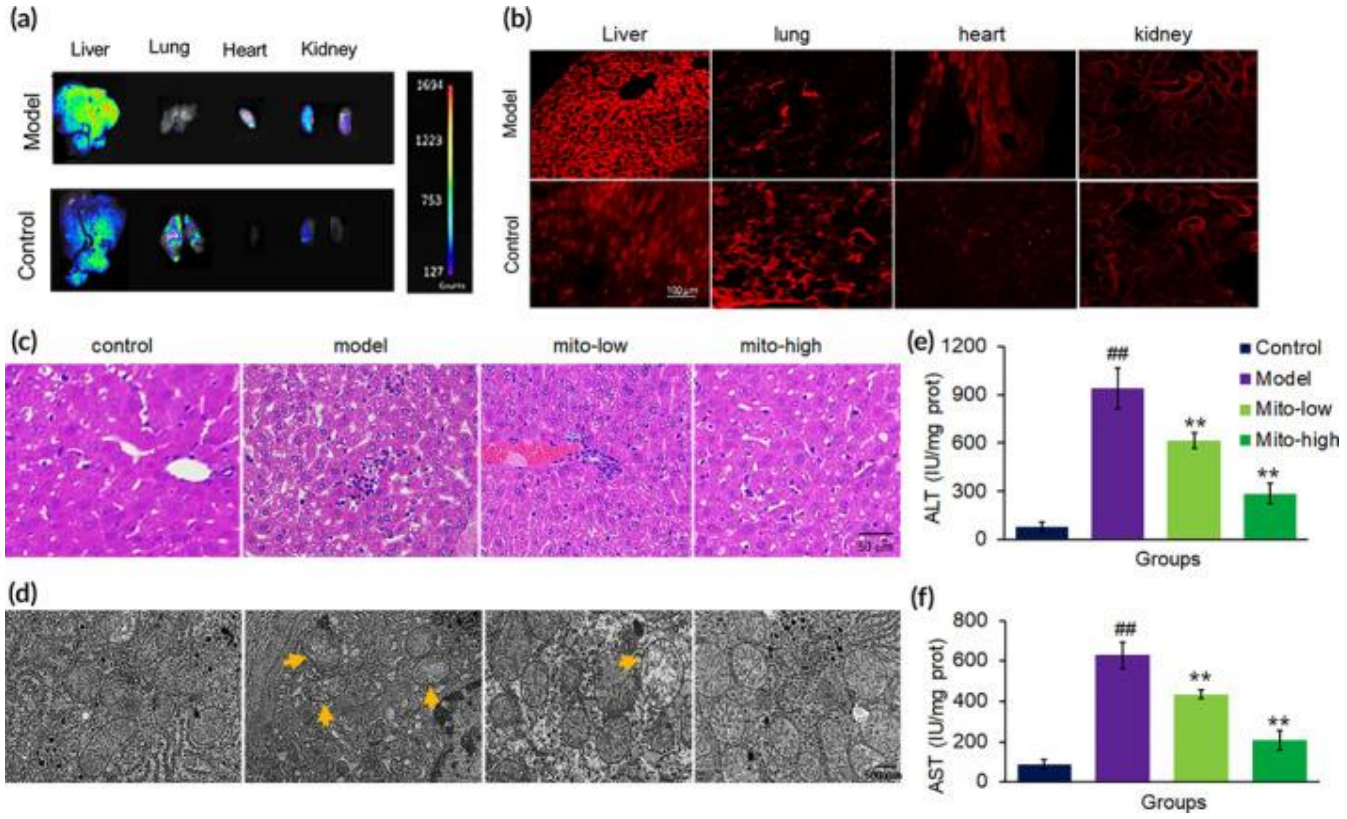
DONATION OF MITOCHONDRIA (TRANSPLANTATION)

Mechanisms of mitochondrial transport

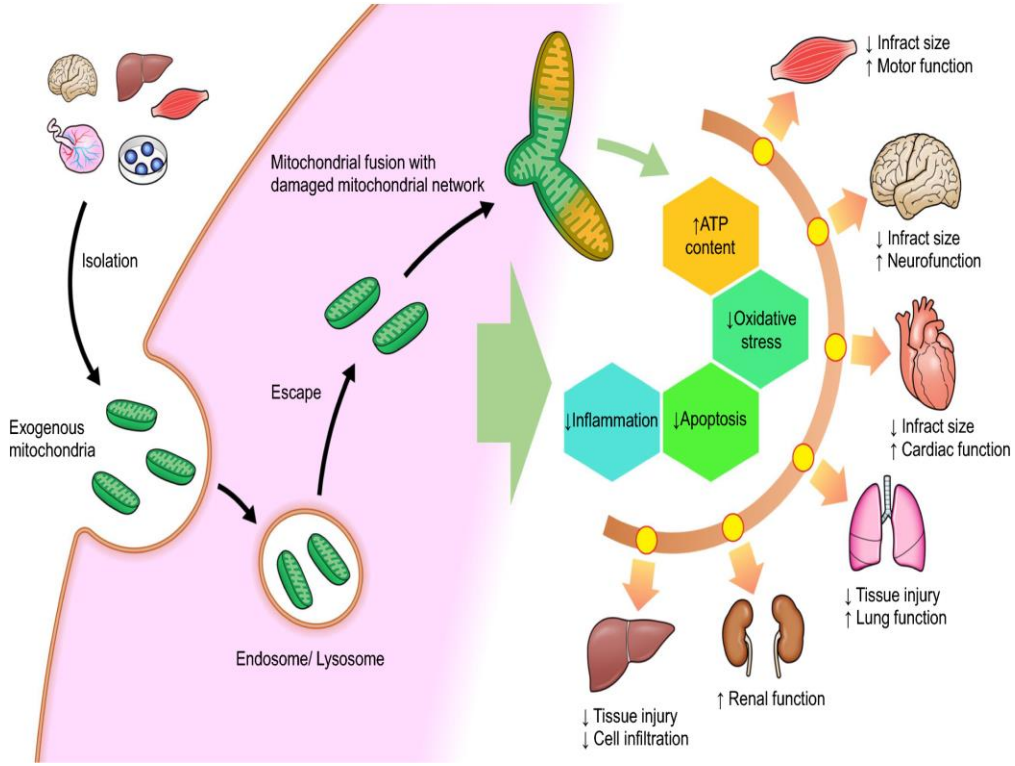


Journal of Clinical and Translational Hepatology **2022** vol. 10(2) | 321–328
DOI: 10.14218/JCTH.2021.00093

Mitochondrial transplantation alleviates organ disease via multiple mechanisms.

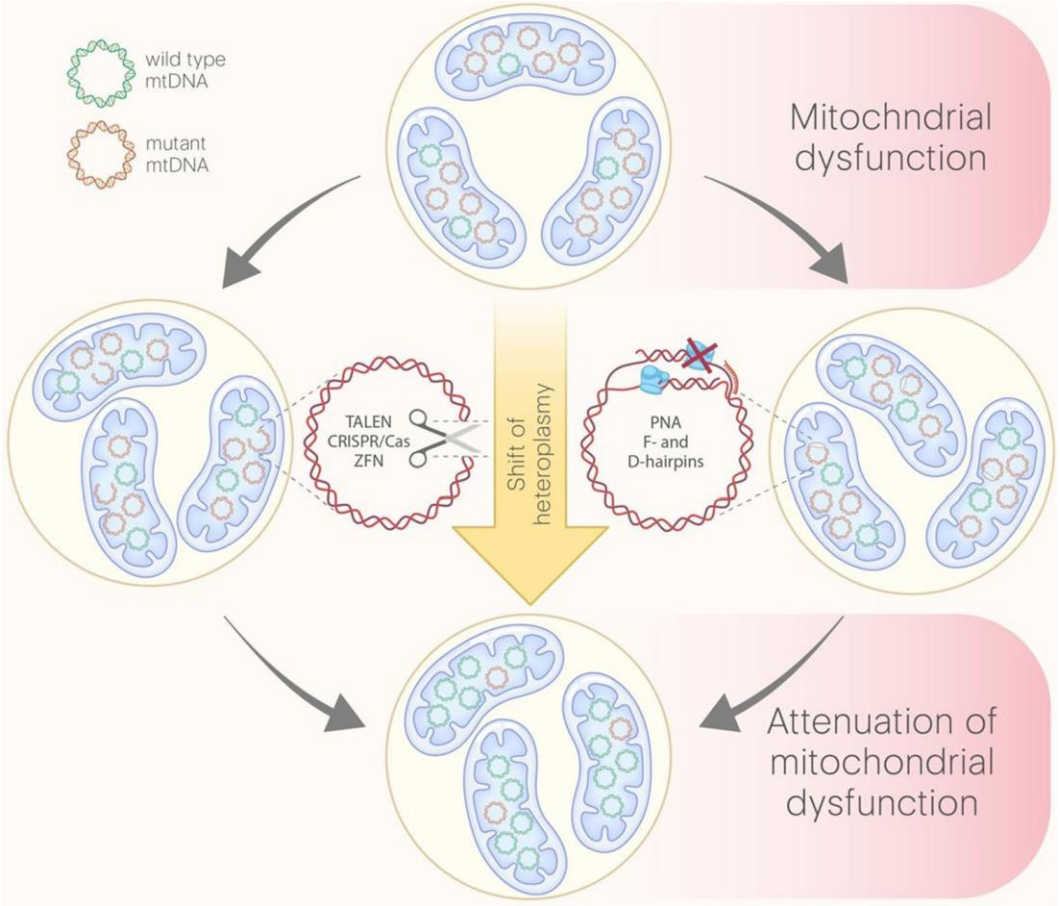


https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8126821/



https://translational-medicine.biomedcentral.com/articles/10.1186/s12967-021-02878-3

Shift of heteroplasmy to eliminate the mutated mtDNA and favor the normal mtDNA



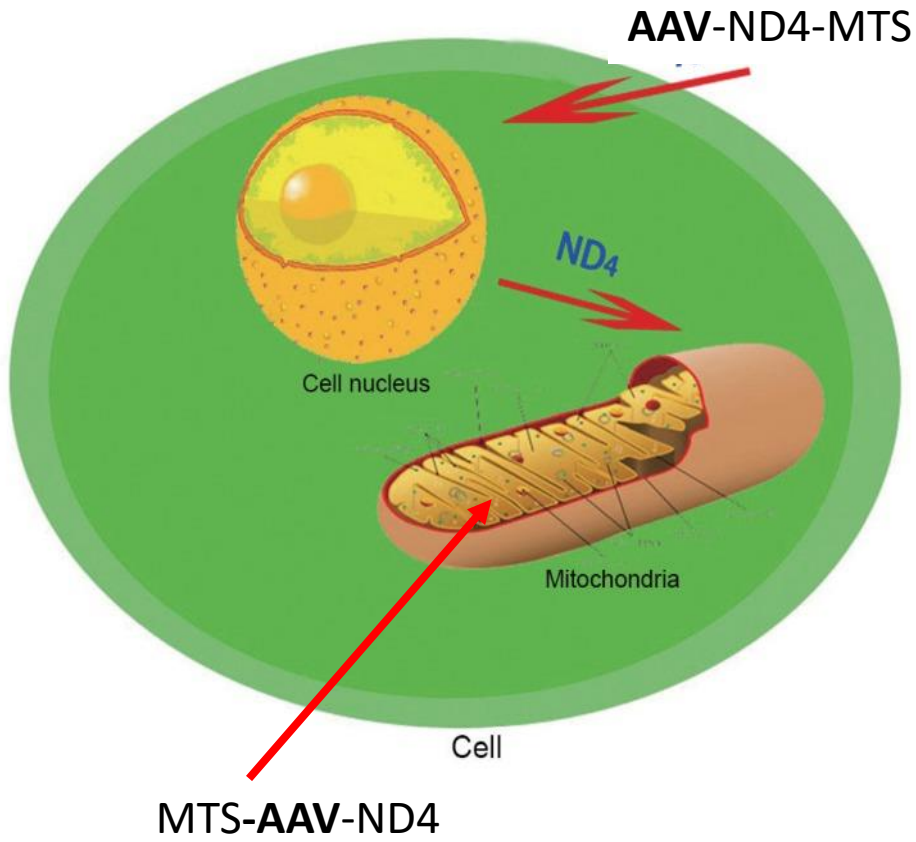
1. **Anti-replicative methods:** peptide nucleic acid oligomers (nucleobases are linked to a peptide backbone) that pair the single stranded mtDNA and inhibits its replication; complementary to mutant mtDNA

2. **Nucleases (gene editing-based therapy):** mitochondrial targeted TALEN and ZFN, CRISPR/Cas9

Gene replacement therapy

1. Utilize the nuclear genome to correct mtDNA defects: a vector, Adenovirus-Associated Virus) containing the **corrected mitochondrial gene** (+ mitochondrial targeted sequence, MTS) is inserted within the nDNA→ corrected protein that will be imported by mitochondria

2. Directed to the mitochondria: AAV modified capsid with an MTS → the correct ND4 is inserted within the mtDNA → corrected ND4 protein is synthesized within the mitochondria



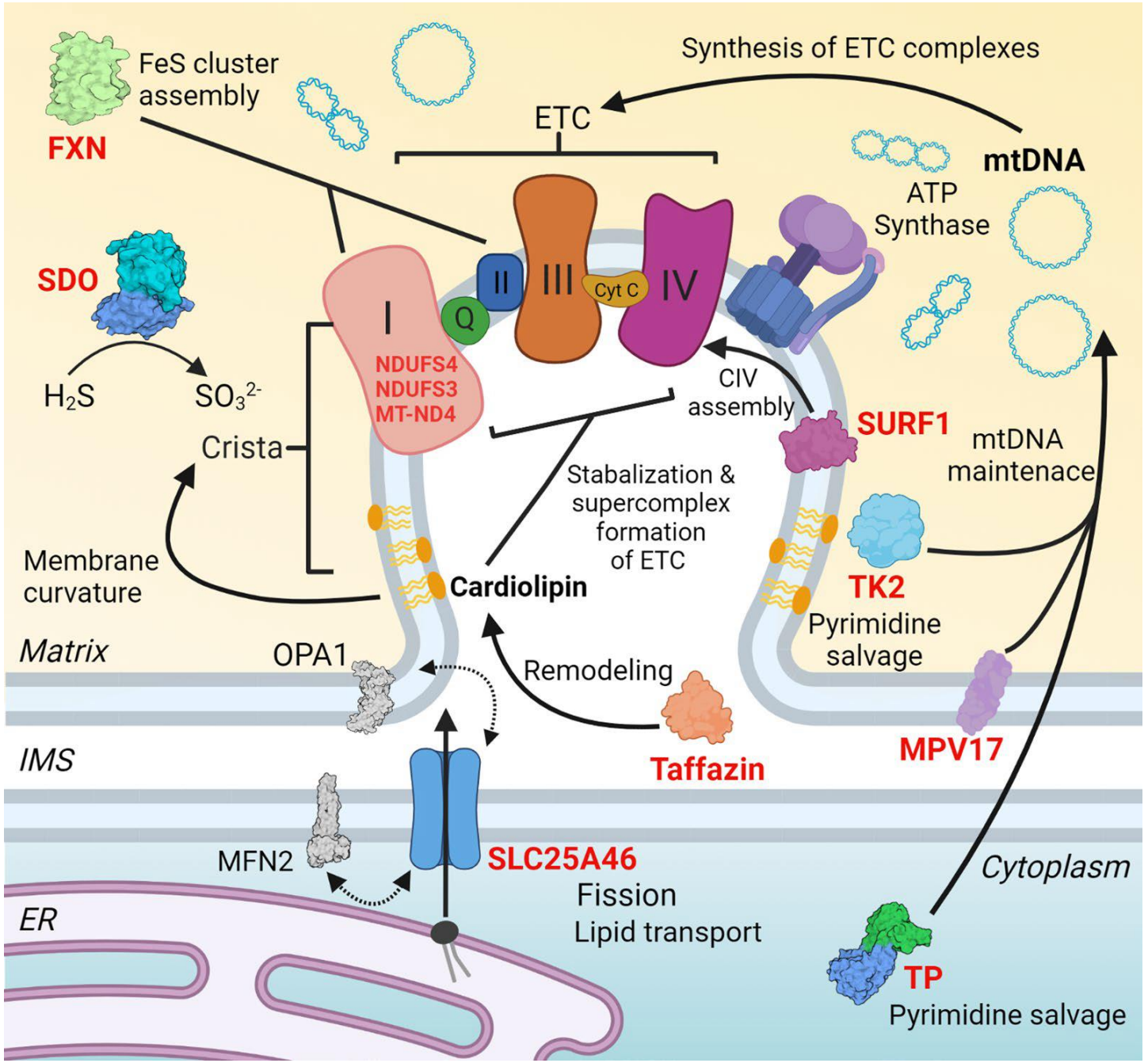
Gene replacement therapy

A. nDNA mutations

1. Taffazin deficiency → ↓ cardiolipin → Barth syndrome
2. Fratraxin deficiency → ↓ Fe-S clusters → Friedreich ataxia
3. NDUFS4, NDUFS3 and SURF1 deficiency → complex I and IV defects → Leigh syndrome
4. Thymidine phosphorylase, Thymidine kinase 2, SLC25A46 → Mitochondrial DNA depletion syndromes

B. mtDNA mutations

NADH dehydrogenase subunit 4 (ND4) → Leber hereditary optic neuropathy (LHON)



https://doi.org/10.1186/s13023-022-02324-7

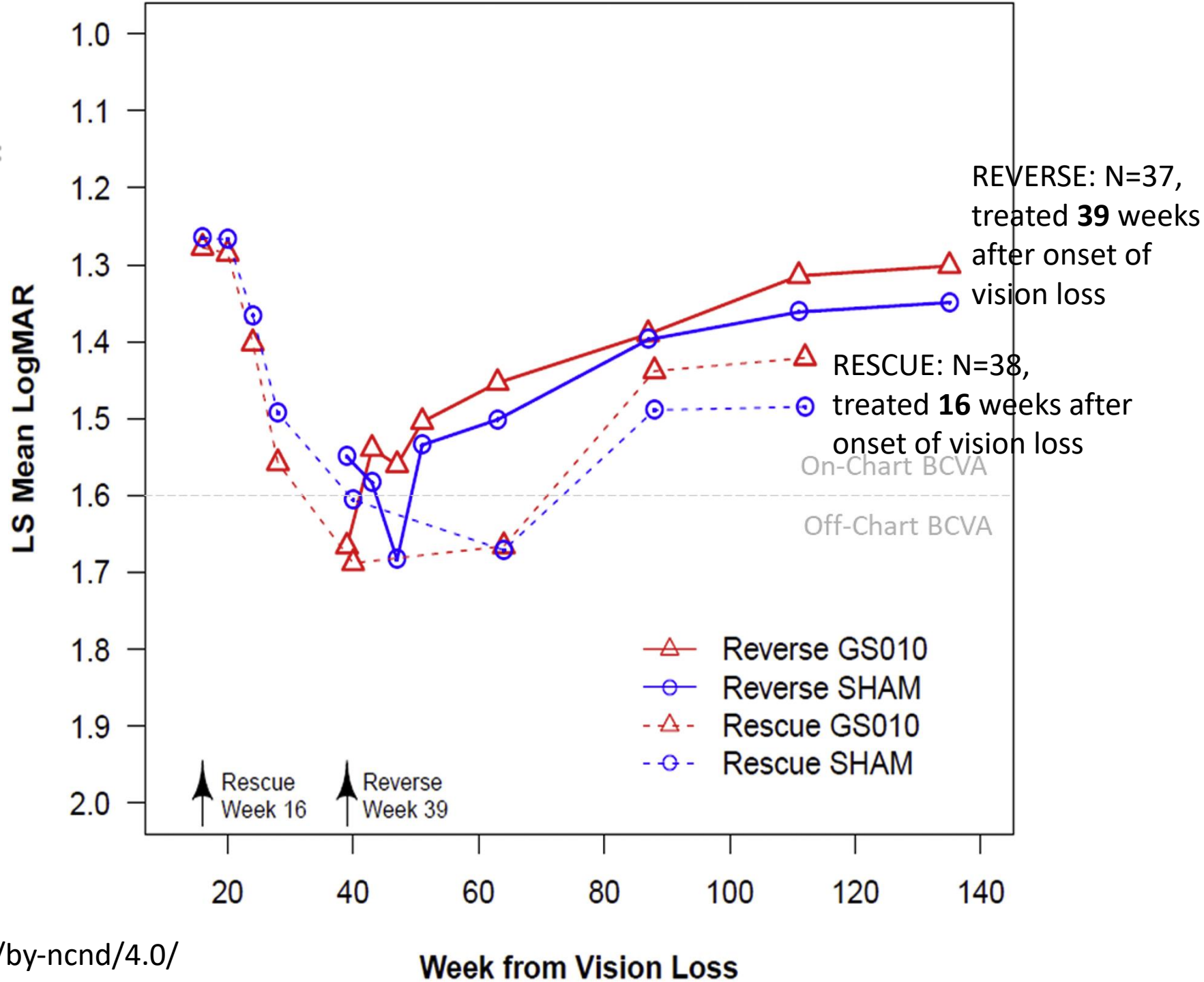
GS010 (Lumevoq, GenSight Biologics):

AAV-ND4 to treat ND4-LHON

Phase III USA clinical trials and submitted for European Approval 2020:

REVERSE (NCT02652780)

RESCUE (NCT02652767)



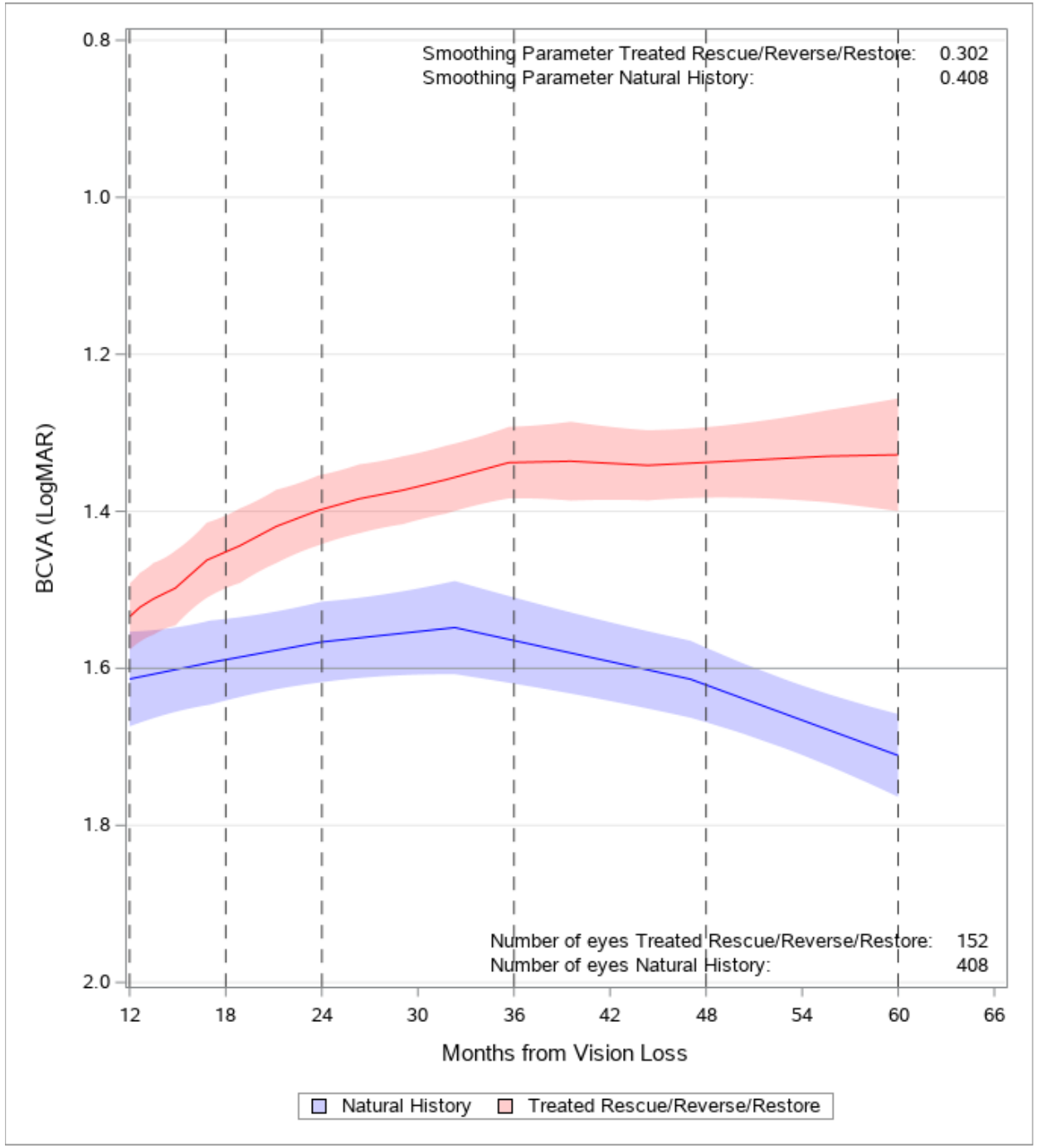
REVERSE: N=37, treated **39** weeks after onset of vision loss

RESCUE: N=38, treated **16** weeks after onset of vision loss

GenSight Biologics: sustained efficacy and safety of one-time treatment with LUMEVOQ (AAV-ND4)

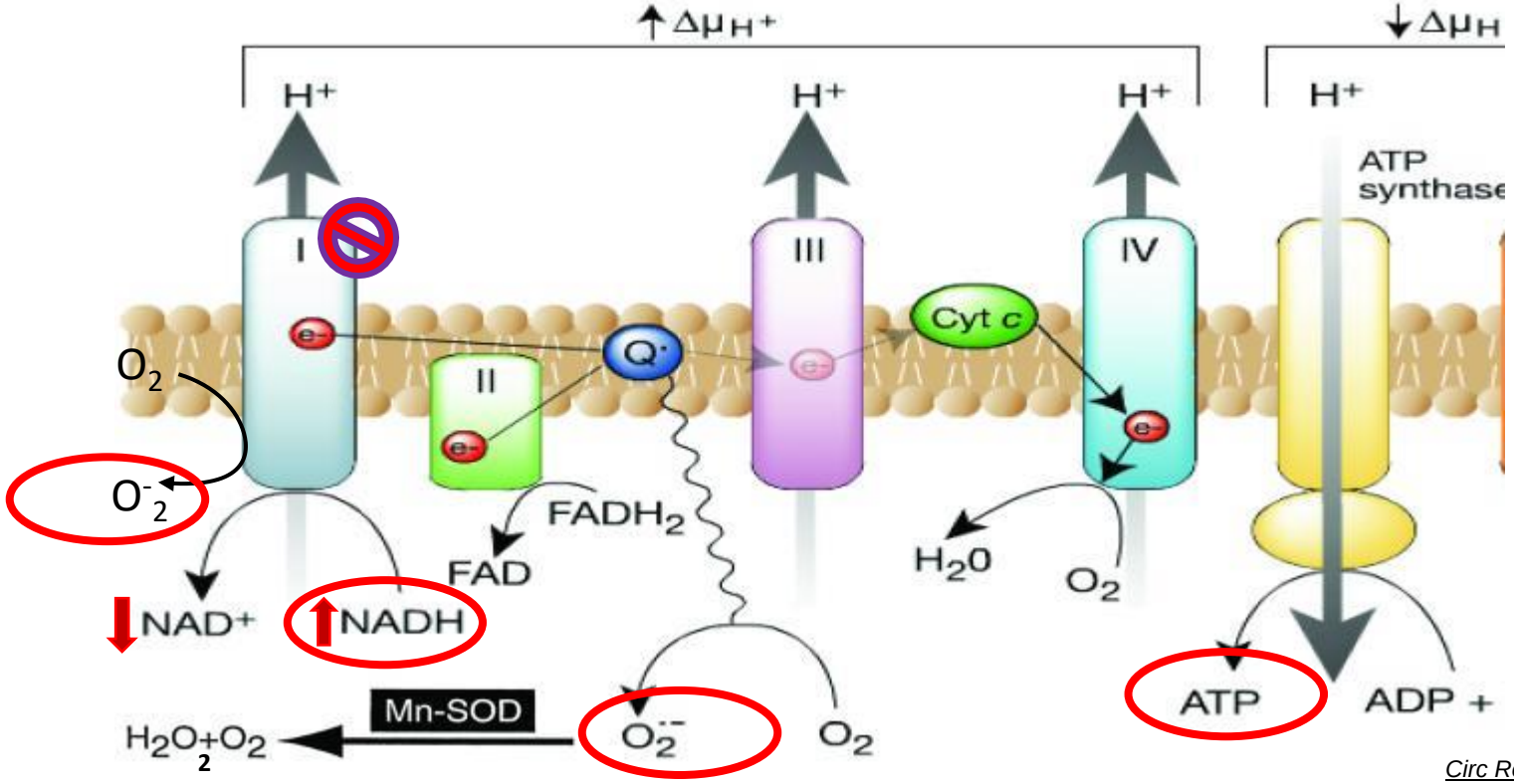
Phase III USA clinical trials and submitted for European Approval 2020:

- REVERSE (NCT02652780)
- RESCUE (NCT02652767)
- RESTORE



BCVA: Best Corrected Visual Acuity

Mechanisms of how mitochondrial defects cause organ disease

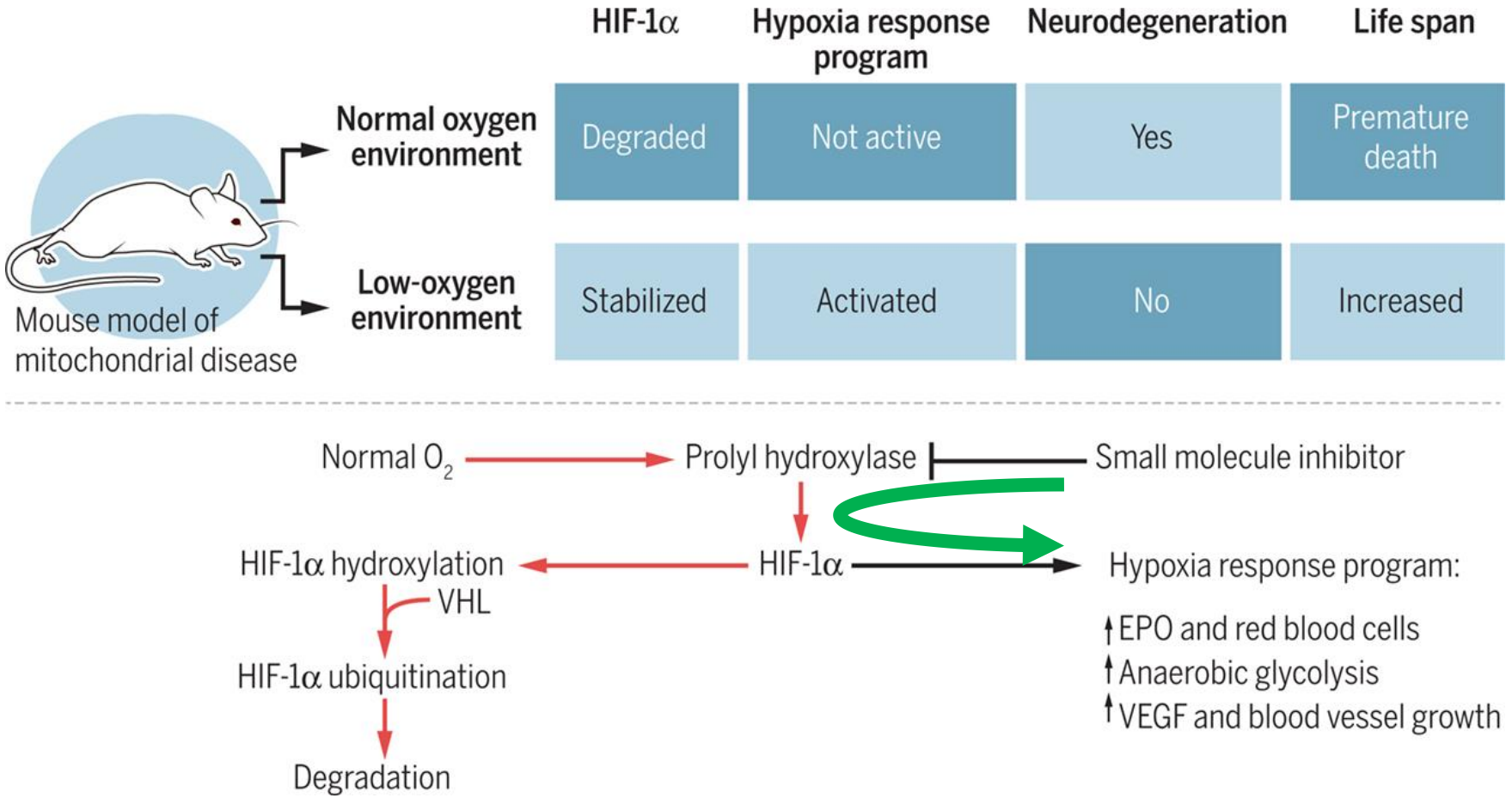


Circ Res. 2016 27;118(11):1808-29. doi: 10.1161/CIRCRESAHA.116.306923.

1. Oxygen toxicity
2. Oxidative stress
3. ↑ NADH with ↓ NAD^+
4. ↓ ATP production

NDUFS4 mouse models of complex I defect treated with hypoxia

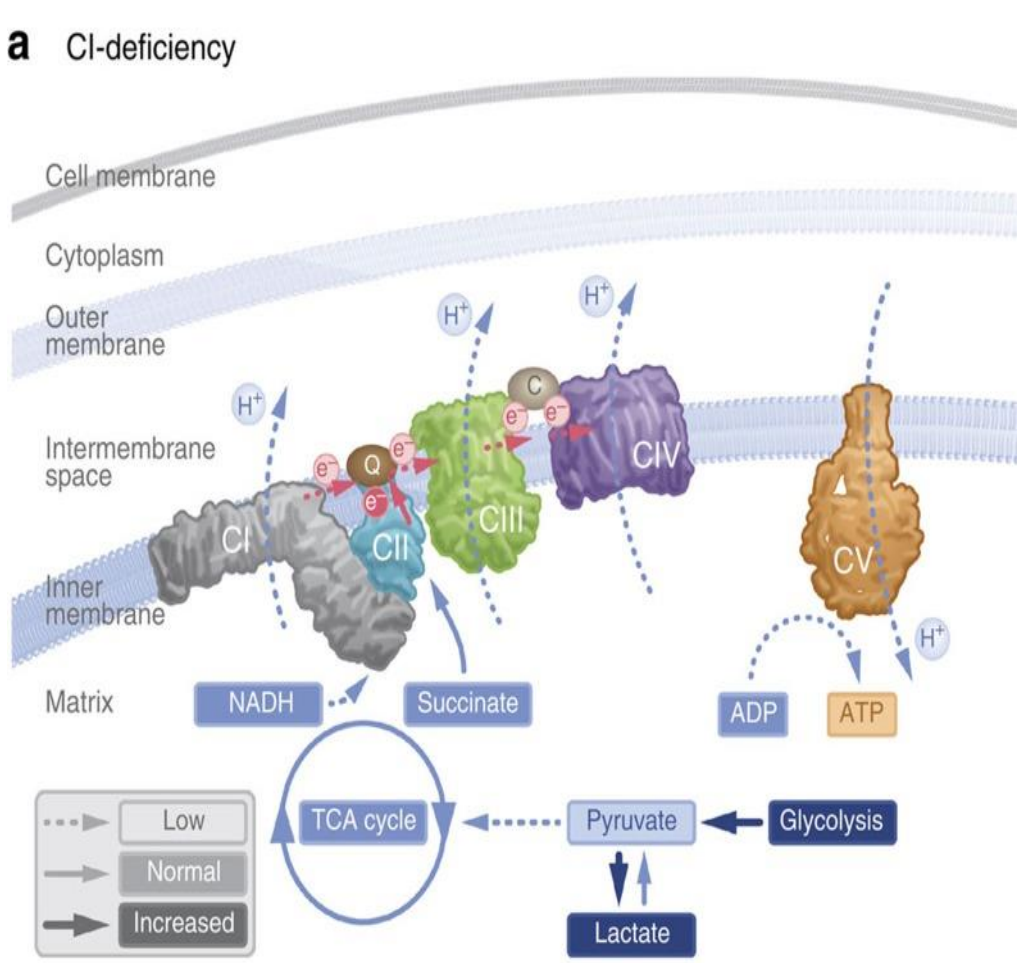
Hypoxia Inducing Factor-1α (HIF-1α)

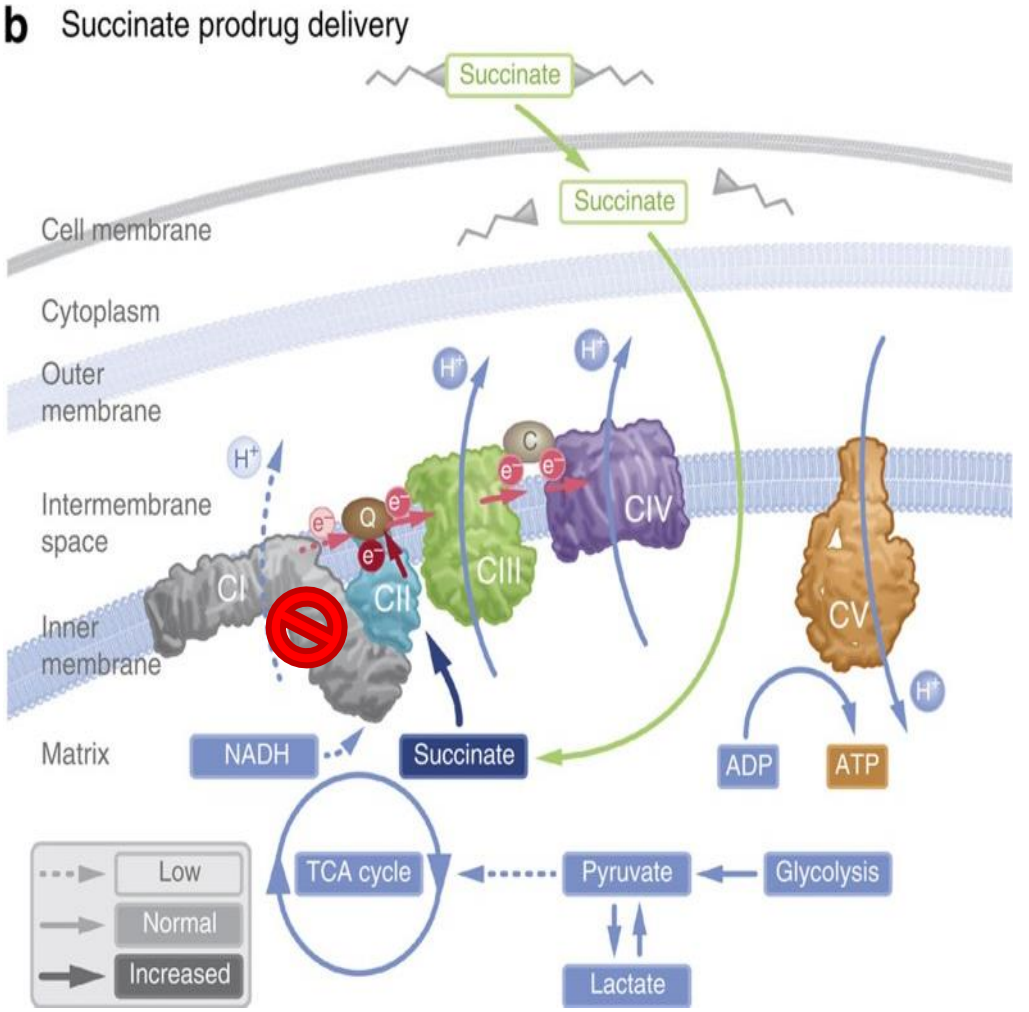


https://www.science.org/doi/10.1126/science.aaf5248?url_ver=Z39.88-2003&rfr_id=ori:rid:crossref.org&rfr_dat=cr_pub%20%200pubmed

Facilitate the electron transport

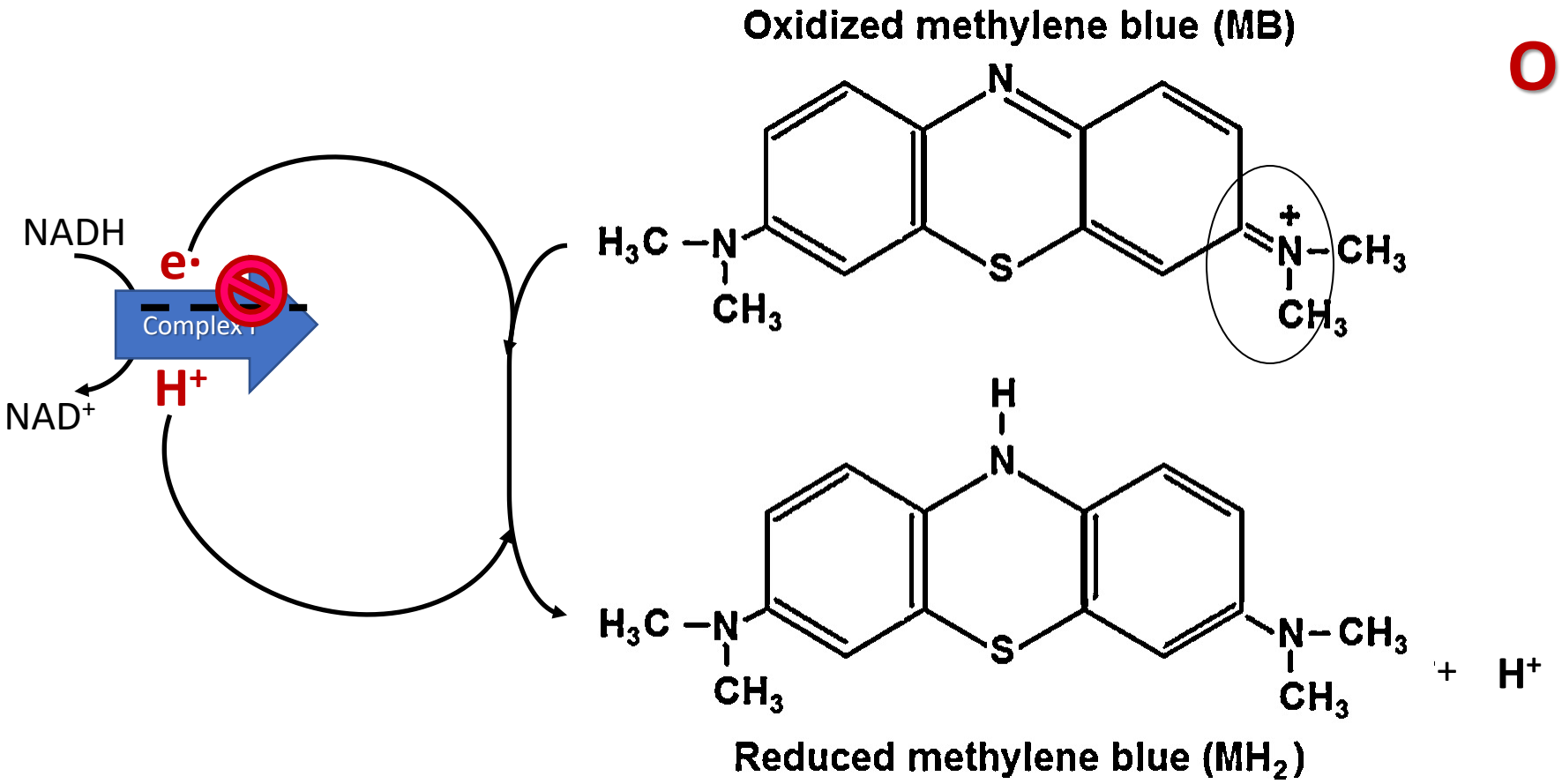
A cell- and mitochondrial-permeable **succinate** prodrug bypasses complex I deficiency and improves cardiac bioenergetics.





Nat Commun. **2016** Aug 9;7:12317. doi: 10.1038/ncomms12317.

Our lab contribution



Rojas et al, Progress in Neurobiology, 2012

FDA approved drug
Lipophilic
Concentrates in mitochondrial membranes
MB accepts electron from components in complex I

HUMAN SUBJECTS

***Acute administration*:** FDA-approved drug

- **Methemoglobinemia**
- **Antidote for cyanide poisoning**

***Chronic administration*:**

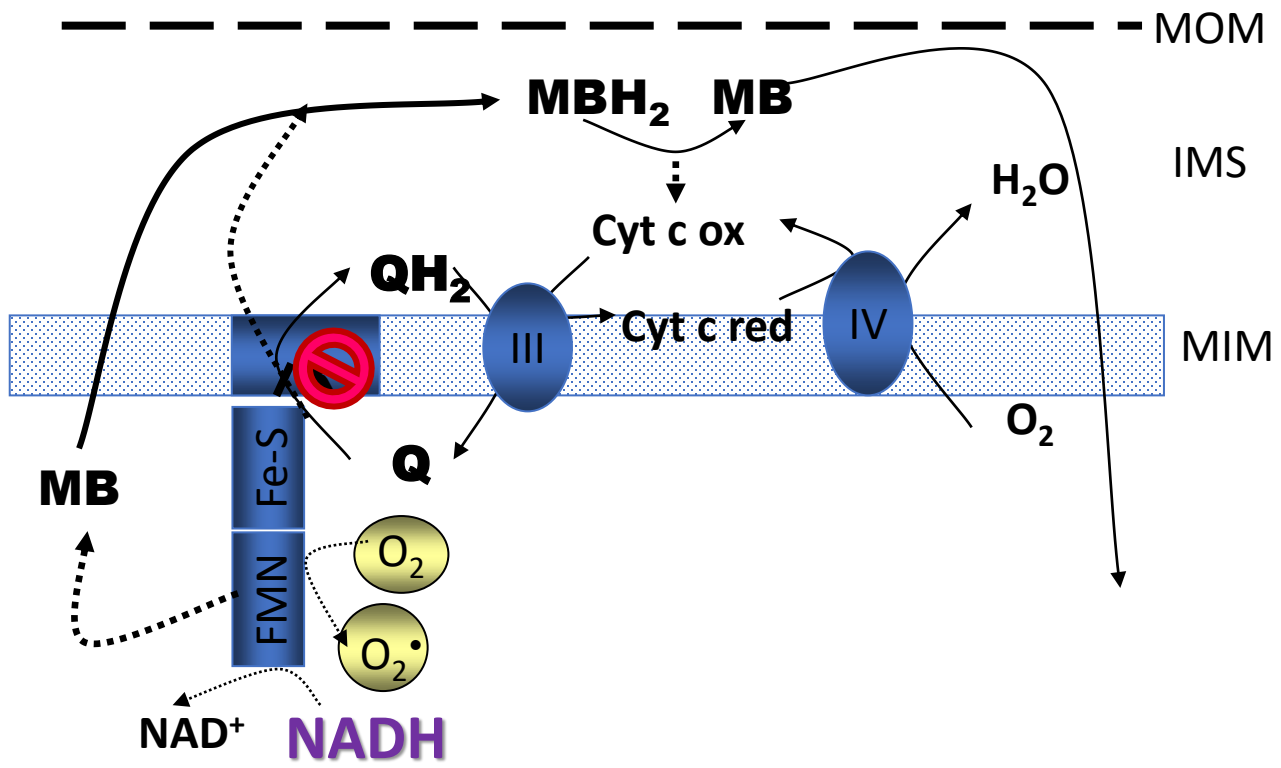
- Experimental treatment in **schizofrenia** (*Allexsaht et al, Psychiat Quart, 1938)*
- **Septic shock** (*Schneider et al, Intensive Care Med, 1992)*
- **Alzheimer disease** and **Postraumatic Stress Disorder**
- **Anxiety disorders** *(unpublished observations in Rojas et al, Progress in Neurobiology, 2012)*
- Neuroprotection against isofosfamide-induced **encephalopathy** *(Kupfer et al, Lancet 1992 and Eur J Clin Pharmacol 1994)*

EXPERIMENTAL MODELS

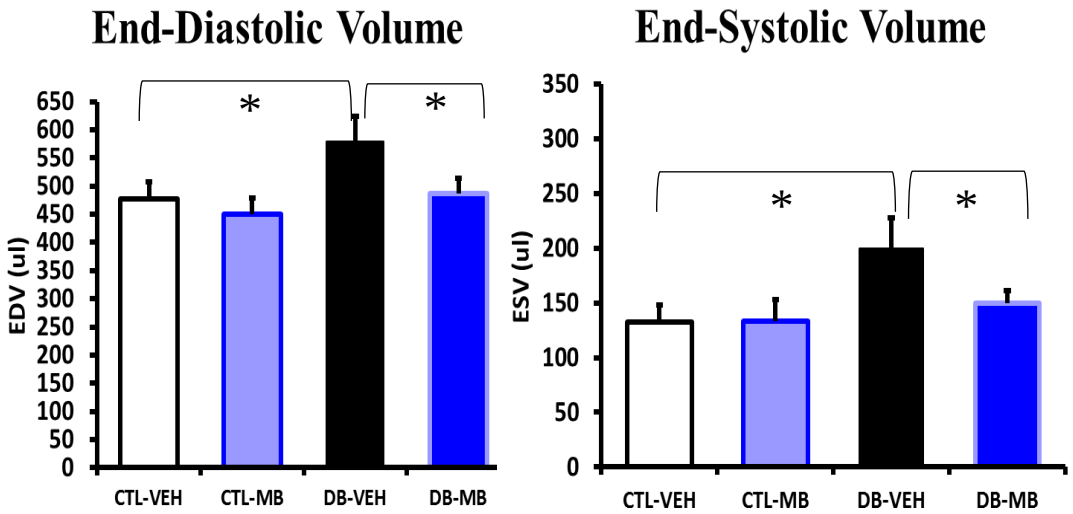
1. **Increases oxygen consumption** in cultured cells on the expense of glycolysis *(Guzman-Barron et al, J Gen Physiol 1930)*
2. **Delays cellular senescence**, and enhances oxygen consumption and **cytochrome c oxidase** amount in cultured human fibroblasts *(Atamna H, FASEB J, 2008)*
3. Enhances consolidation phase in **memory** processing *(Martinez et al, Physiol Psychol 1978; Callaway et al, Neurosci Lett 2002)*
4. Avoids **loss of memory in azide-induced cytochrome c inhibition** *(Riha et al, Neurobiol Learn Mem 2008)*
5. Provides neuroprotection against:
    - Experimental **optic atrophy** induced by rotenone-induced complex I inhibition *(Rojas et al, Neurotox Res 2009)*
    - Experimental **Parkinson disease** induced by rotenone-induced complex I inhibition *(Yi Wen et al, J Biol Chem 2011)*
    - **Cerebral ischemia-reperfusion** *(Yi Wen et al, J Biol Chem 2011)*
    - **Cardiac arrest-induced brain damage** (Miclescu et al, Crit Care Med 2006)

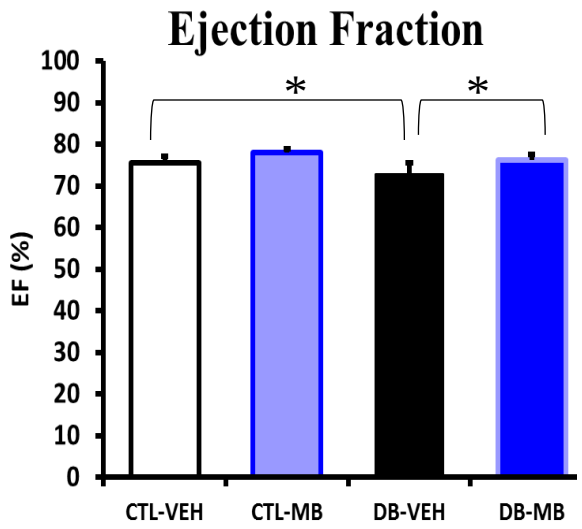
Enhancing Extinction Learning in Posttraumatic Stress Disorder With Brief Daily Imaginal Exposure and Methylene Blue: A Randomized Controlled Trial. J Clin Psychiatry 2017. DOI: 10.4088/JCP.16m10936

MB acts as an alternative electron carrier in complex I defective cardiac mitochondria from type 1 diabetic rats.



MB improved cardiac function in a T1D rat model.

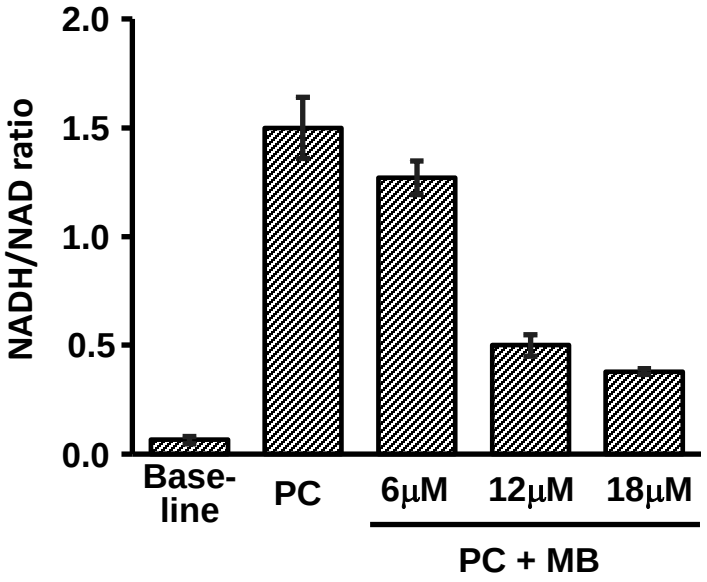




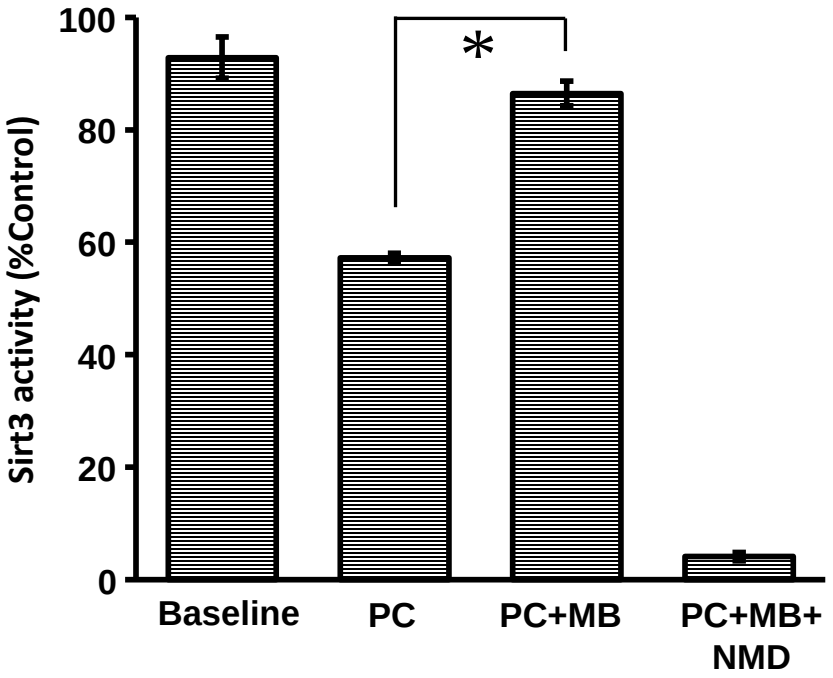
Cardiovasc Res. 2015 Sep 1;107(4):453-65. doi: 10.1093/cvr/cvv183. Epub 2015 Jun 22.
Mitochondrial complex I defect and increased fatty acid oxidation enhance protein lysine acetylation in the diabetic heart.
Vazquez EJ, Berthiaume JM, Kamath V, Achike O, Buchanan E, Montano MM, Chandler MP, Miyagi M, Rosca MG.

Mol Cell Biochem. 2017 Aug;432(1-2):7-24. doi: 10.1007/s11010-017-2993-1. Epub 2017 Mar 16.
Methylene blue decreases mitochondrial lysine acetylation in the diabetic heart.
Berthiaume JM, Hsiung CH, Austin AB, McBrayer SP, Depuydt MM, Chandler MP, Miyagi M, Rosca MG.

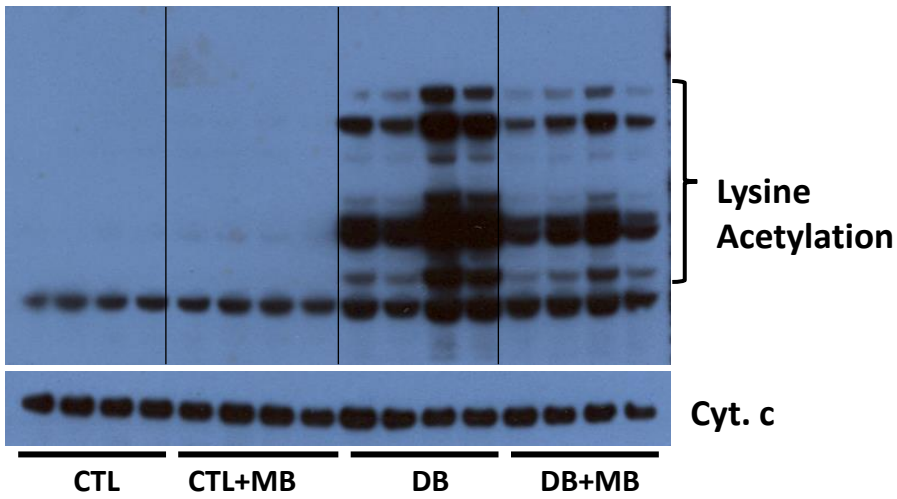
MB decreases NADH and increases NAD+



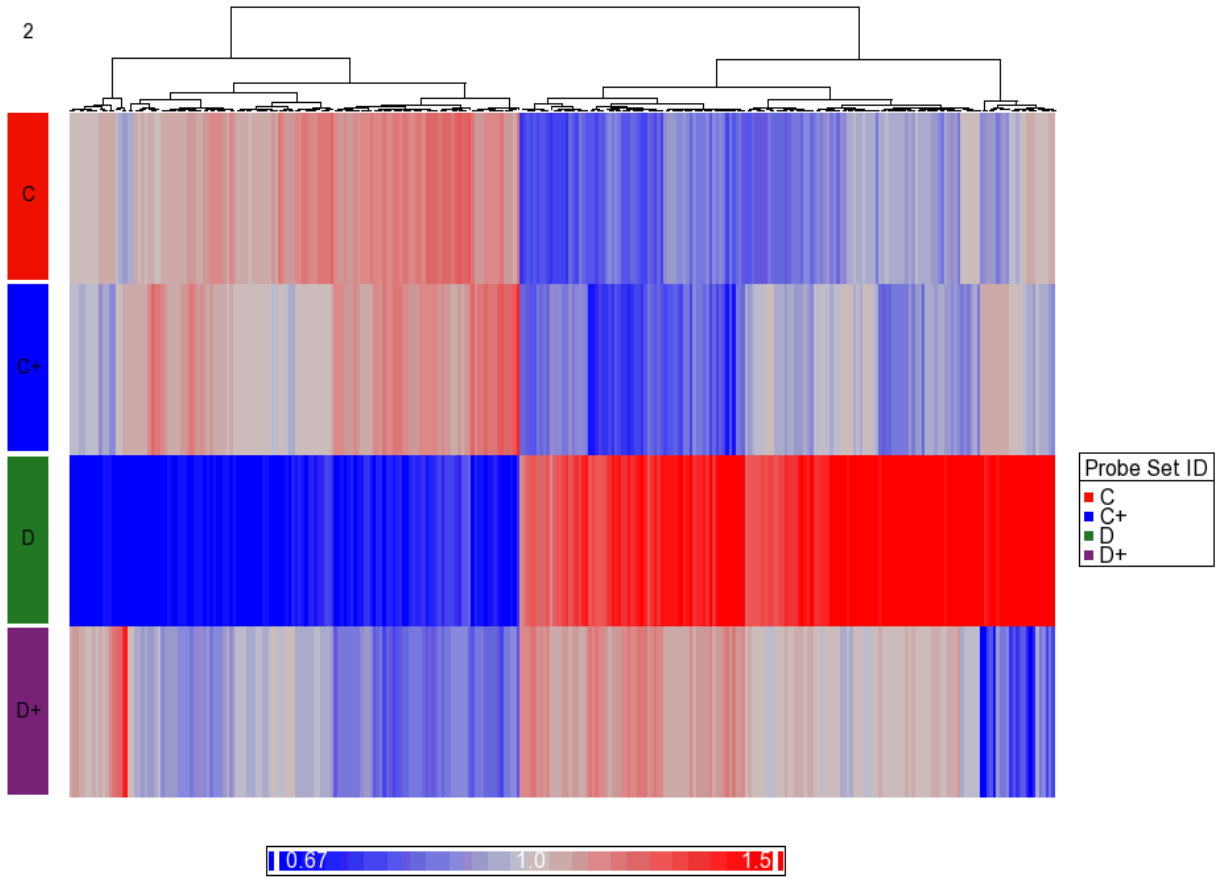
MB increases the activity of mitochondrial deacetylase sirtuin3.

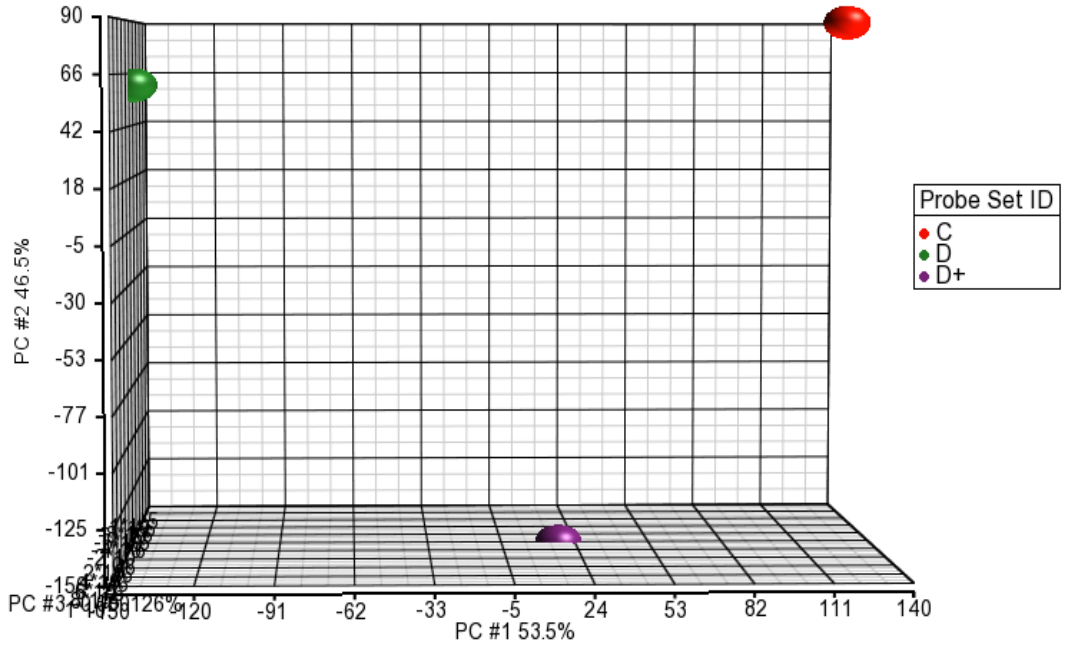


MB decreases protein lysine acetylation.



MB treatment reversed pathologic gene transcription in the diabetic heart.





Transcriptional changes during diabetic cardiomyopathy, and the effect of MB. A. Heatmap of differentially expressed transcripts. B. Principal component analysis plot using samples from control, diabetic and diabetic+MB hearts. The analysis includes all genes (668 genes with altered transcription in diabetes).

Functional pathway analysis of diabetes-induced genes that were reversed by MB

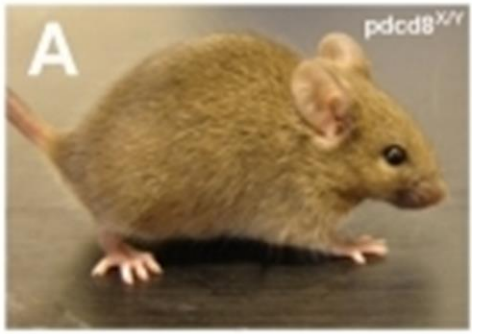
Pathways	P value
G-protein coupled receptors signaling pathway	1.02E-46
Translation	0.0010
Positive regulation of cell proliferation	0.0300
Regulation of cell growth	0.0250
Atrial muscle cell development	0.0001
Ventricular muscle cell development	0.0004
Ventricular septum morphogenesis	0.0019
TOR signaling cascade	0.0029
Ribosomal small subunit: biogenesis assembly	 0.0096 0.0072
Embryonic heart development	0.0080
Negative regulation of translation	0.0316
Heart morphogenesis	0.0422

Functional pathway analysis of diabetes-repressed genes that were reversed by MB

Pathways	P value
Protein phosphorylation	0.0002
Response to DNA damage	0.0001
Proteasomal ubiquitine-dependent protein catabolic process	1.35E-5
Cardiomyocyte contraction	0.0003
Insulin receptor signaling pathway	0.0008
Negative regulation of apoptosis	0.0014
Response to hypoxia	0.0293
P53-induced cell cycle arrest due to DNA damage	0.0033
Positive regulation of ryanodine calcium channel activity	2.3E-5
Autophagic vacuole assembly	0.0041

Assessment of differentially expressed transcripts revealed that expression of 668 genes is altered in the diabetic heart, and 474 of these gene events (increases and decreases in gene expression) are corrected in the diabetic group treated with MB. Global analysis of gene expression profile revealed that MB treatment abrogated induction of 299 diabetes-induced genes and corrected the diabetes-induced decrease of 175 genes

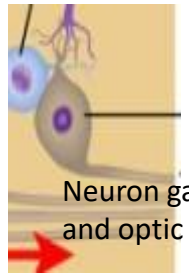
MB protects retinal photoreceptor degeneration in a murine complex I defect model



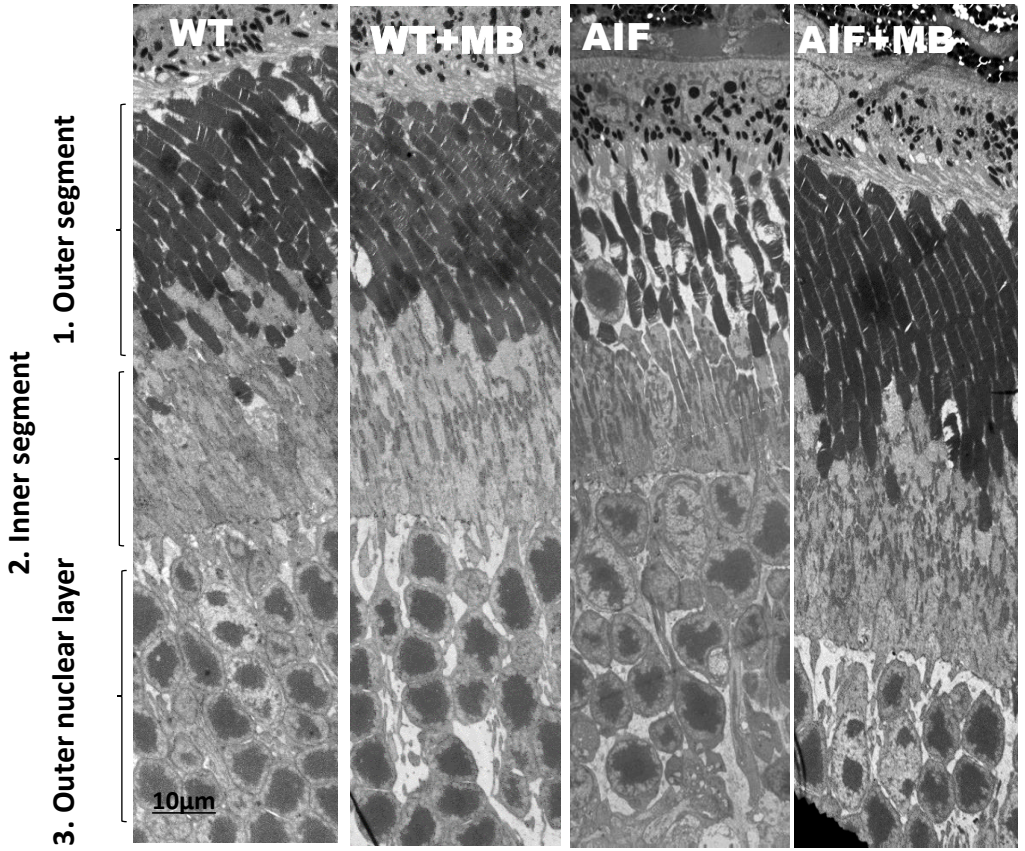
(A) Normal mice

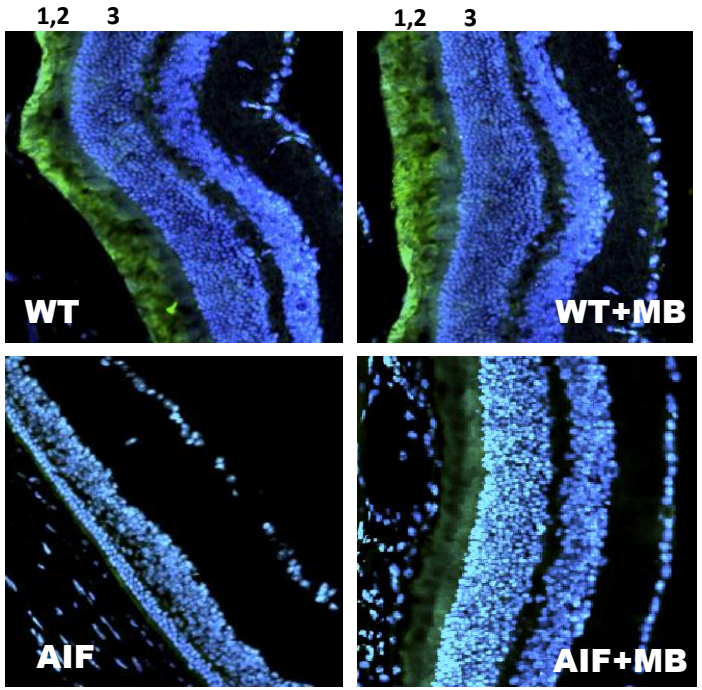
(B) Mitochondrial Complex-I deficient mice

(Armand et al, PLoS One 2011;6(11):e27283)



Neuron ganglion cells and optic nerve

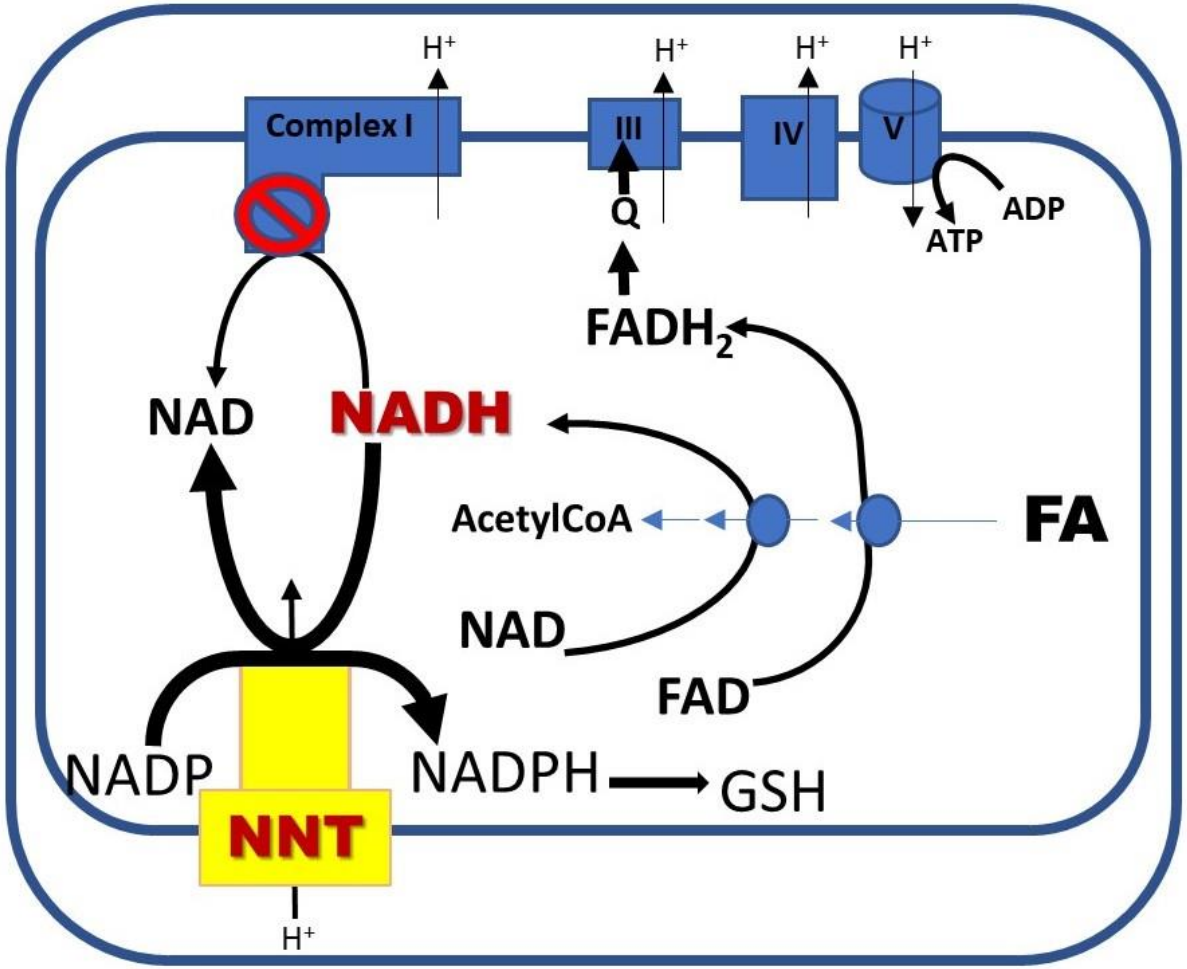




Confocal microcopy, DAPI (blue for nuclei) and rhodopsin (green)

Apoptosis inducing factor deficiency causes retinal photoreceptor degeneration. The protective role of the redox compound methylene blue.Mekala NK, Kurdys J, Depuydt MM, Vazquez EJ, **Rosca MG.** Redox Biol. 2019 Jan;20:107-117. doi: 10.1016/j.redox.2018.09.023. Epub 2018 Sep 29.

Nicotinamide Nucleotide transhydrogenase (NNT) regulates bioenergetic metabolism in complex I defective cardiac mitochondria

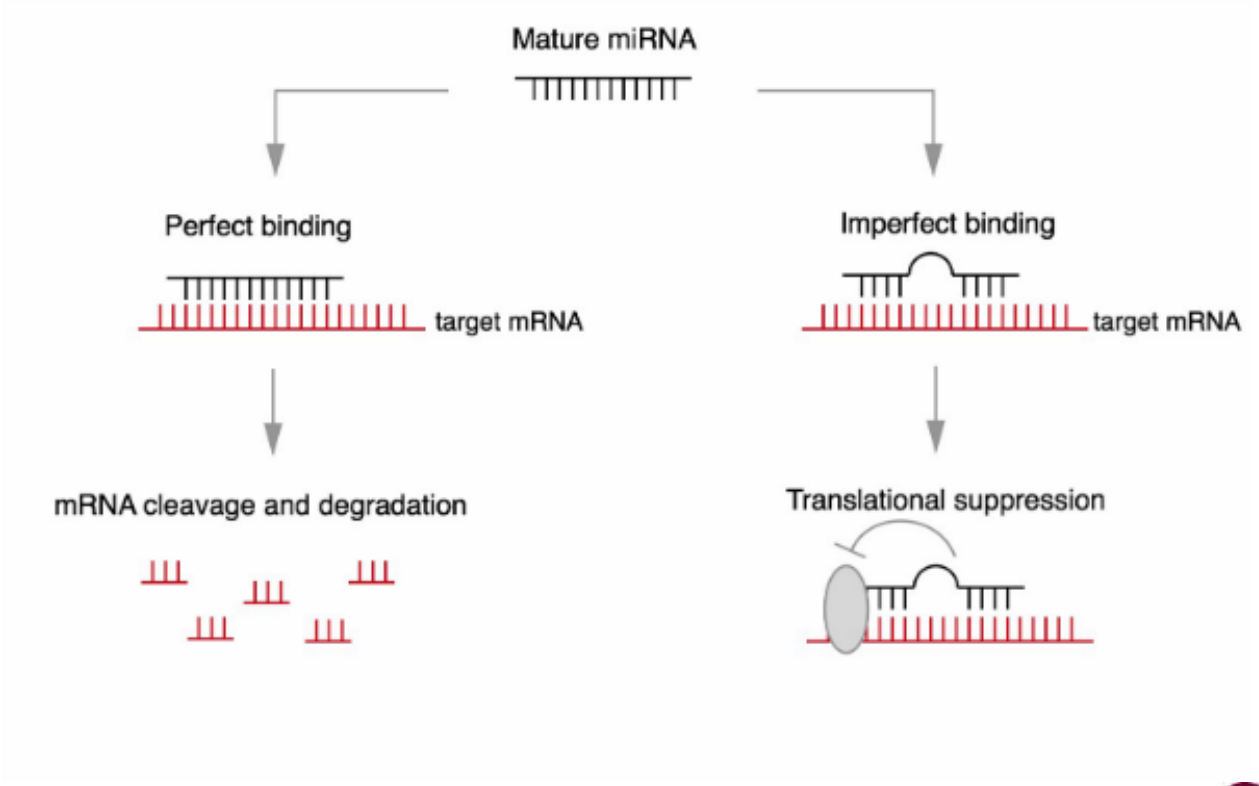


Under complex I defect, NNT oxidizes NADH to NAD and allows FADH2 generation within FA β-oxidation.

FADH2 bypasses the inhibited complex I, donates electrons to Q-Complex I and maintains ATP synthesis.

MicroRNAs

- are short (20-25bp) non-coding RNA sequences
- inhibit gene expression by:
  - Binding to 3’-untranslated regions of mRNA (silencing)
  - Destabilizing the mRNA (degradation)



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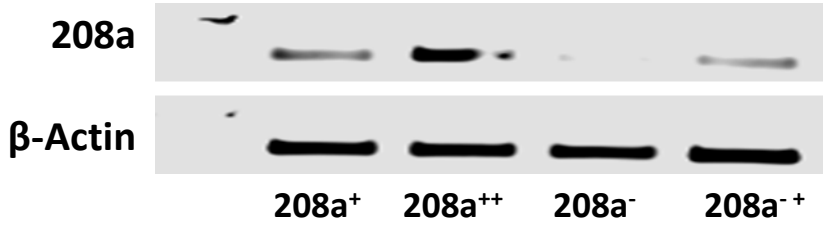
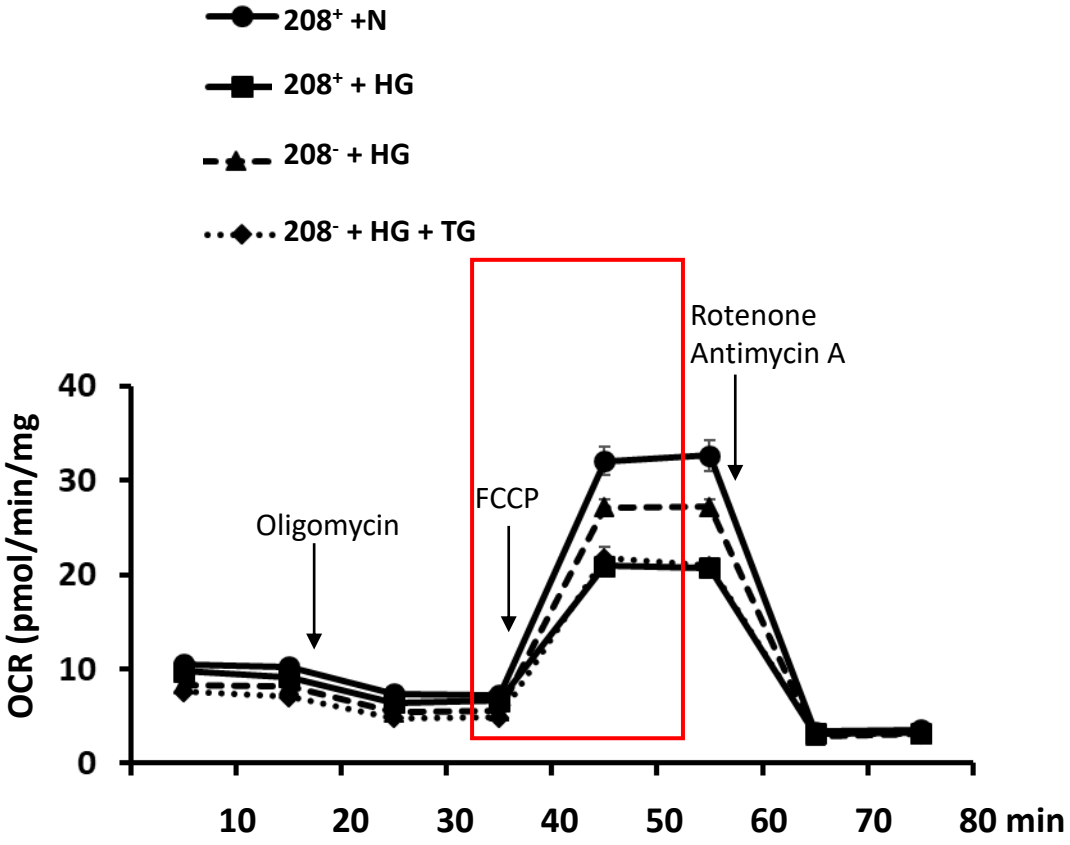
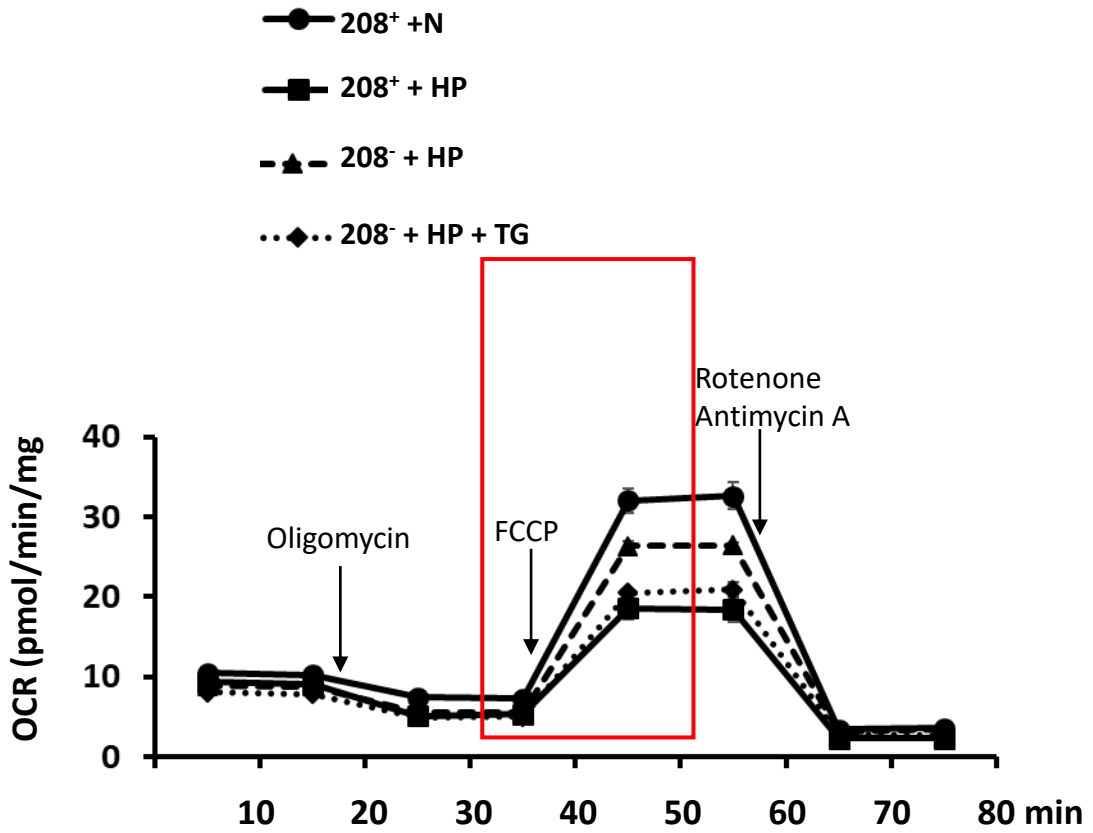
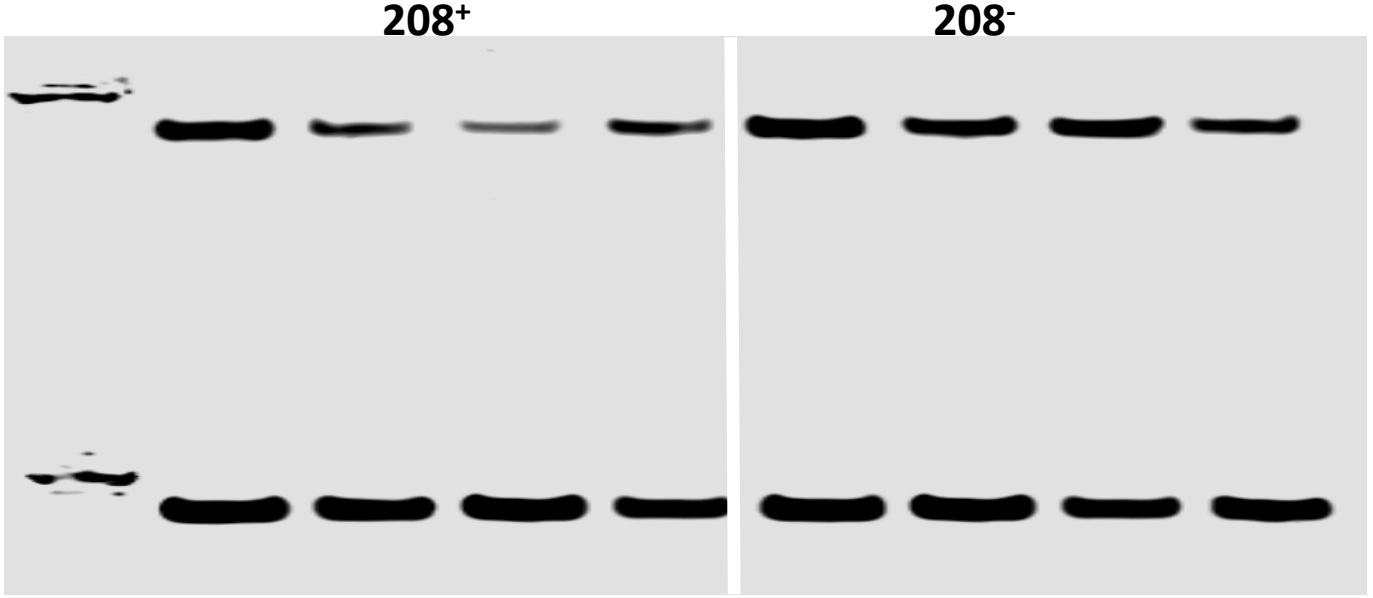
MiR 208a deficiency protects against the decrease in mitochondrial function induced by diabetic conditions in cultured cardiomyocytes.



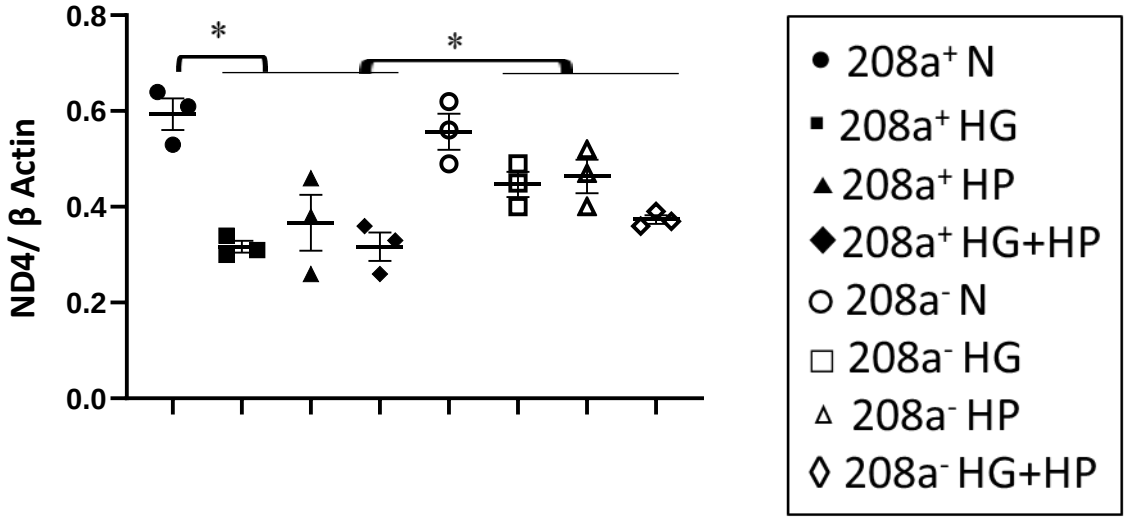




Cells. 2021 Nov 13;10(11):3152. doi: 10.3390/cells10113152



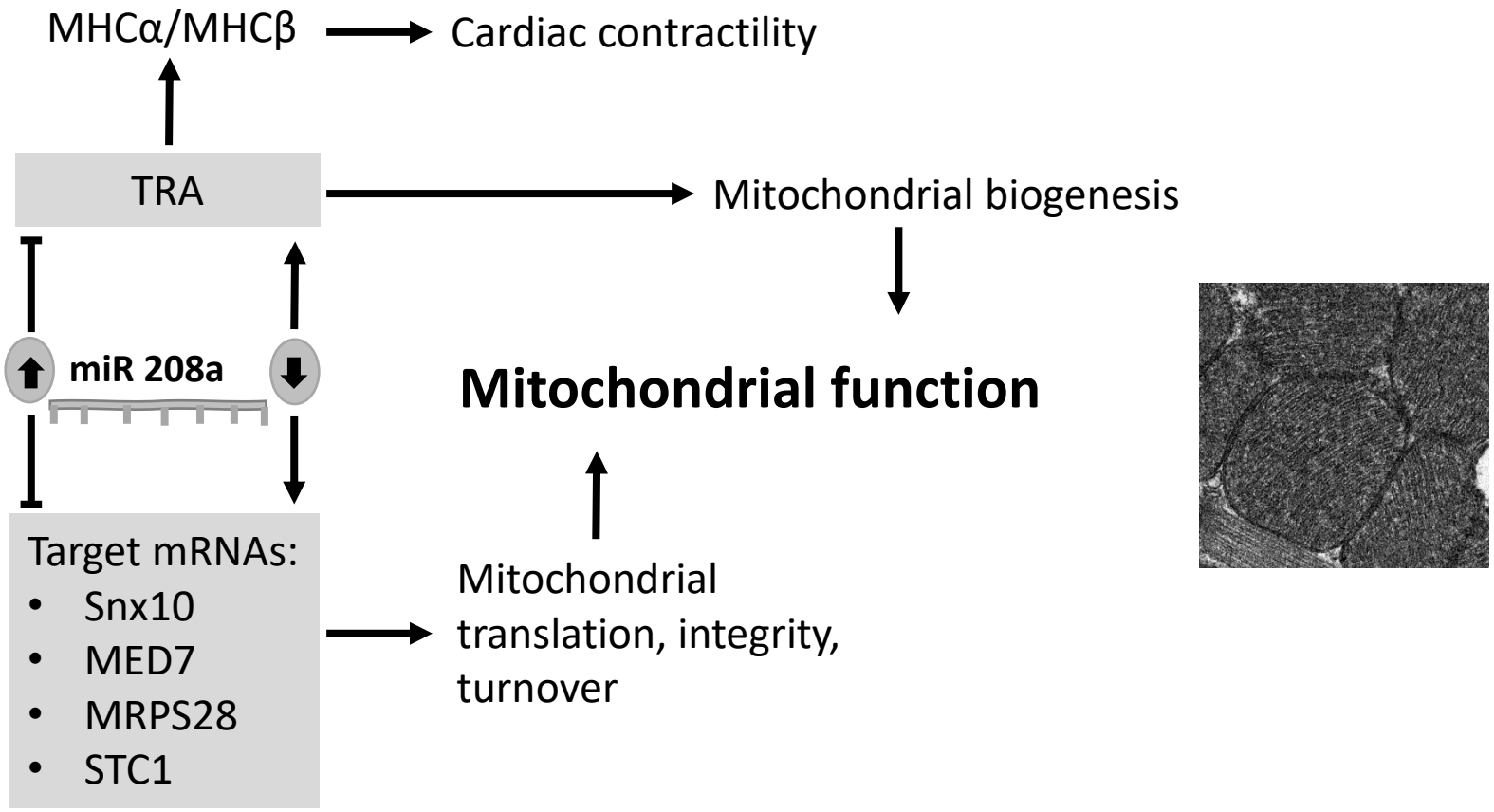
HG	-	+	-	+	-	+	-	+
HP	-	-	+	+	-	-	+	+



MiR 208a deficiency protects against the decrease in mtDNA induced by diabetic conditions in cultured cardiomyocytes.

Cells. 2021 Nov 13;10(11):3152. doi: 10.3390/cells10113152

miR 208a regulates mitochondrial function in cardiomyocytes.



MHC: Myosin Heavy Chain

TRA: Thyroid hormone receptor A

SNX10: Sortin nexin 10

MED7: Mediator Complex subunit 7

MRPS28: Mitochondrial Ribosomal Protein S28

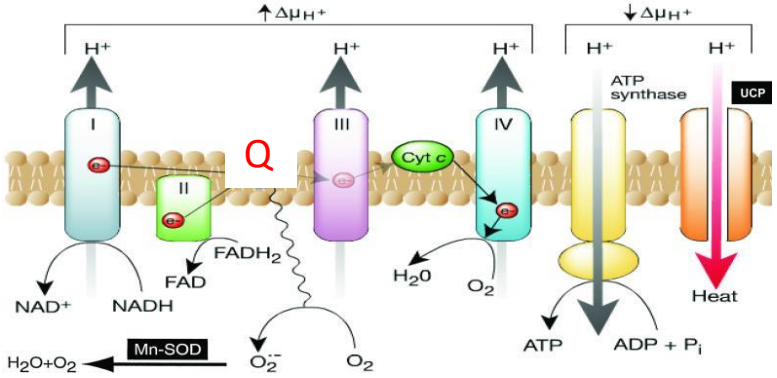
STC1: Stanniocalcin 1

Cells. 2021 Nov 13;10(11):3152. doi: 10.3390/cells10113152

THERAPEUTIC APPROACHES FOR MD

Oxidative stress modulators	**Mechanisms**	**Clinical Trials**	**Outcomes**
KH176 (Sonlicromanol)	Water soluble form of vitamin E	KHENERGY phase IIA trial	Fail primary outcome (cognition)
RP103	Cysteamine bitartrate Glutathione synthesis	Interrupted	Fail
MTP131/SS31	Bendavia, Elamipretide, cationic tetrapeptide, binds and protects cardiolipin	MMPOWER-3 (international phase III trial 200 patients with MD)	Fail to improve 6 min walk (outcome) and the Primary Mitochondrial Myopathy Symptom total fatigue score
EPI-743/PTC-743	Alpha-tocotrienol quinone, vatiquinone, glutathione synthesis		
Increase mitochondrial biogenesis			
Bezafibrate	PPAR agonist		Concerns about increasing metabolic markers on chronic administration
Resveratrol	Plant polyphenol activator of AMPK and sirtuins, upstream of PGC1α		Results not yet available
Omaveloxone (RTA408)	Prevents ubiquitination of NRF1-2		Phase II trial: no change in exercise tolerance but improved lactate and heart rate
Physical exercise	Increase mitochondrial mass Shift in heteroplasmy		Endurance and resistance exercise have been shown to be a safe method for patients with PD, but no specific exercise routine is indorsed by the UMDF
Taurine	Increases PGC1a and mitochondrial translation		Phase III clinical trial: less strokes in MELAS
Restauration of the nucleotide pool			
Deoxynucleotide supplementation	Increase mitochondrial nucleotide concentration and mtDNA	Compassionate use program with 38 participants	Active
Restauration of NAD pool			
Nicotinamide riboside (NR)	NAD precursor		Active
Acipimox			Active
Niacin	Restore systemic NAD		Increase muscle strength
Mitochondrial augmentation therapy	transplanting healthy mitochondria derived from donor white blood cells or placenta into affected patients through enrichment of the patient's own peripheral stem cells.		Autologous CD34+ cells enriched with blood derived mitochondria

CLINICAL TRIALS IN LEBER HEREDITARY OPTIC NEUROPATHIES (LHON)

Topical (eye drops)	Mechanisms	Outcomes
Brimonidine	Decrease intraocular pressure in glaucoma Re-purposed, neuroprotective	Fail
Elamipretide (MTP-131)	Small peptide, antioxidant	Active
Oral		
EPI-743 (α-tocotrienol quinone)	Natural analogue of vitamin E	Promising, stop in progression
Idebenone (Raxone)	Synthetic, less lipophilic analogue of coenzyme Q10 Antioxidant Facilitates electron transport 	RHODOS: Rescue of Hereditary Optic Disease Outpatient Study Positive effects that persist after the drug is discontinued International Expanded Access Program: clinically relevant recovery proportional with the treatment duration 2015: conditional approval for LHON in the European Union Phase IV open-label intervention study assigned by the European Medicine Agency at 31 sites in 9 European countries and USA Safe and effective in LHON
Intravitreal with AAV-ND4	Retina is an immune-privileged organ. Approach: recoding the mtDNA gene to fit the nuclear genetic code, add a MTS to be imported into the mitochondria, allotopic nuclear expression of a corrected mt gene	USA: safe and improved visual acuity at 12 month follow up Europe, USA: RESCUE/REVERSE/RESTORE REFLECT (injected bilaterally)

Conclusions and Future Perspectives

- therapeutic strategies predominantly symptomatic/restorative and not curative
- > 50 clinical trials that interrogate various strategies to alleviate organ diseases in mitochondrial defects. The evidence for good outcomes is missing for most.
- quality of clinical trial impacted by:
  - lack of study power (limited sample sizes)
  - variable endpoint selection (single primary, multiple, or composite)
  - dichotomy between statistical significance and clinically meaningful results
  - lack of established biomarkers to substitute for a clinical efficacy endpoint (“surrogate endpoint”)
- Idebenone licensed to treat visual impairment in adolescent and adult patients with LHON
- need for increased awareness of mitochondrial involvement in chronic diseases
- genetic therapies closer to clinic
- novel strategies for redox therapy

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Thank you for your attention!