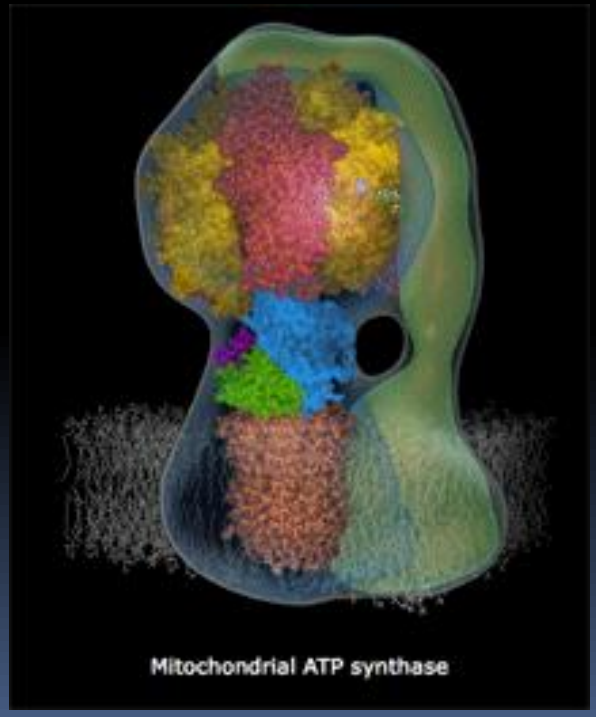
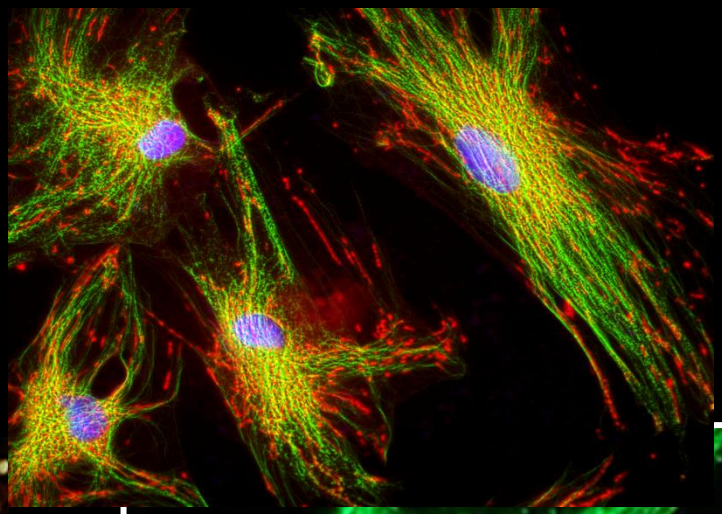
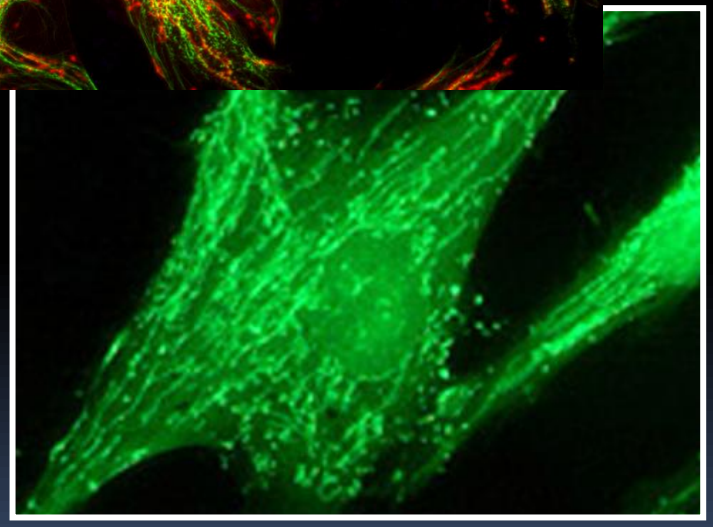
Mitochondrial disorders and defects of mitochondrial beta oxidation of fatty acids

Institute of Inherited Metabolic Disorders
1.LF

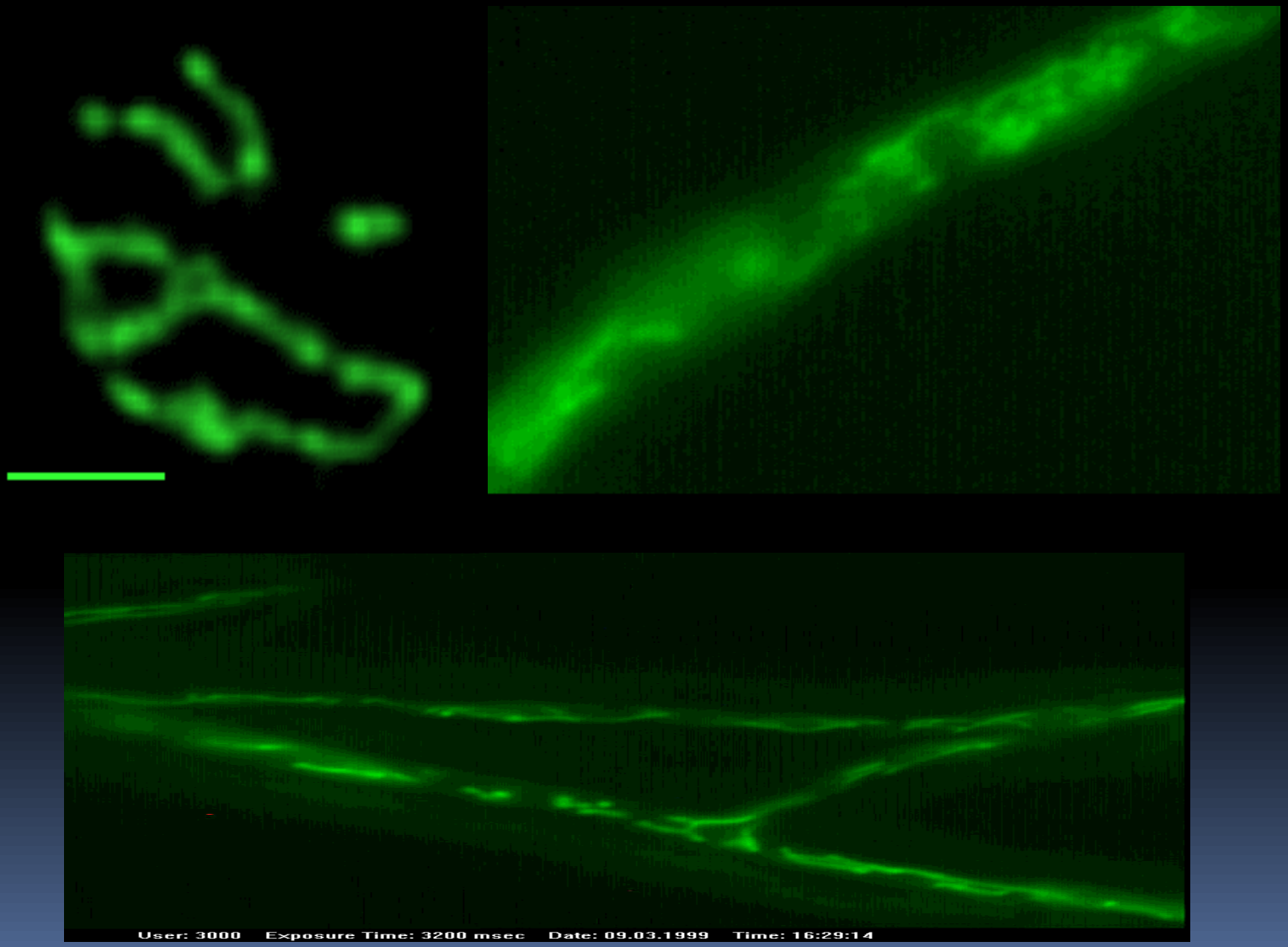


Mitochondria



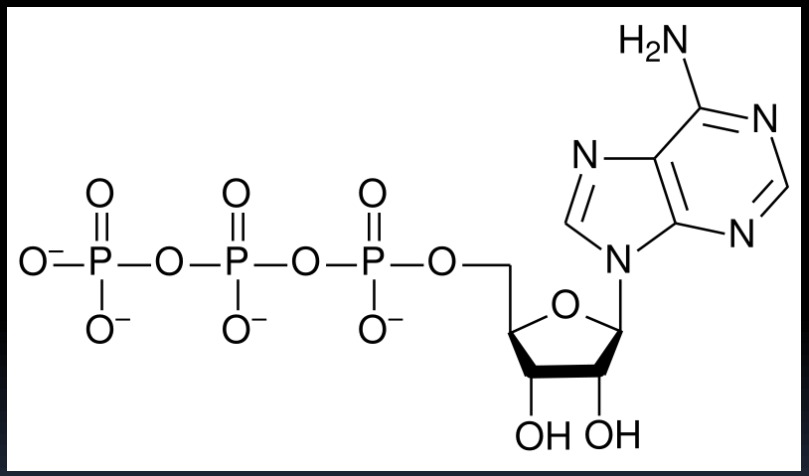


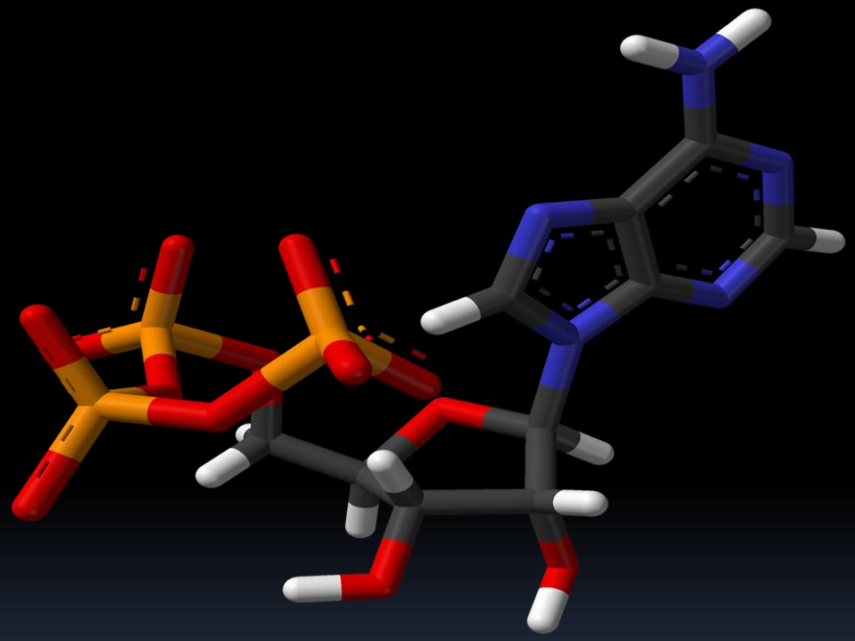
Mitochondria structure



OXPHOS (oxidative phosphorylation systeme)
Respiratory chain
ATP production – molecule for energy transport in cells
Aerobic oxidation in eukaryotic mitochondria

(glycolysis and Krebs cycle)

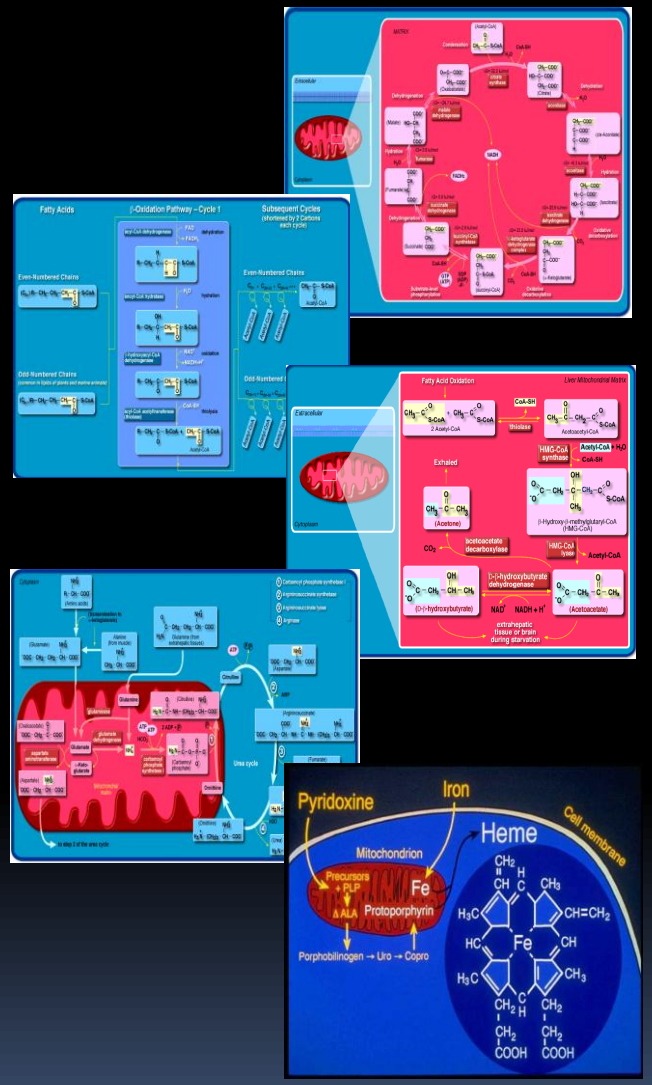




ATP – adenosine triphosphate

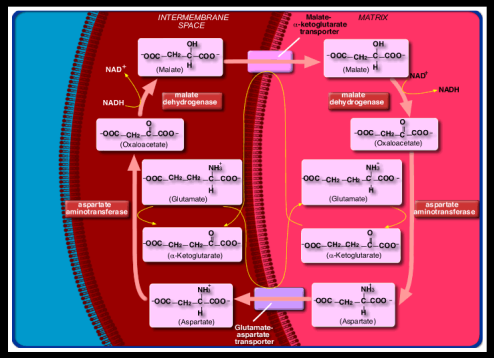
Metabolic pathways in mitochondria

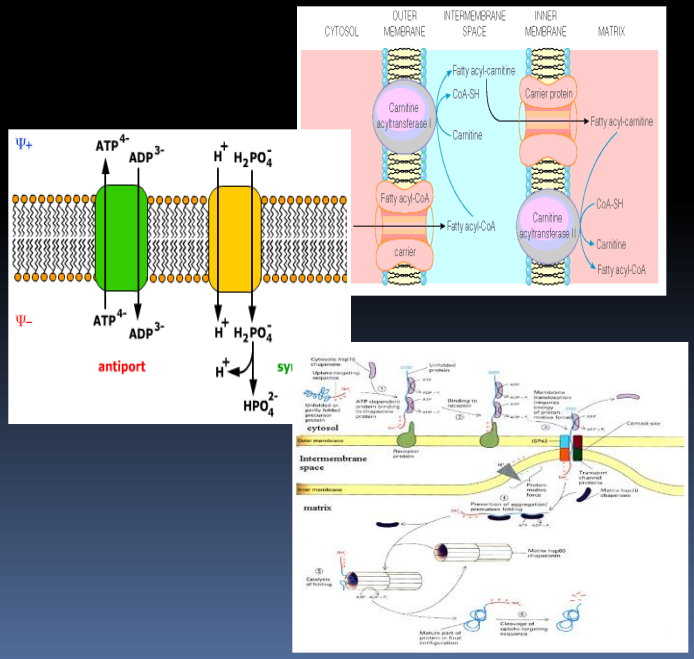
- Krebs cycle
- β oxidation FA
- Ketogenesis
- Urea cycle
- Syntesis of haem and phorphyrine
- …



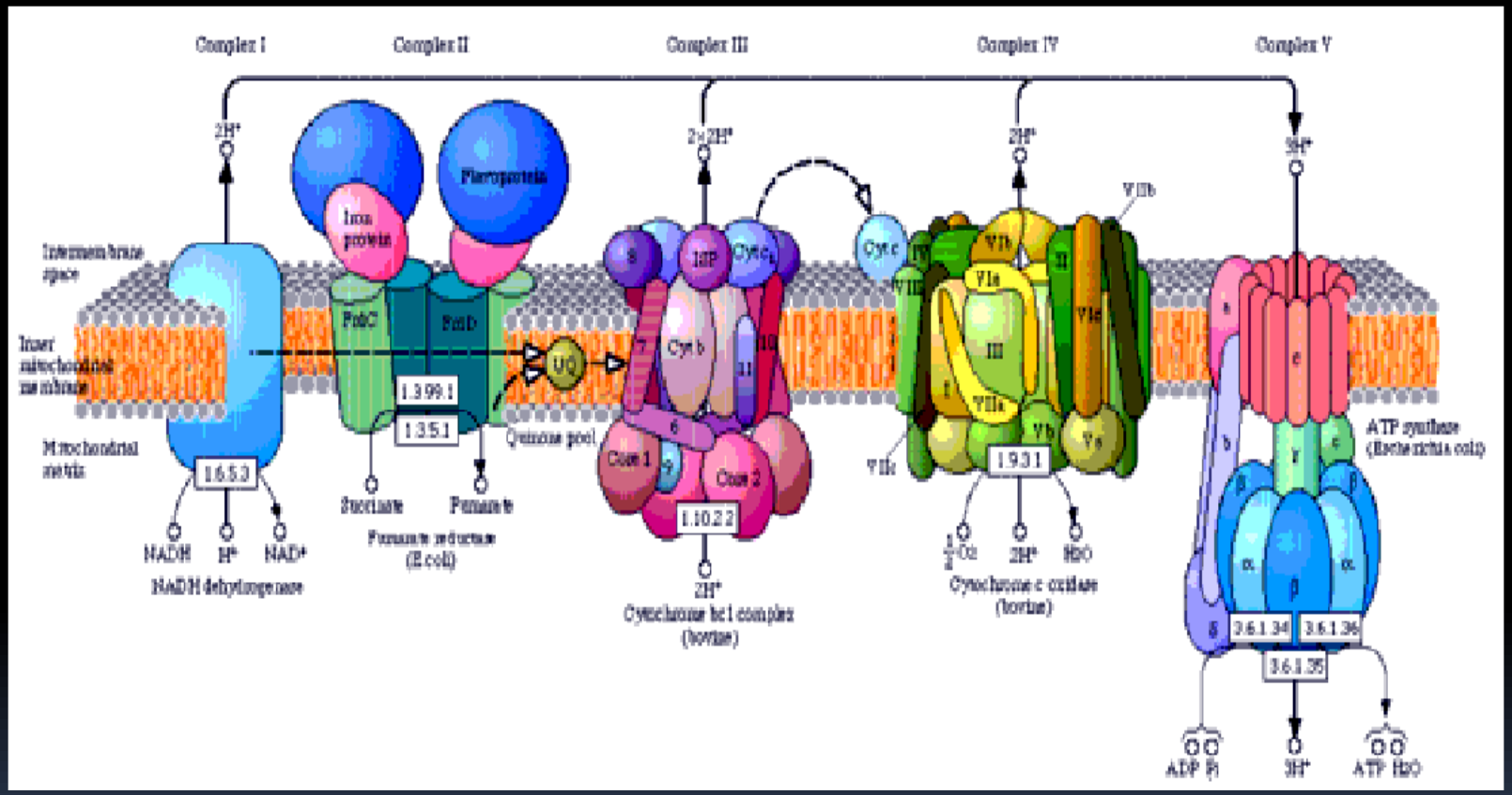
Mitochondriální transportní systém

- shuttle for NADH.H$^+$ transport
  - malate-aspartate shuttle
  - glycerolphosphate shuttle
- substrates and products transporters
- ADP/ATP transocator
- protein import



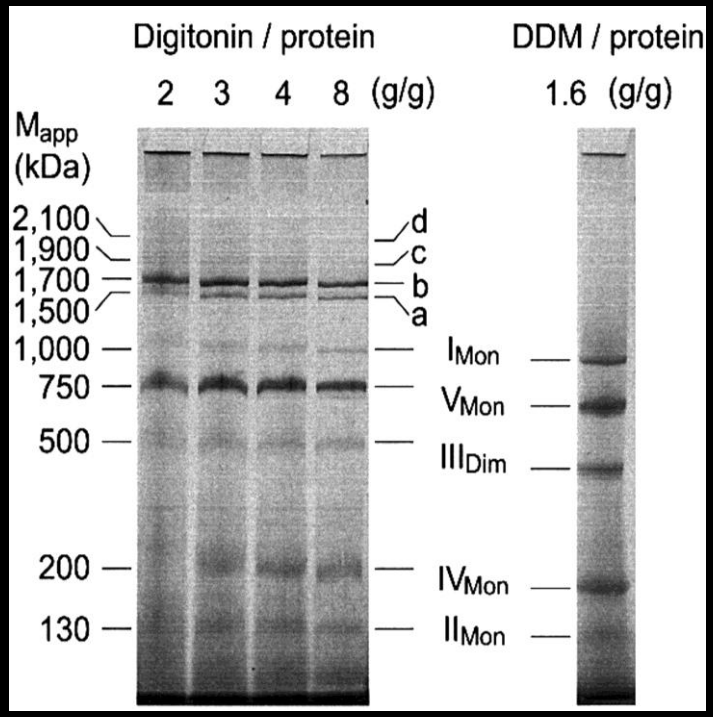


Oxidative phosphorylation

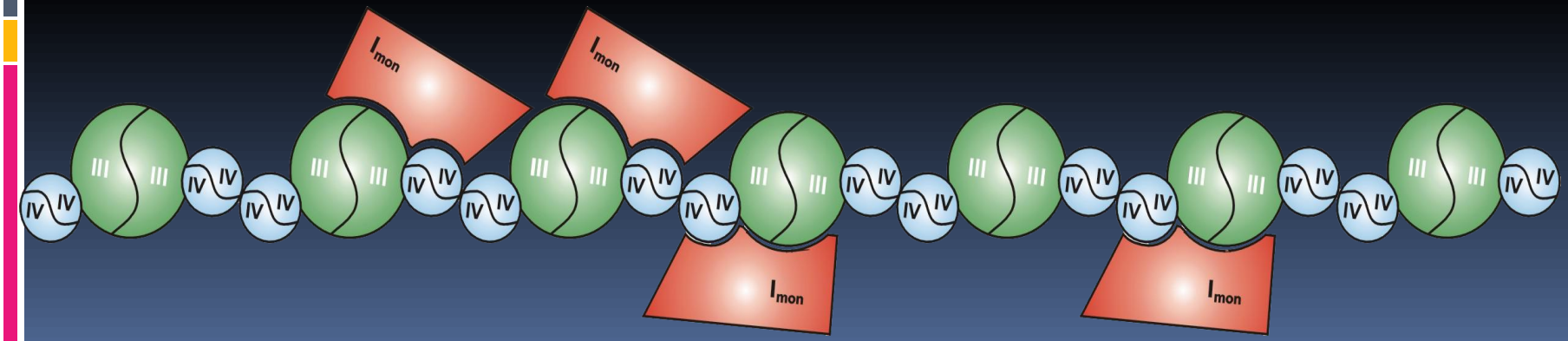


> 43 4 11 13 16 subunits

Supercomplexes



Tissue specifity
(liver, haert, brown adipose tissue …)
Ontogenetic changes
(perinatal developement, …)



Mitochondrial Energetics

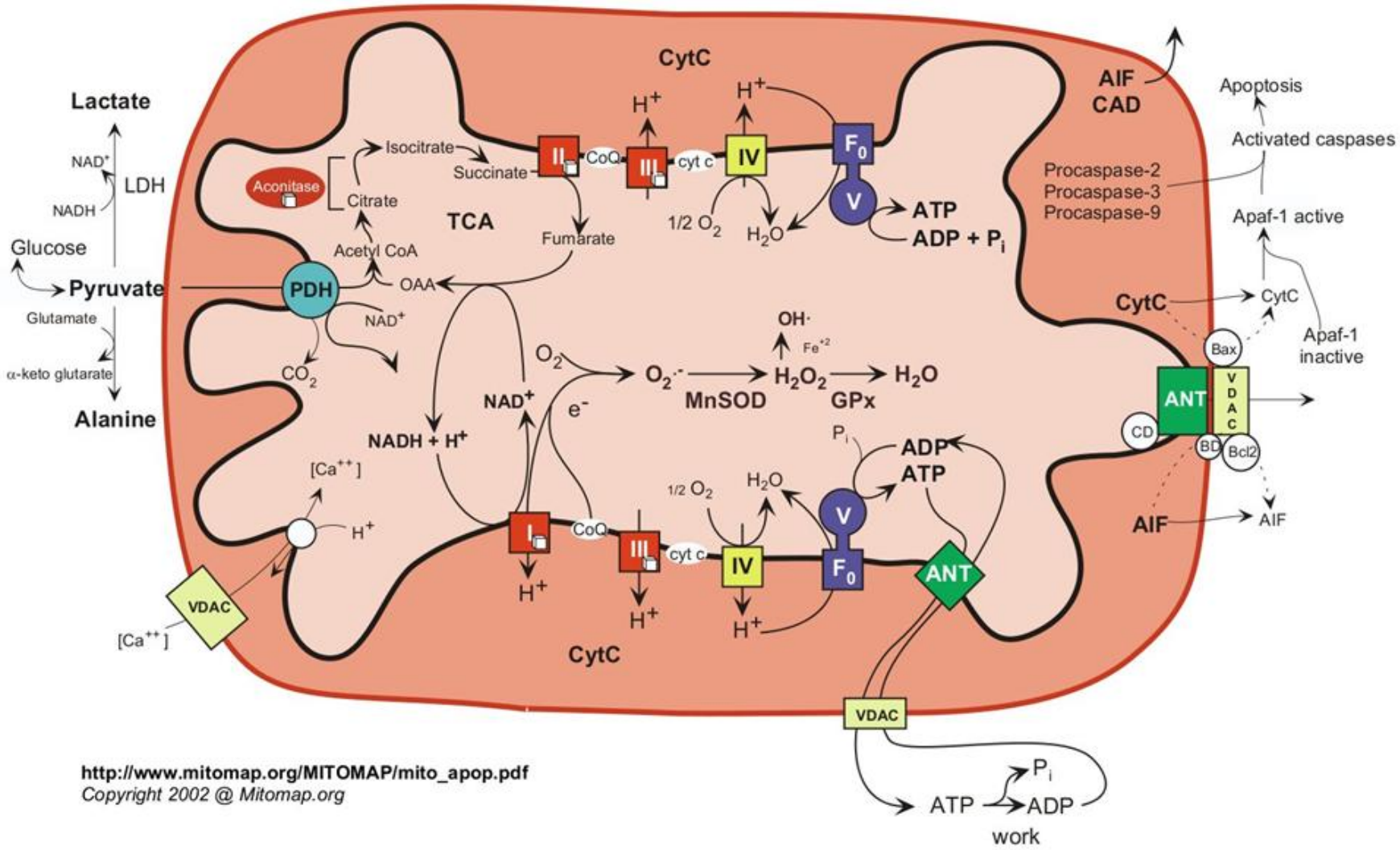


Diagram of the mammalian mitochondrion showing the relationship between energy production, ROS generation, and regulation of apoptosis.

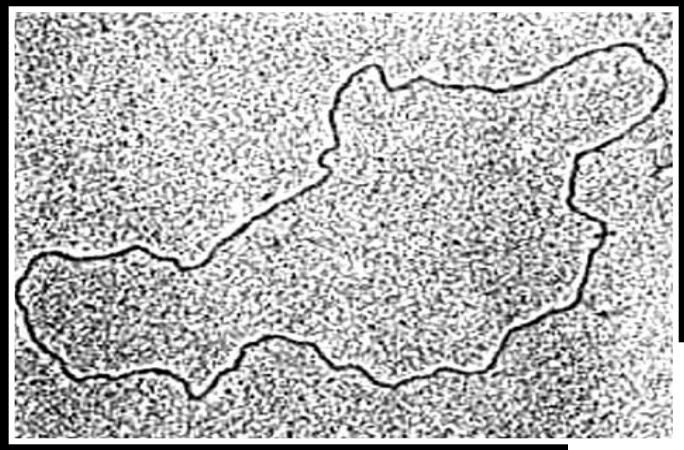
Mitochondrial disorders

Mitochondrial energy defets
- nuclear genes
- mitochondrial genes (mtDNA)

- Deficiency of respiratory chain complexes; OXPHOS
- Deficiency of Krebs cycle enzyme and pyruvate dehydrogenase metabolism
- Defects of mitochondrial replication, transcription, translation, repair, assembly or structural proteins…; in fusion and fission processes

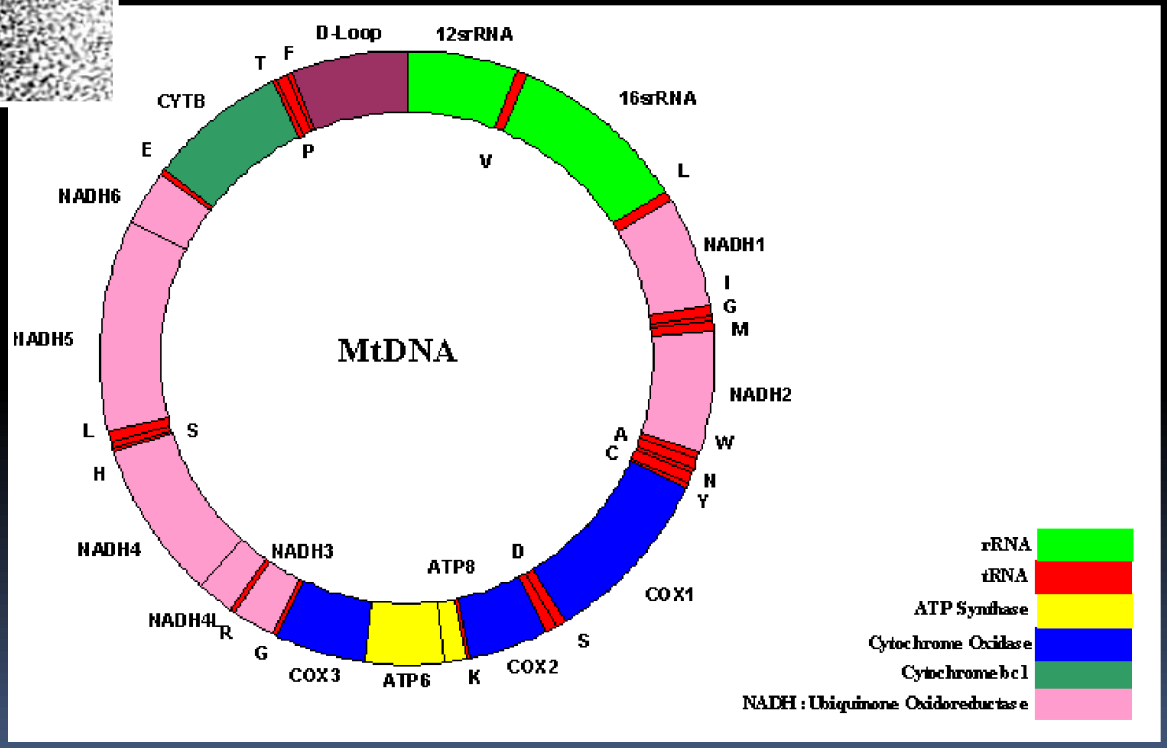
Incidence 1/3000-4000

Mitochondrial DNA



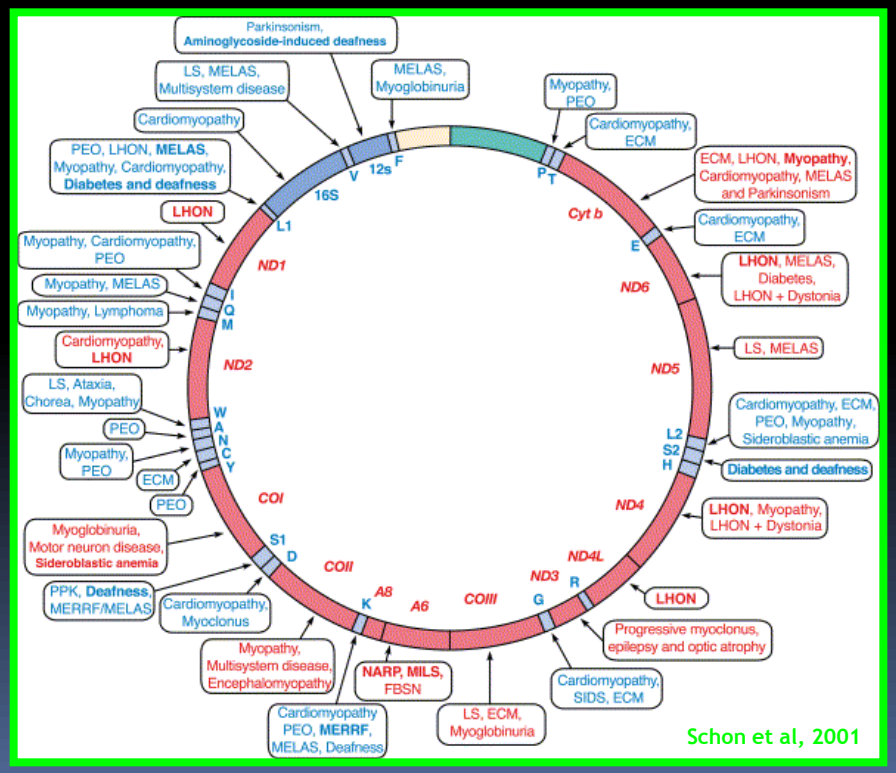
- 16 569 base pairs
- 13 mRNA, 2 rRNA; 22 tRNA

- 2-20 / mito
- 10-10 000 / bb



Mitochondrial disorders

- Incidence 1:3500 – 1:4000
- High energy dependend tissues
- Mutation in ncDNA and v mtDNA



Schon et al, 2001

- 13 mRNA, 2 rRNA
- 22 tRNA - >100 mutation
  - ✓ Leu; Lys; Ser
  - ✓ MELAS; MERRF

Mitochondrial disoders

- 1962 Luft R, et al — Luft disease
- 1963 Nass S, Nass MHK — DNA v mitochondria
- 1970 Spiro AJ, et al. — Respiratory chain defects
- 1974 Berk AS, Clayton DA — replication of mtDNA
- 1979 Barrell BG, et al — genetic code of mtDNA
- 1981 Andersson S, et al. — Sequence in mtDNA
- 1988 Holt IJ, et al. — Deletion of mtDNA
  Wallace DC, et al — point mutation of mtDNA
- 2000 www.gen.emory.edu/mitomap.html
  > 130 point mutations
  > 70 deletions/duplications

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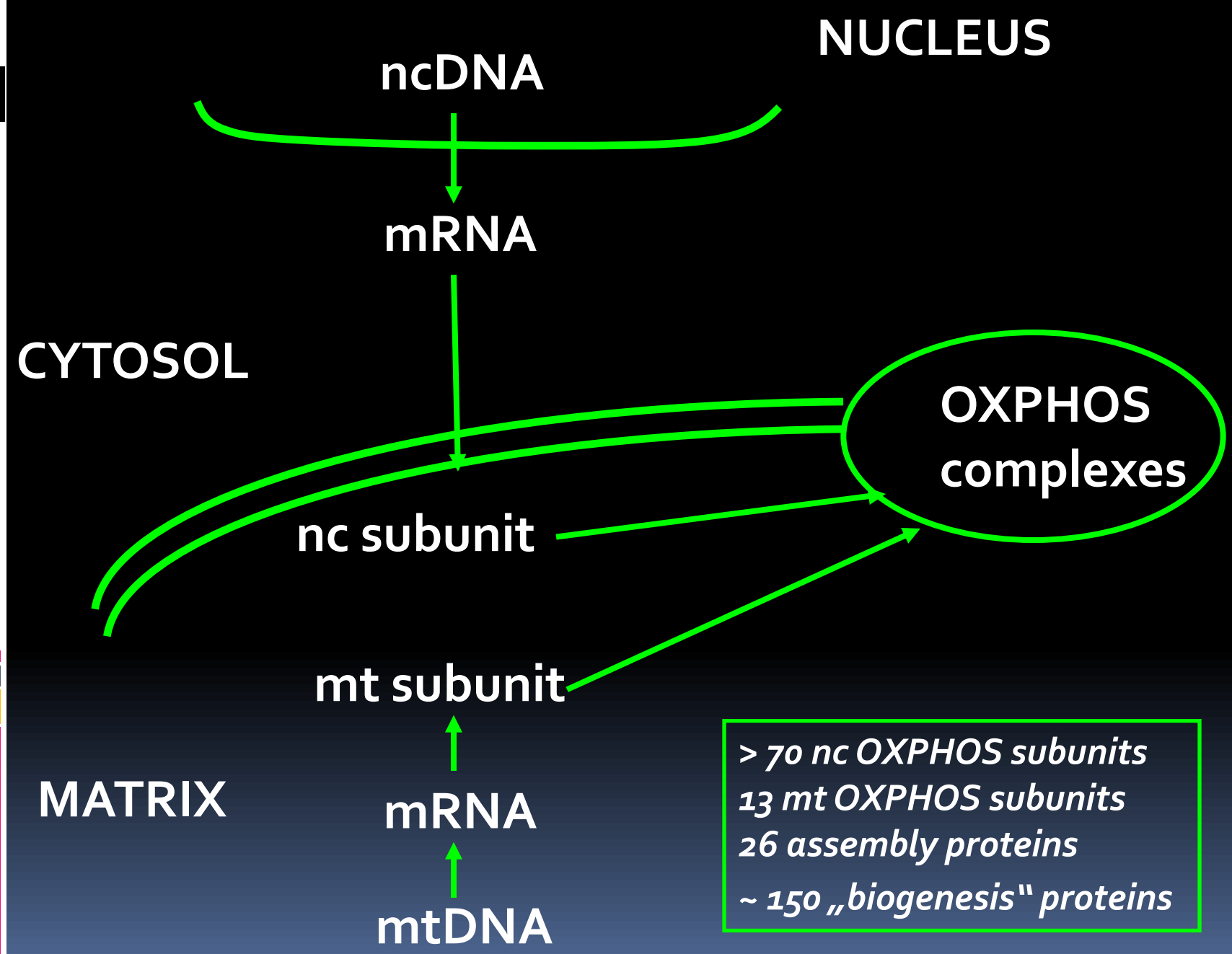

NUCLEUS
ncDNA
mRNA
CYTOSOL
OXPHOS
complexes
nc subunit
mt subunit
MATRIX
mRNA
mtDNA
> 70 nc OXPHOS subunits
13 mt OXPHOS subunits
26 assembly proteins
~ 150 „biogenesis" proteins

Genetic of mitochondrial disorders

mtDNA

- OXPHOS subunits
- rRNA
- tRNA
- Amount of mtDNA

nDNA

- OXPHOS subunits
- mt biosyntetis systeme (replication, transcription, translation)
- Protein import and modification
- Assembly factors

OXPHOS defects

Hereditary mtDNA mutation

- Sporadic mtDNA defects
  single deletion/duplication

- Maternally inherited mtDNA mutation
  point mutations
  single deletion/duplication

- Autosomal inherited mtDNA mutations
  mulriple deletions
  depletions

Point mutations in mtDNA

Homoplasmic form of mtDNA

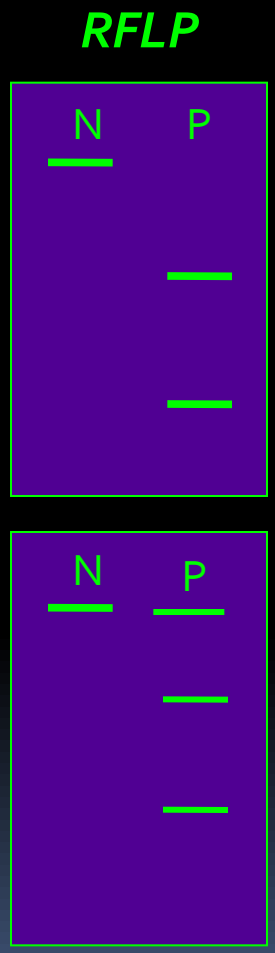
- OXPHOS proteins (LHON)

Heteroplasmic form of mtDNA

- tRNA (MERRF, MELAS)
- rRNA (cardiomyopaty)
- OXPHOS proteins

  Leigh, NARP

  LHON



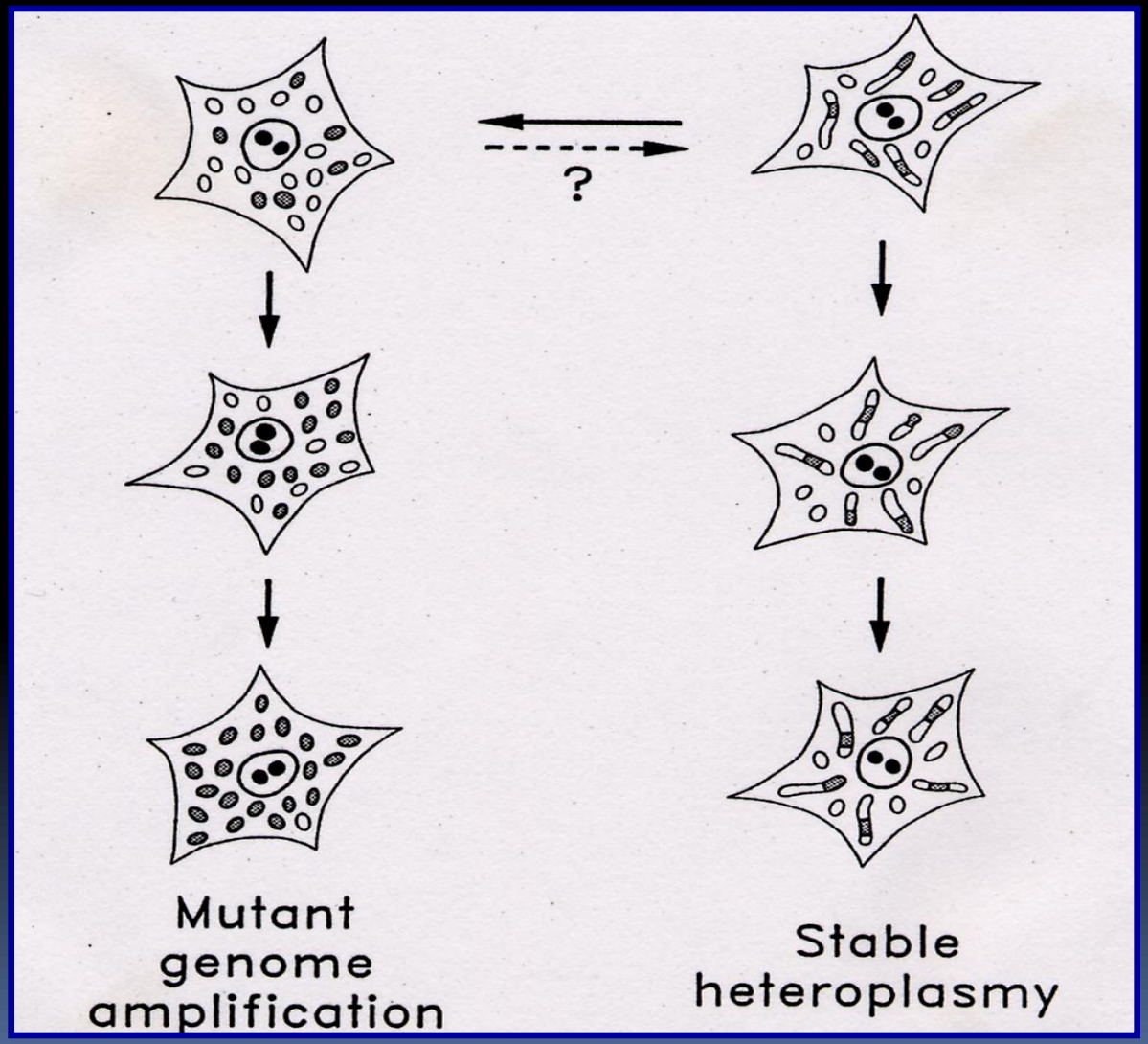
Heteroplasmy, mtDNA a diseases

The symptoms of mtDNA diseases often progressively worsen with age
bioenergetic threshold is breached that results in mitochondrial dysfunction.

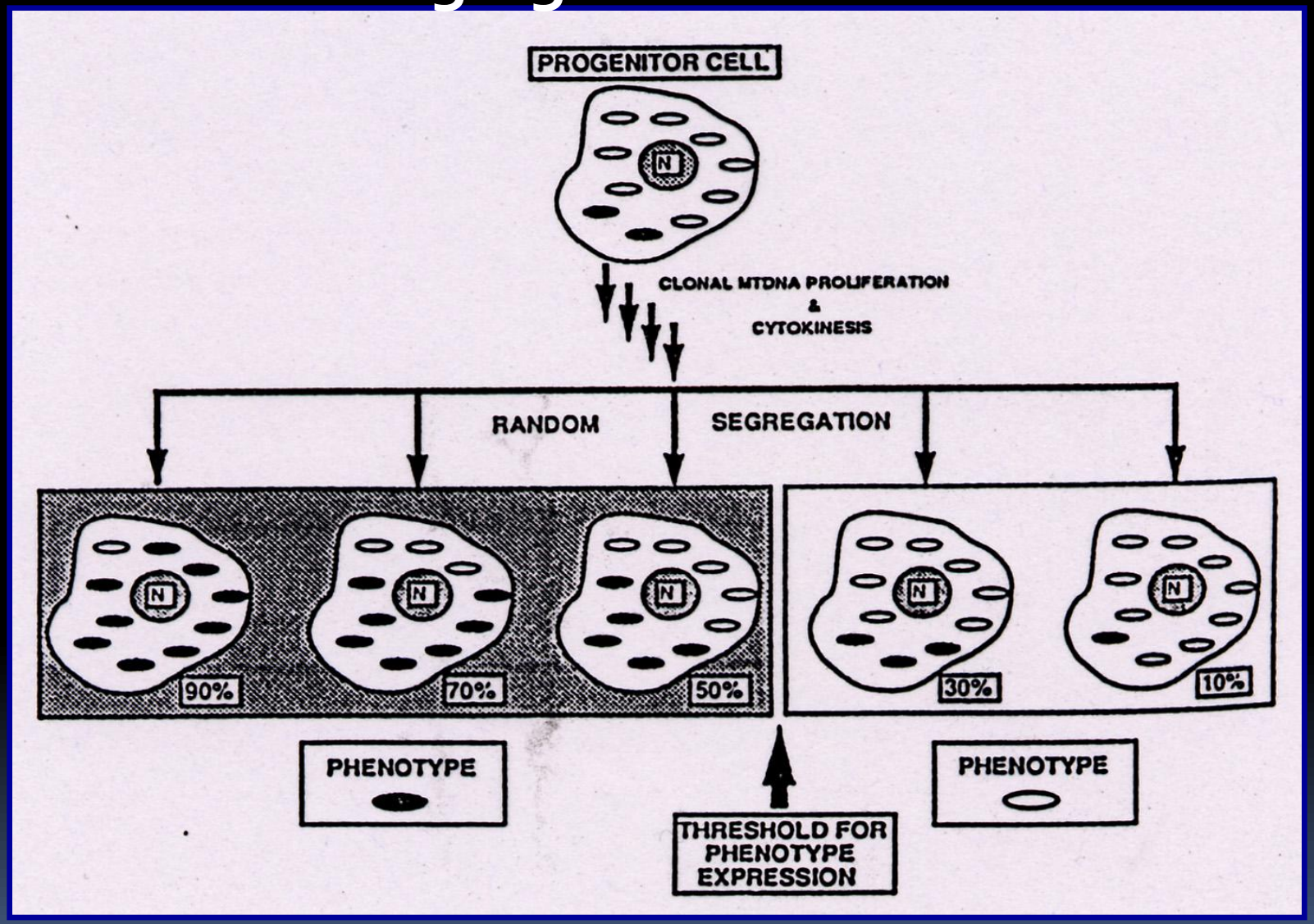
Some organs are particularly dependent on respiratory function: brain, skeletal muscle, heart muscle, and endocrine glands are particularly dependent on respiratory function.

Cells do not lose respiratory function until high loads of pathogenic mtDNA are present, ranging from 60% to 90% depending on the specific mutation.

Inter- a intramitochondrial heteroplasmy



Segregace mtDNA



Mitochondrial genetic bottleneck

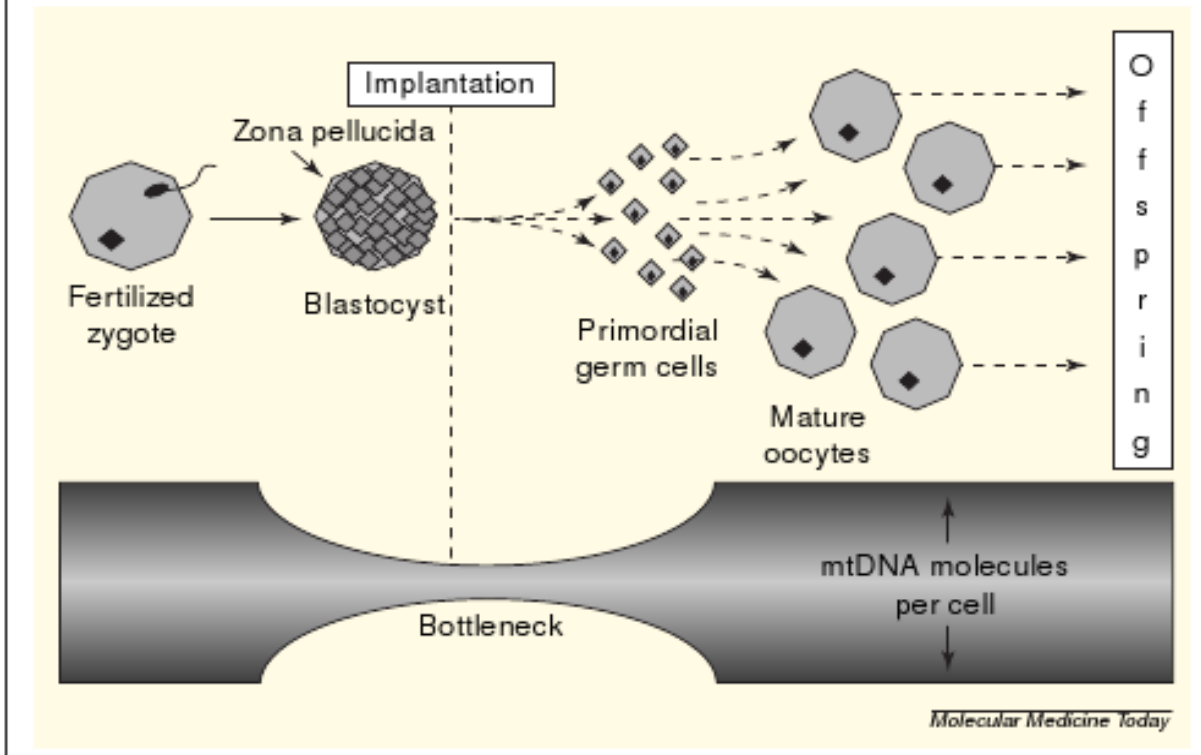
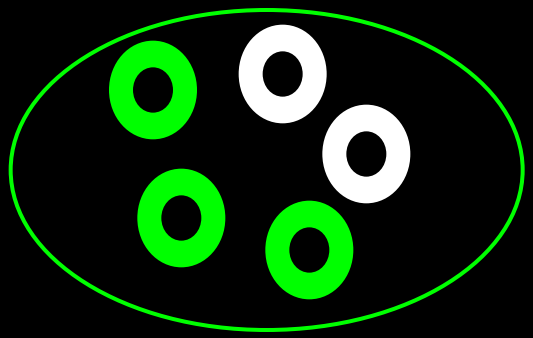


Figure 1. The mitochondrial genetic **bottleneck**. The mitochondrial genetic bottleneck provides an explanation for the different percentage of mutant mtDNA that can occur in siblings. It is thought that there is a restriction in the number of mtDNA molecules within the cell early in the development of the female germ line. This leads to marked differences in the level of heteroplasmy between primary oocytes within the same female and accounts for the variation amongst offspring.



Segregations and distribution od mtDNA mutations

Germinative cells

(„bottleneck" effect

Speed segregation

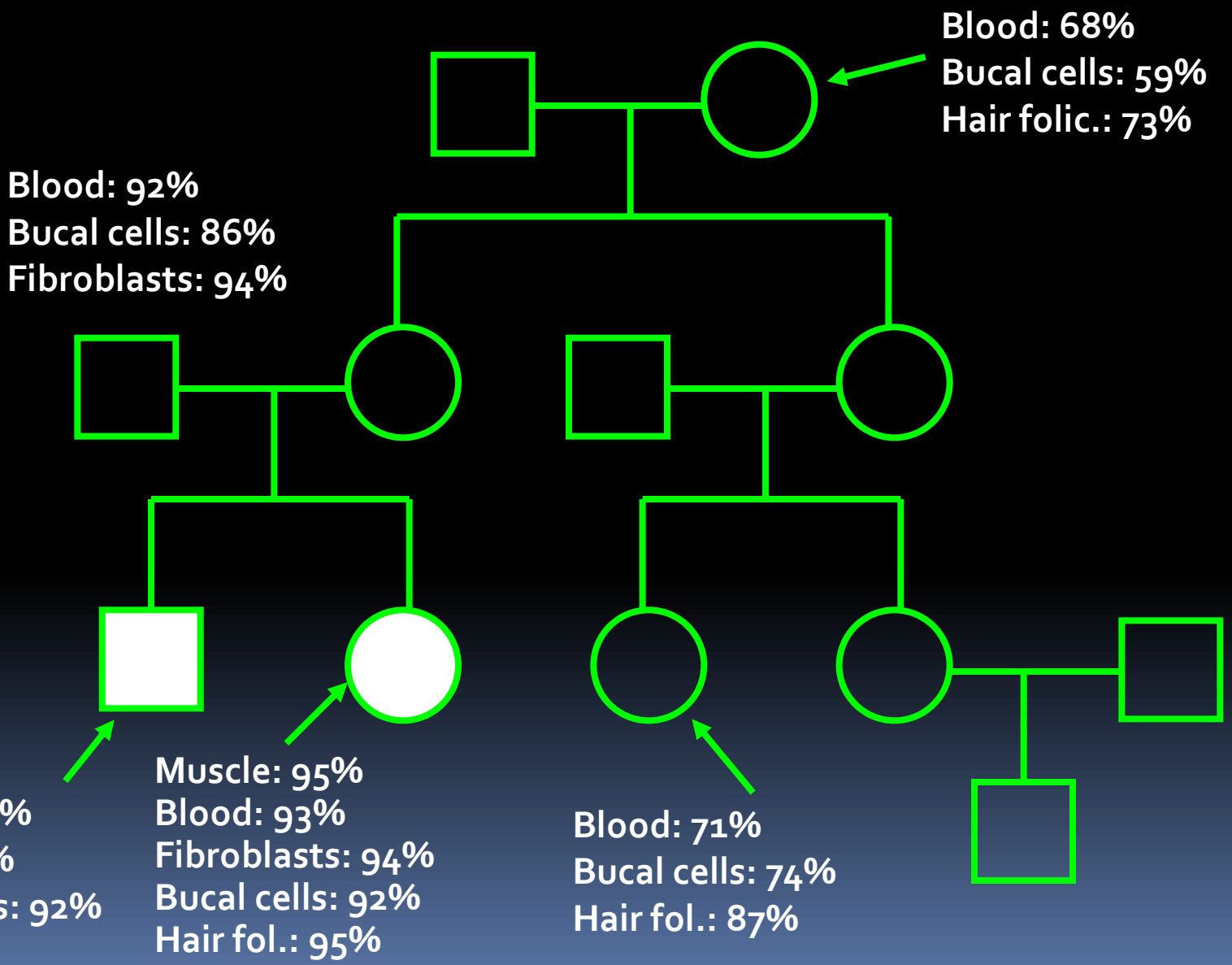
(*homoplasmy trends*)

Somatic cells

slow segregation

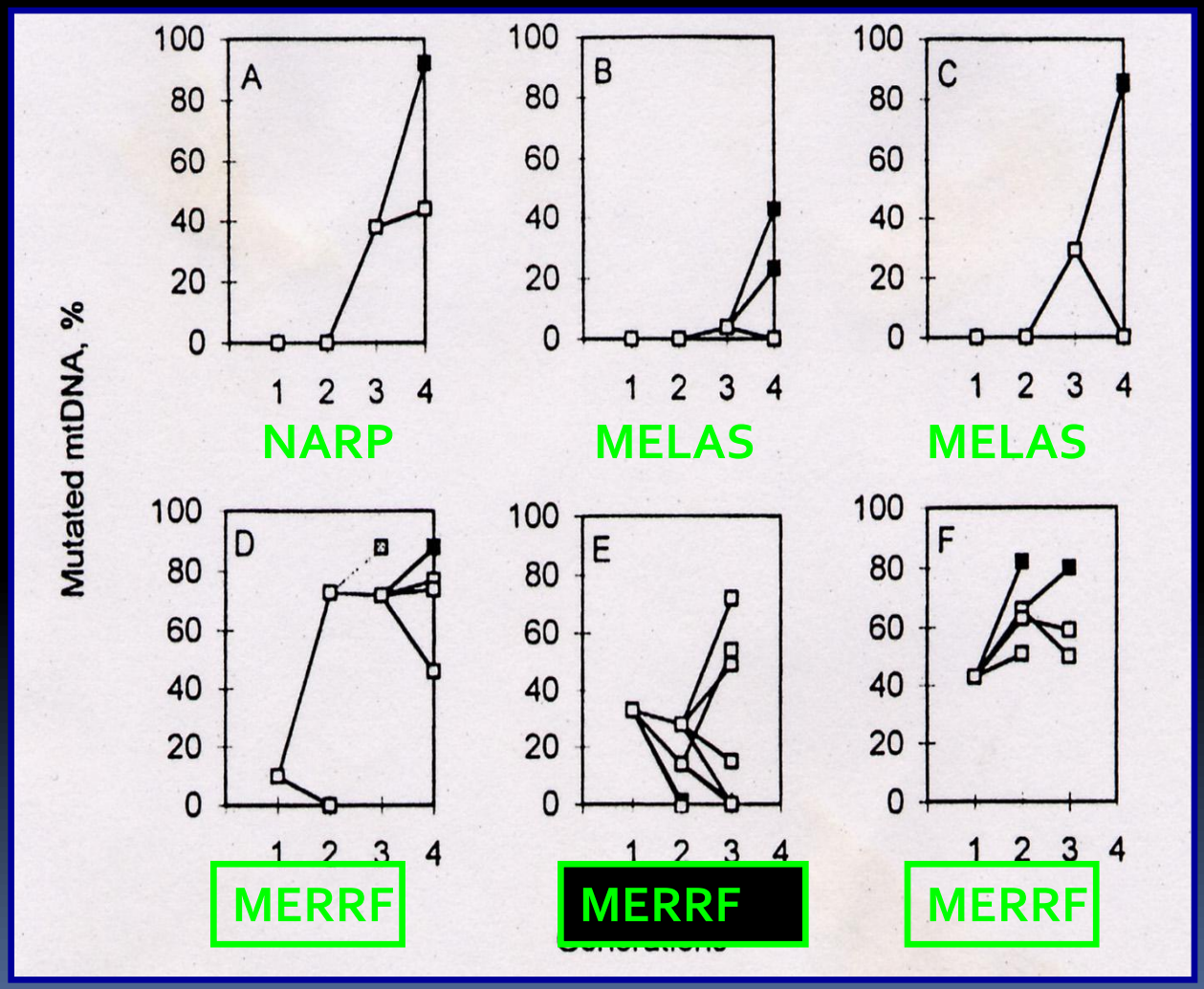
tissue heteroplasmy

7512T>C mutation in mtDNA



Segregation of mtDNA

NARP (8993), MELAS (3243) a MERRF (8344). Of

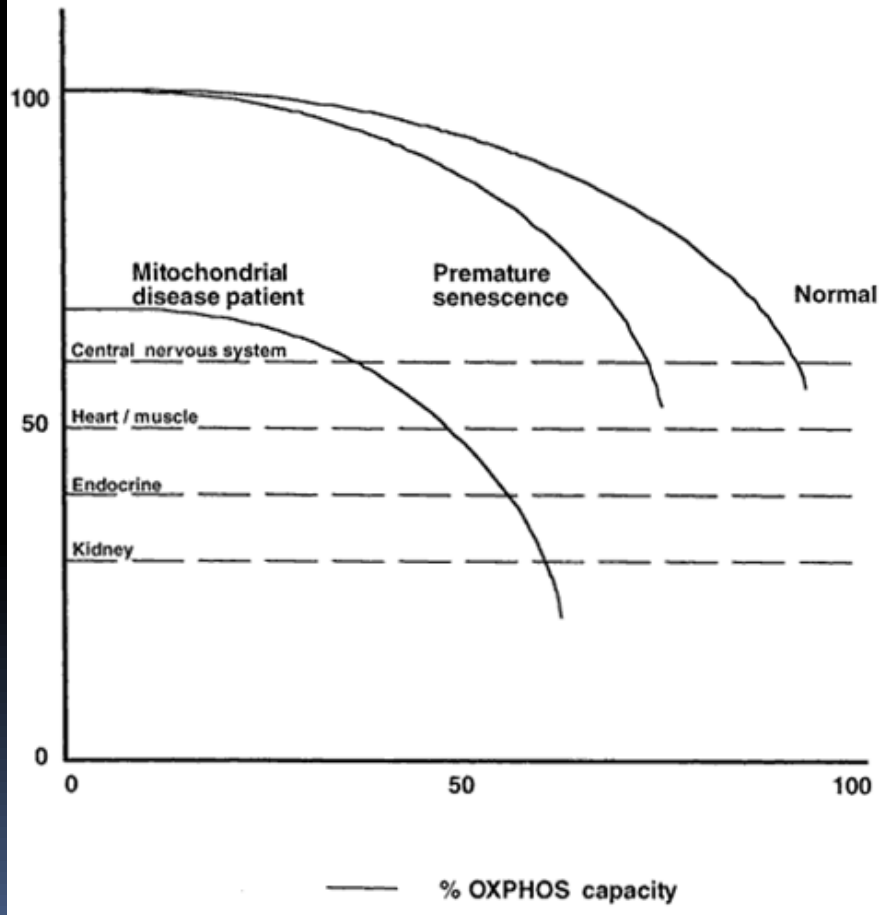


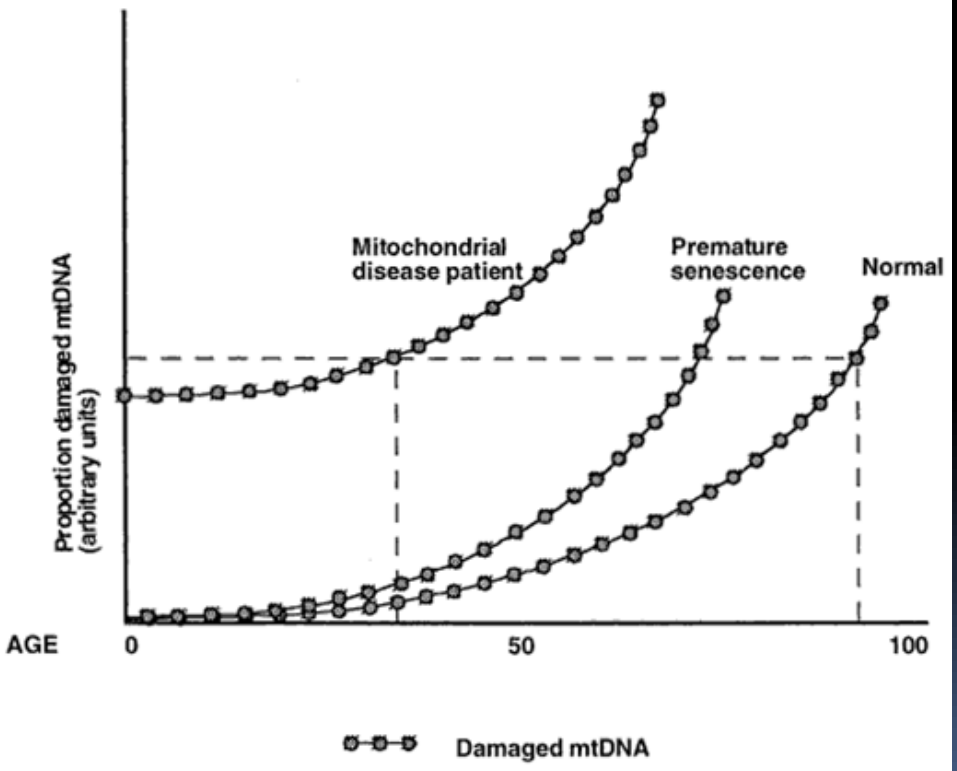
Threshold efect of OXPHOS defects

- 0-100% of mutated mtDNA
- Non-linear relation between heteroplasmy and dysfunction - 90%
- Age dependent OXPHOS activity
- Tissue and cell specifity –energetic demands

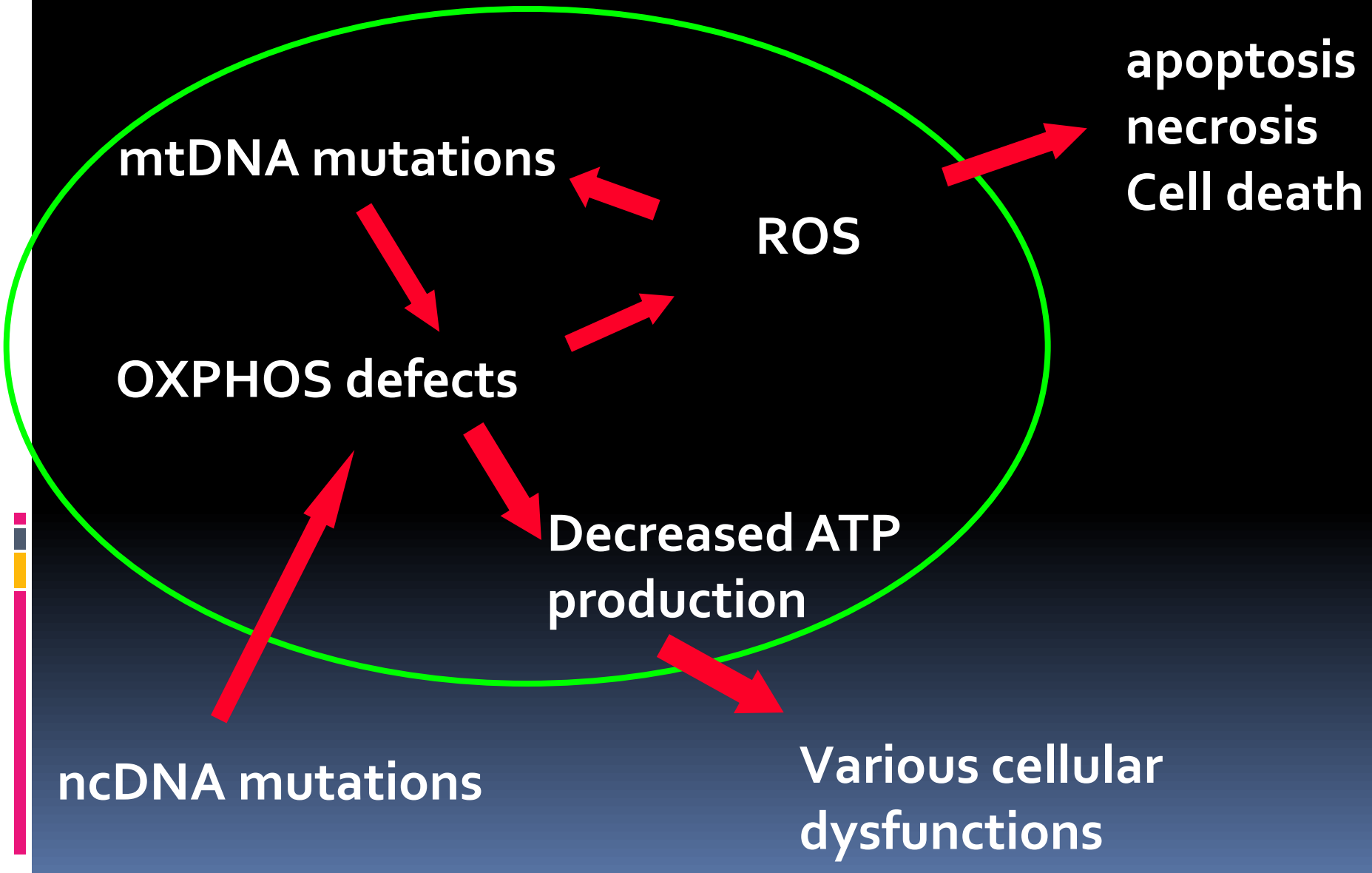
Threshold Hypothesis

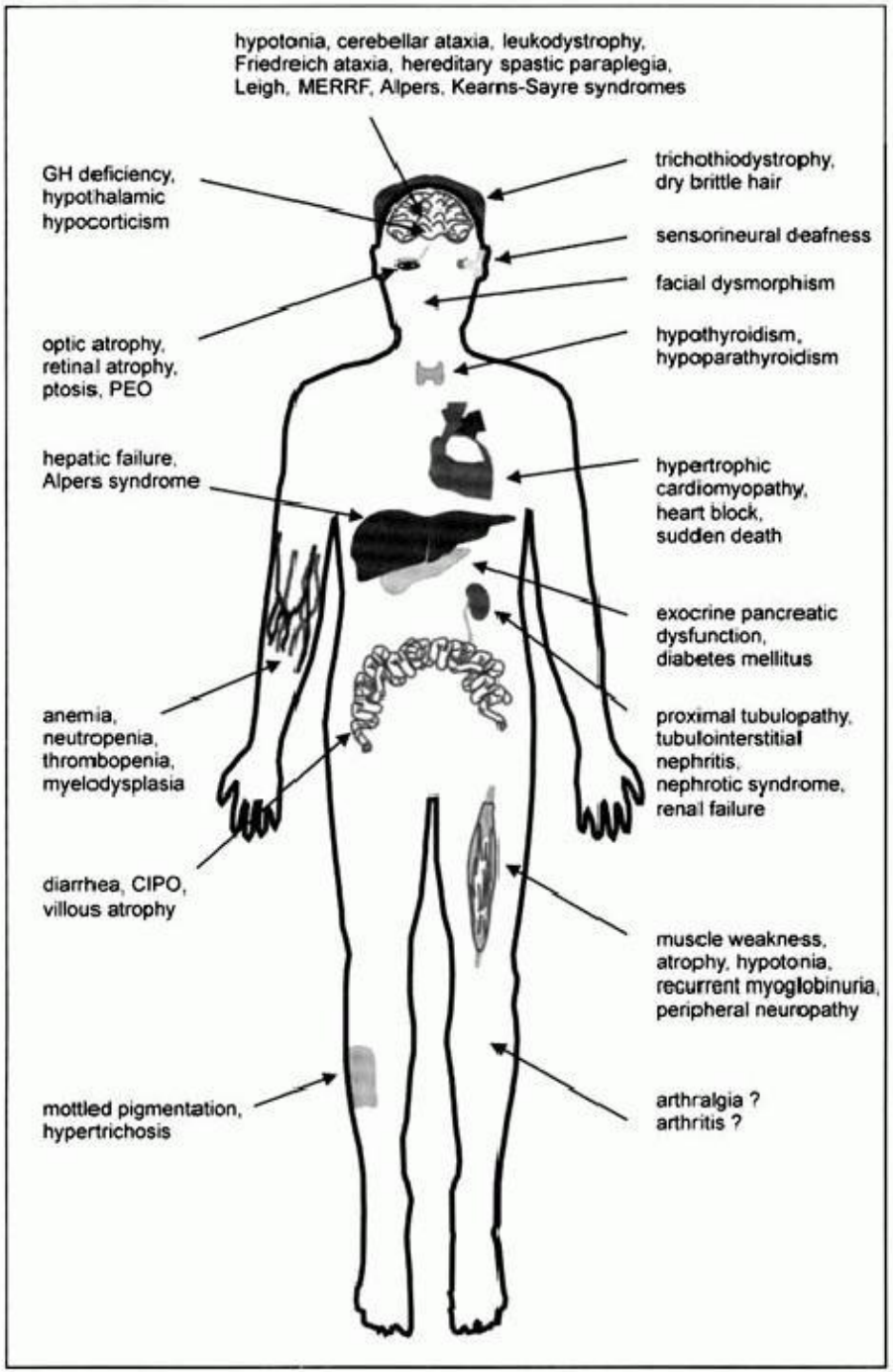
OXPHOS Capacity vs. mtDNA Damage





Patogenetic mechanisms





*"**Any symptom, in any organ, at any age**, and with any mode of inheritance"*

Munnich *et al*, OMMBID, Ch 99

MtDNA defects

LHON – Leber hereditary optic neuropathy

- 11778 G>A; 3460G>A; 14484 T>C – complex I subunits
- akute/subakute blindess in adults
  - 4x in men

NARP/Leigh syndrome – neuropathy, ataxy, retinitis pigmentosa

- 8993 T>G and others
- psychomotor retardation, lactic acidosis, basal ganglia nekrosis

MERRF – myoclonic epilepsy, ragged red fibres

- 8344 G>A (tRNA pro Lys) and others
- deafness sensorineural

MELAS – mit. encephalomyopathy, laktic acidosis, stroke-like episodes

- 3243 A>G (tRNA pro Leu) and others
- diabetes mellitus

.....

Leigh syndrome

A neurodegenerative disorder usually starting before 1 year of age and leading to death within months or years.

„subacute necrotizing encephalomyelopathy“

Degeneration of basal ganglia, progressive course with motor and developmental decline („plateaus“), irregular breathing, ataxia, hyperlactacidemia, muscle weakness, seizures

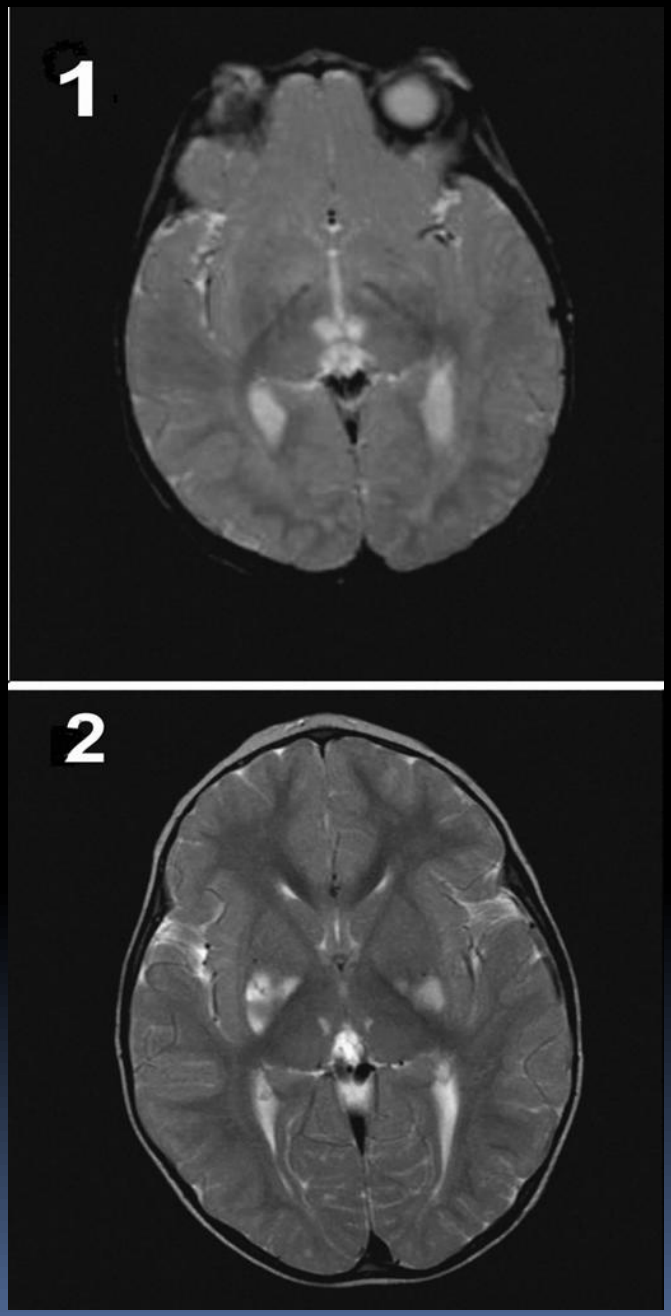
Intermediate phenotypes

Defects of OXPHOS:
pyruvate dehydrogenase complex (PDH)(E1α gene),

cytochrome c oxidase (complex IV) -often putative complex IV assemply gene SURF-1

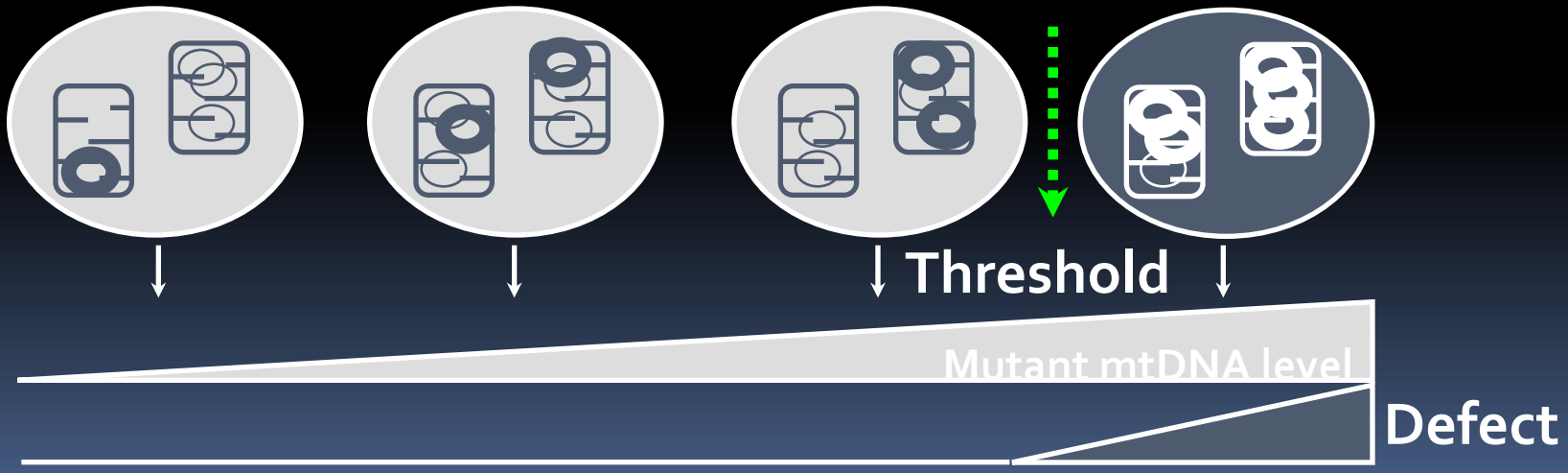
NADH-ubiquinone oxidoreductase (complex I).
Both nuclear gene defects and mtDNA mutations

(other complexes of respiratory chain)

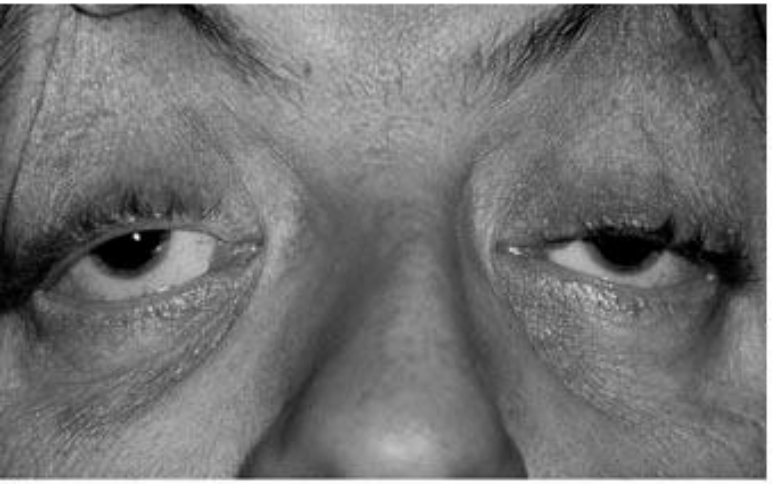


Threshold efekt

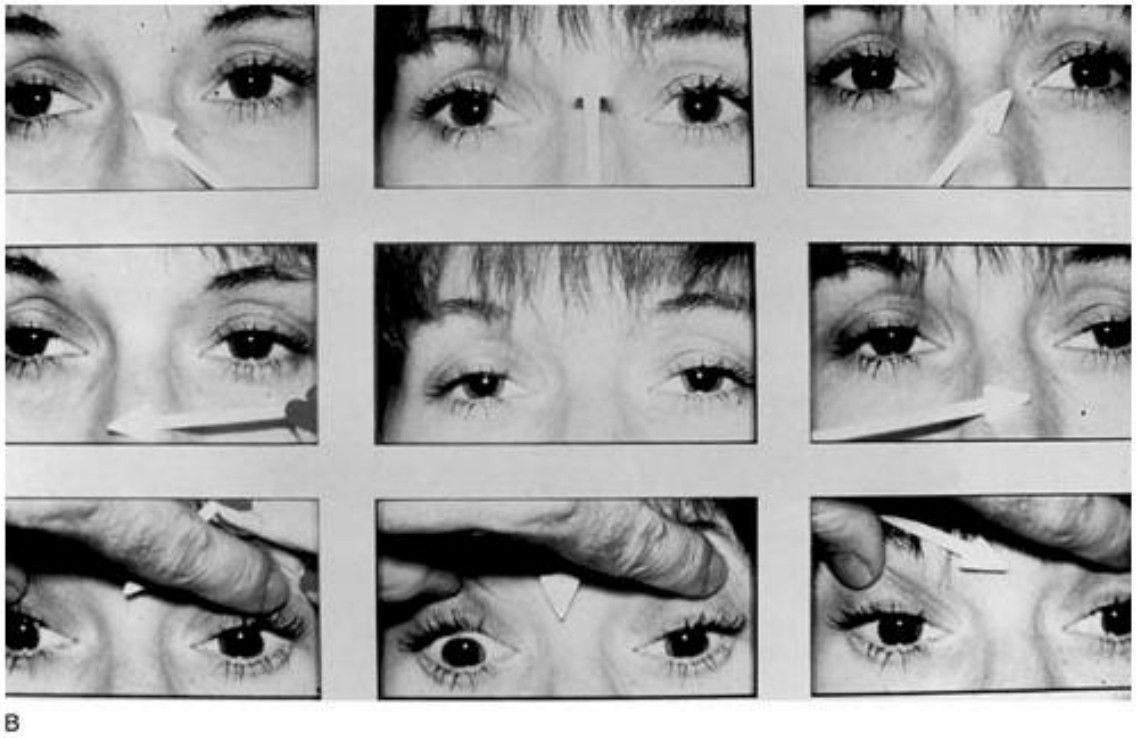
mut mtDNA	31 %	82 %	93 %	> 95 %
onset	-	adult	infancy	newborn
symptomes	healthy	ataxy retinopathy	NARP syndrome	Leigh syndrome



CPEO – Chronic progresive external oftalmoplegy



A



B

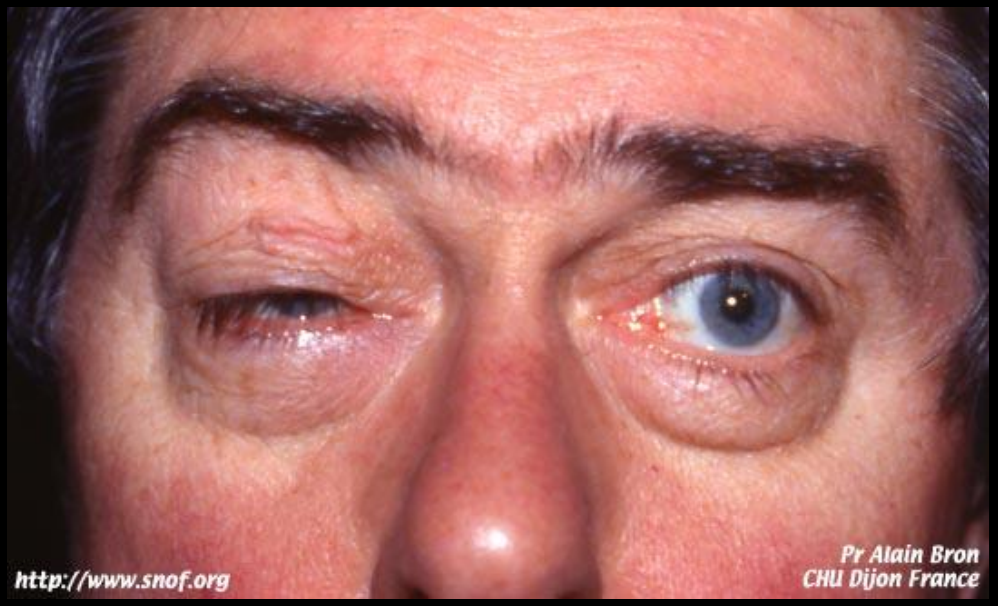
Point mutations in mtDNA

Table 5.2 MtDNA point mutations associated with CPEO

Gene	MtDNA mutation	Reference
$tRNA^{Leu(UUR)}$	A3243G	21
$tRNA^{Ile}$	T4274C	142
	T4285C	143
	G4309A	144
$tRNA^{Asn}$	A5692G	145
	G5703A	146
$tRNA^{Leu(CUN)}$	T12311C	147
	G12315A	22

Abbreviations: tRNA = transfer RNA, Leu = Leucine, Ile = Isoleucine, Asn = Asparagine.

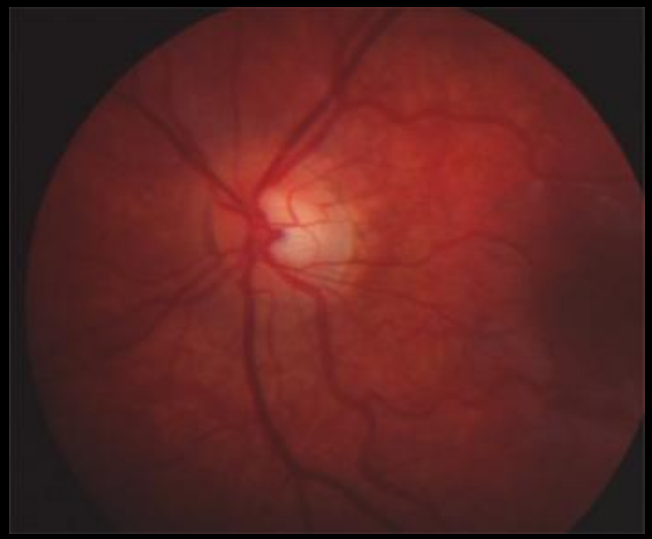
Kearns-Sayre syndrome



http://www.snof.org/maladies/kearnsSayre.html

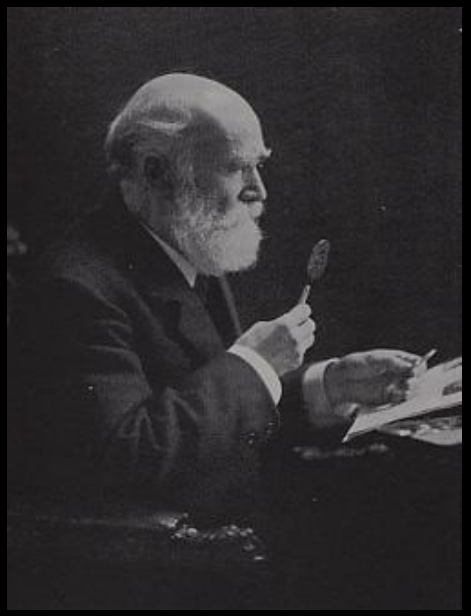
Ophthalmoplegia, ptosis, and mitochondrial myopathy prior to age 20
additional symptoms: retinitis pigmentosa and at least one of the following: cardiac conduction defects, cerebellar ataxia, or elevated cerebral spinal fluid protein above 100 mg/dl.
Commonly caused by mtDNA deletions

Leber hereditary optic neuropathy-LHON



http://www.snof.org/maladies/leber.html

Theodore Leber

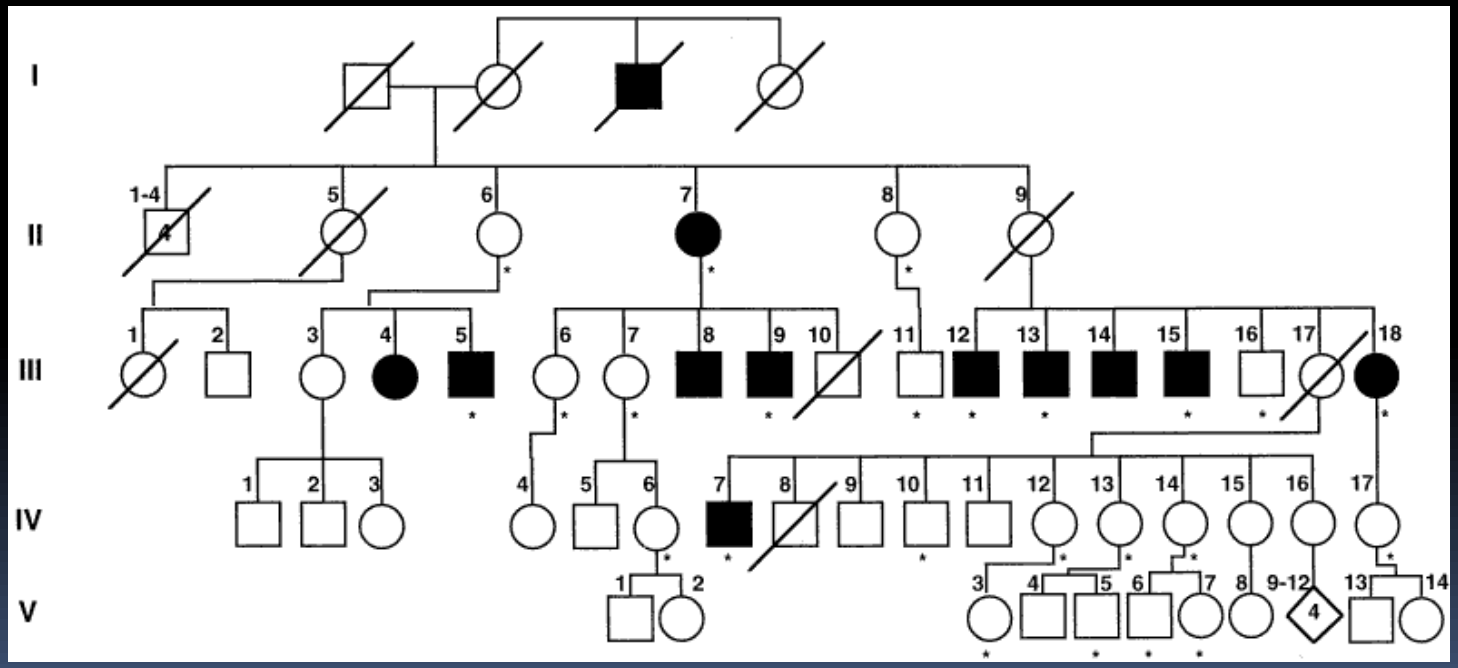


LHON is a **maternally inherited, late-onset, acute, optic atrophy.** In some families also there is also optic neuritis.
Incomplete penetrance (40% males, 10% females develop symptoms)
Caused by homoplasmic missense mutations in mtDNA (complex I).

More than 90 percent of European and Asian LHON cases result from three mtDNA missense mutations.

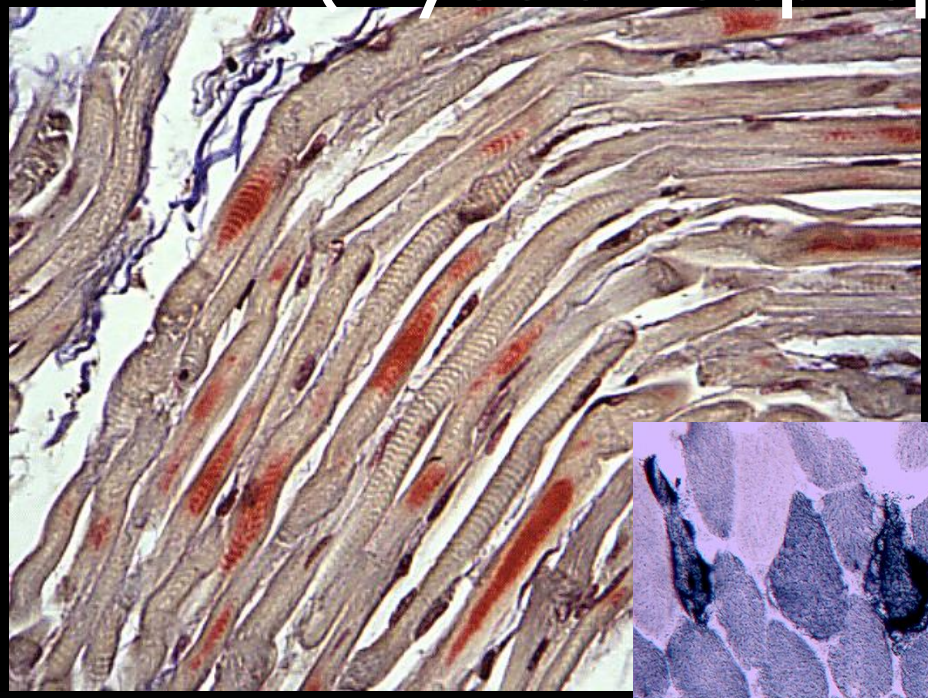
G to A mutation in the MTND4 gene at nucleotide 11778 (MTND4*LHON11778A)
about 50 percent of European cases and about 95 percent of Asian LHON patients.
MTND1*LHON3460A (ND1 Ala52Thr) and MTND6*LHON14484C (ND6 Met64Val) .
A number of rare mutations also appear to cause LHON.

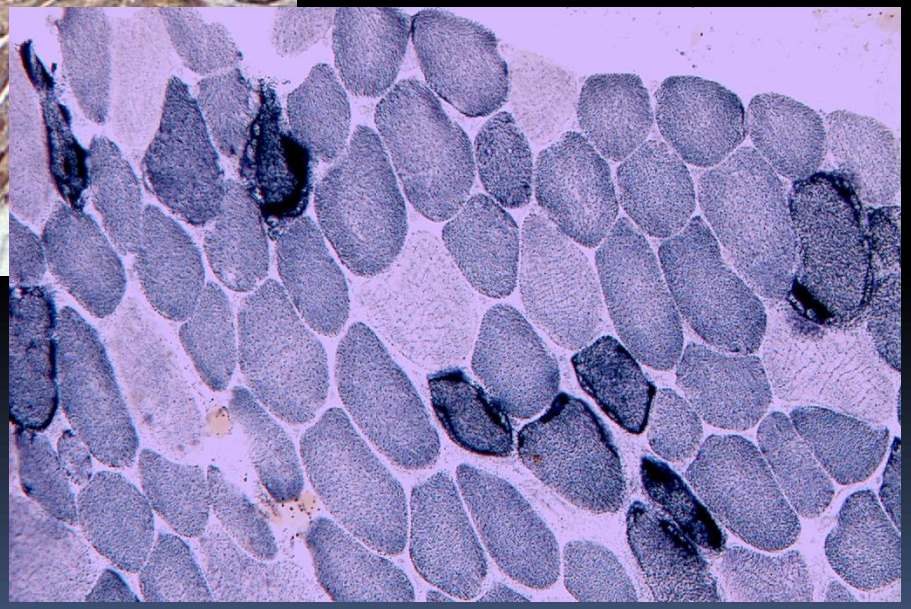
LHON pedigree - maternal inheritance



MtDNA defects– MERRF (myoclonic epilepsy, RRF)

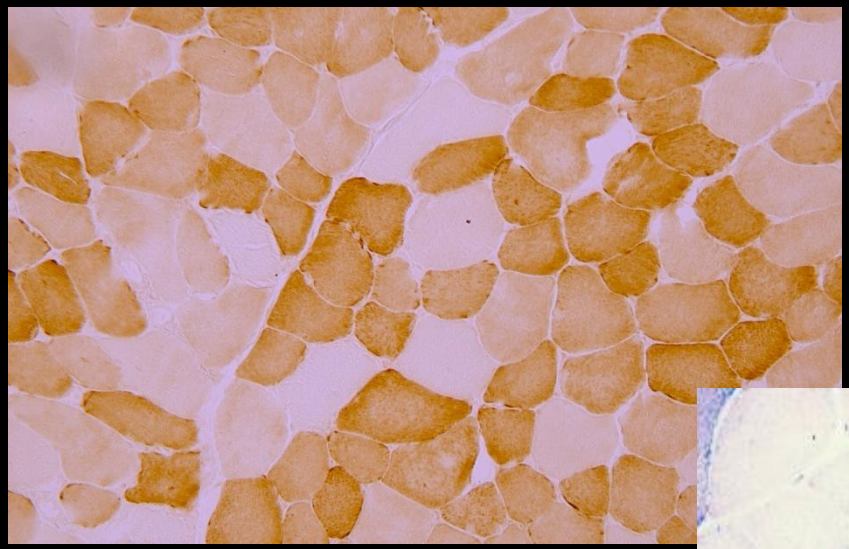
Muscle biopsy





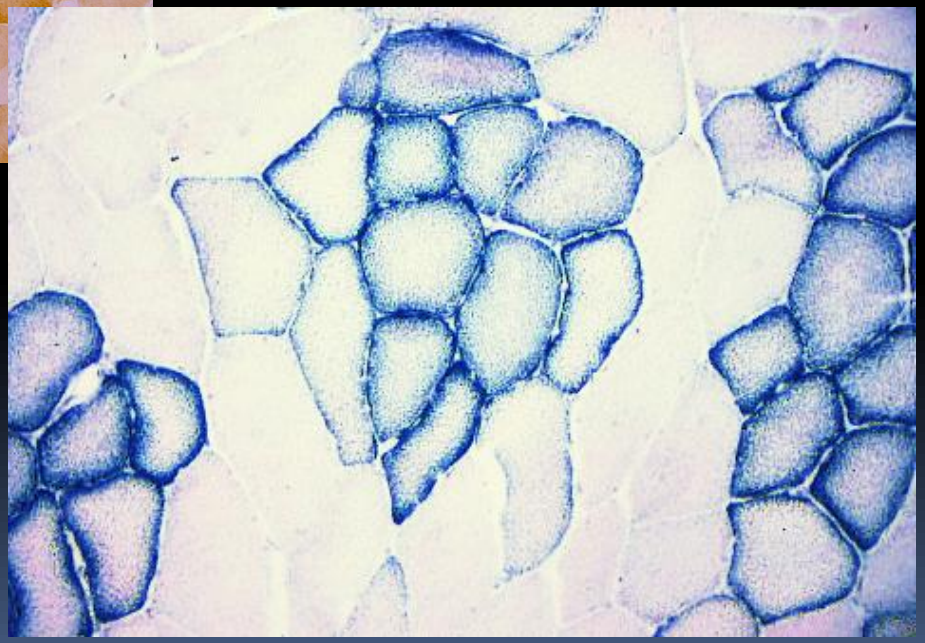
Ragged red fibres

MtDNA defects
Muscle biopsy



COX negative fibres

Subsarcolemal
accummulation of SDH



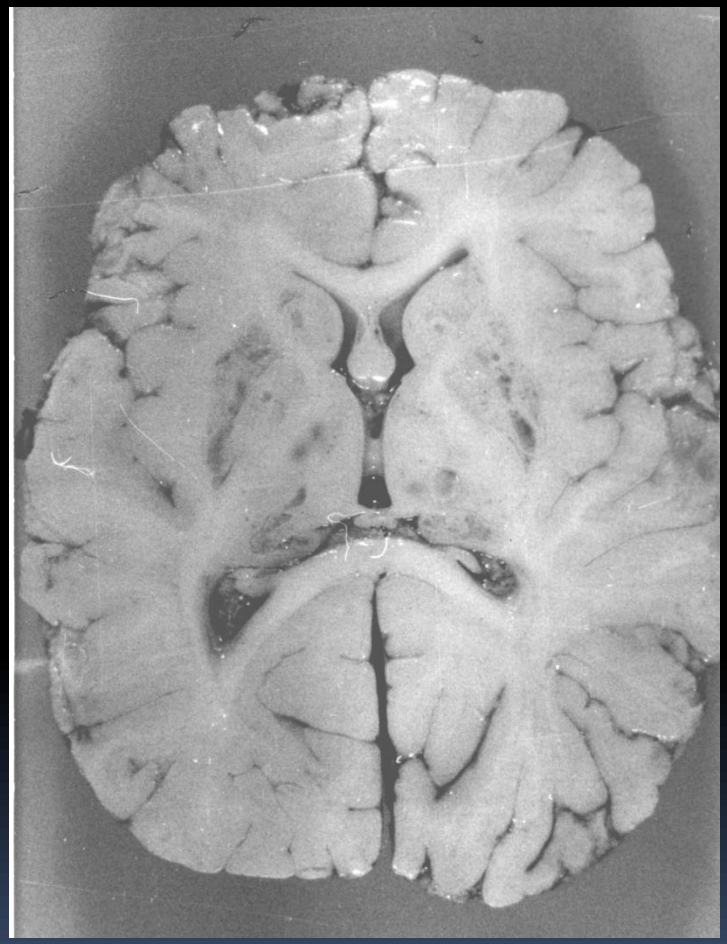
Nuclear defects of the mtDNA

nuclear DNA - replication, transcription, translation, repair
assembly or structural proteins…

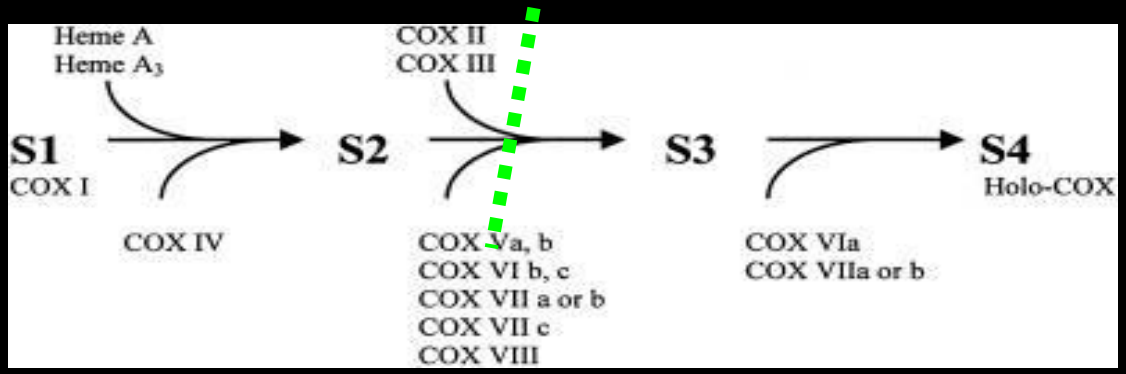
- defects in genes for structural subunits
  - complex I – Leigh syndrome, cardiomyopathy, encefalomyopathy, myoclonic epilepsy
  - complex II – ataxy, optical atrophy - heredital paraganglioms
- defects in assembly proteins
  - COX – SURF-1, SCO_2, SCO_1, COX_{10}, COX_{15}, …
  - ATPase – ATP_{12}, $TMEM_{70}$

Leigh syndrome

- SURF1 defect (assembly protein)
- severe neurodegenerativ disease
- newborn, infancy
- basal ganglia necrosis
- fatal prognosis

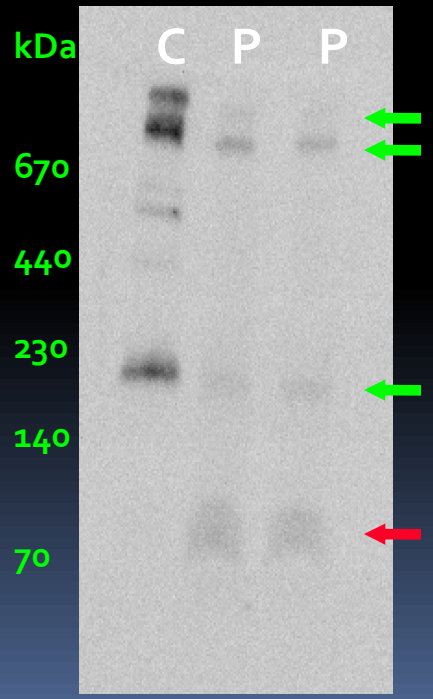


COX (cytochrom c oxidase) deficiency due to Surf1 protein defect



Non-complet froms of COX

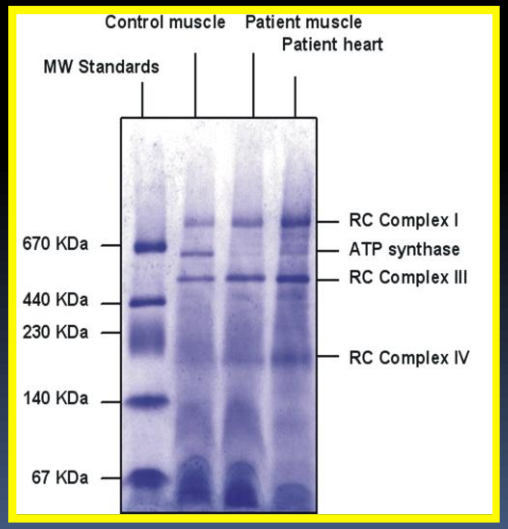
- absence of regulating nc-encoded subunits
- Labilita of subcomplexes
- H+transport defect



ATPase defects – nuclear origin

TMEM70 protein

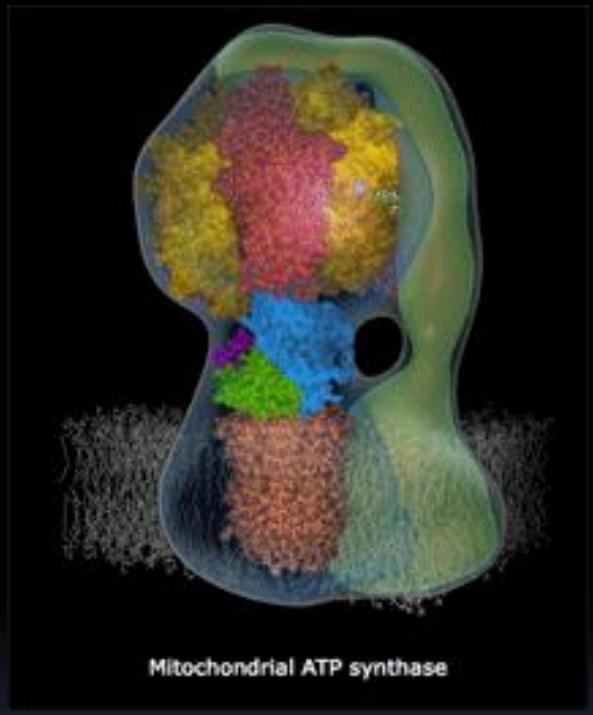
Newborn onset	14/14
Death	7/14 (4; 3)
Alive (≥3;≥5;≥10 let)	7/14 (3;2;3)
Cardiomyopathy	13/14
Hypotony	12/13
Psychomotor retardation	10/10
Hepatomegaly	6/14
Facial dysmorphy	5/14
Hyperlactacidemia	14/14
3-methylglutakon.aciduria	12/12
ATP hydrolysis <30%	13/13
ATP production <30%	4/4
Decrease of ATPase level	13/13



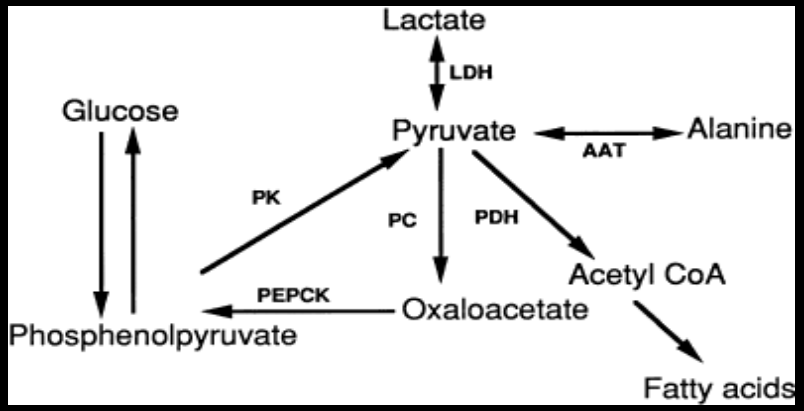
Sperl, Ješina et al, *Neuromuscul.Disord.*, 2006

ATPase defects

Mutations in mtDNA		in nuclear DNA
„qualitative“		**„quantitative“**
Structural modified ATPase complex with dysfuntion	**X**	Decreased content of ATPase
Maternally inherited	**X**	Mendelian inherited
neurologic symptomatology	**X**	cardiomyopathy



Disorders of mitochondrial pyruvate metabolism and citric acid cycle



Pyruvate dehydrogenase deficiency
Dihydrolipoamid dehydrogenase , E3 subunit of PDH, multiple 2-oxo acid dehydrogenase deficiency : PDH, 2-ketoglutarte deficiency, branched-chain 2 oxo-acid deficiency
Pyruvate carboxylase deficiency
Phosphoenocarboxykinase deficiency
Fumase deficiency, - In heterozygotes: predisposition to leiomyomas of skin and uterus, kidney carcinoma
Succinate dehydrogenase deficiency
Pyruvate transporter deficiency

Lactic acidosis, progressive course, frequent neurological symptoms, muscle symptoms
Autosomal recessive disorders, deficiency of α-subunit of PDHE1 is X-linked

Pyruvate dehydrogenases complex

Pyruvate → acetyl-CoA

Dehydrogenase component :E1, subunit α is X-linked
PDHE1α
Psychomotor retardation, ataxia, seizures
Phenotypes:
neonatal lactic acidosis,
Leigh encephalopathy : abnormal breating, apnoe, , ataxia, muscle, developmental delay,
Females: facial dysmorphy, seizures, subscortical and cortical atrophy,
Deficiencies of other subunits are rare

Lactic acidosis, increase of lactate after meals, during fasting lowering of lactate levels

Treatment : ketogenic diet, thiamin, dichloroacetate(inhibition of pyruvate kinase)

Unfavourable prognosis

Defects of nuclear DNA

nuclear DNA - replication, transcription, translation, repair …
assembly, structural subunits…

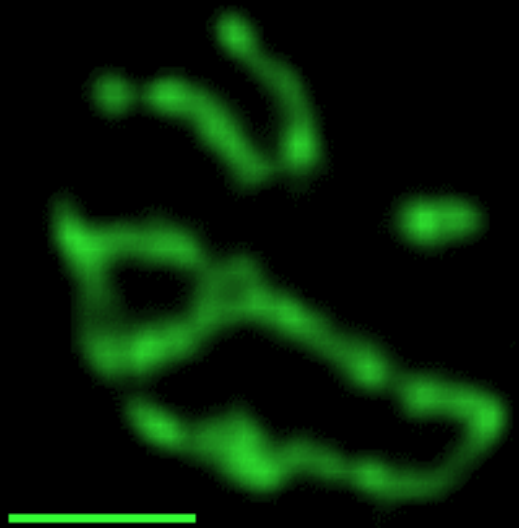
- Integrity and replication of mtDNA – thymidin fosforylase
  ANT1, twinkle, polymerase γ (POLG)
  MNGIE (mitochondrial neuro-gastro-intestinal encephalopathy);
  Alpers-Huttenlocher syndrome

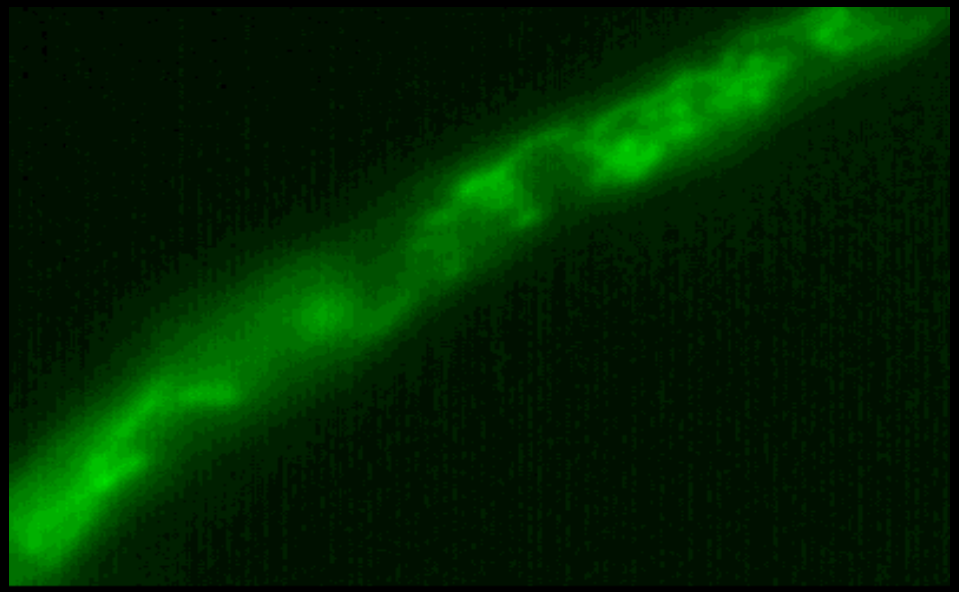
- Transport of proteins

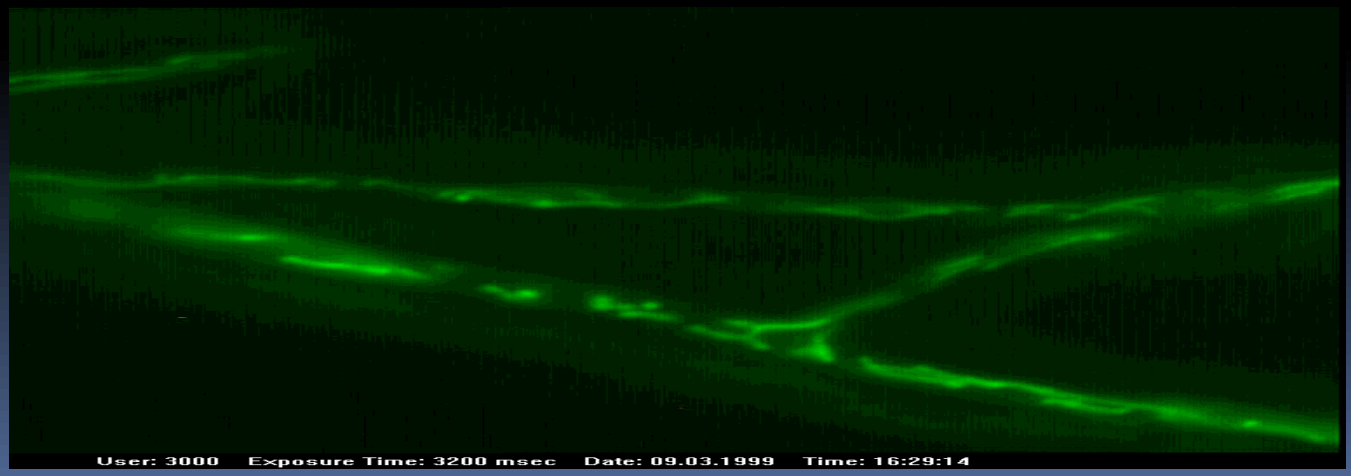
- Fission / fuse of mitochondria – OPA1

- Stability of mitochondrial membrane – Barth syndrome

Mitochondrial fusion and fission







Disorders of mitochondrial-beta oxidation of fatty acids

Carnitine cycle
Beta oxidation
Electron transfer to complex II (glutaric aciduria type II)
Synthesis of ketone bodies, ketolysis

Beta oxidation deficiencies:

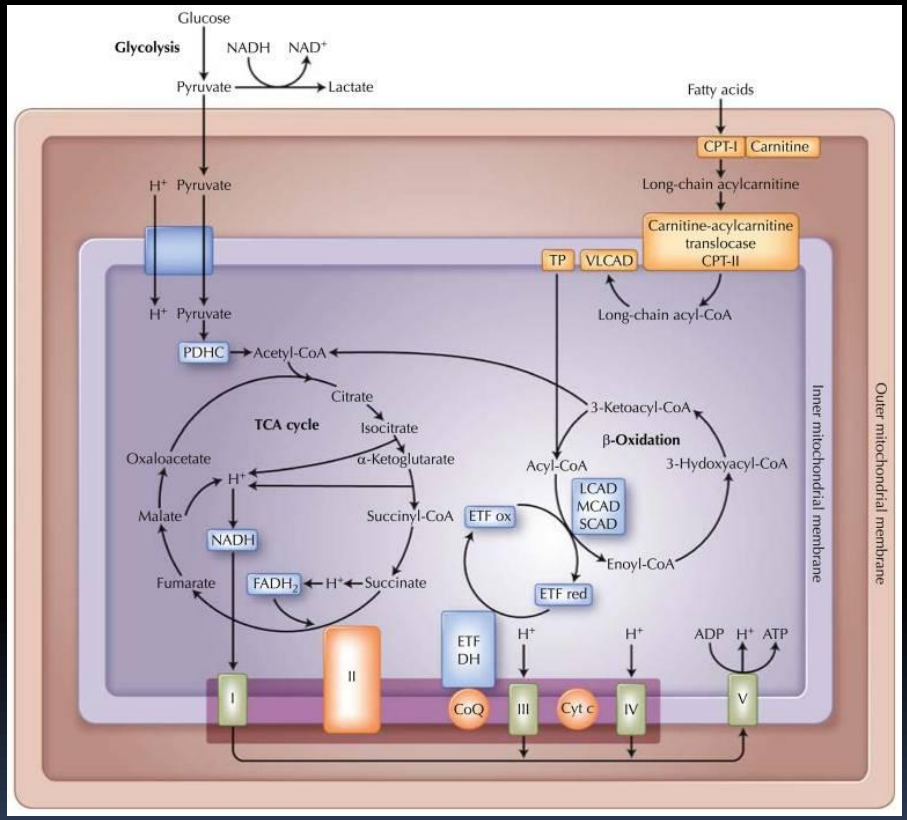
Symptoms often develop after fasting (12-16h)
Hypoglycemia
Low ketones

(In some disorders muscle weakness, rhabdomyolysis ,cardiomyopathy)

Disorders of mitochondrial-beta oxidation of fatty acids

- Disorders of FA trasport
- Disorders of b-oxidation

- Cardiomyopathy
- Hepatopathy
- Nonketot.hypoglycaemia
- Myopathy



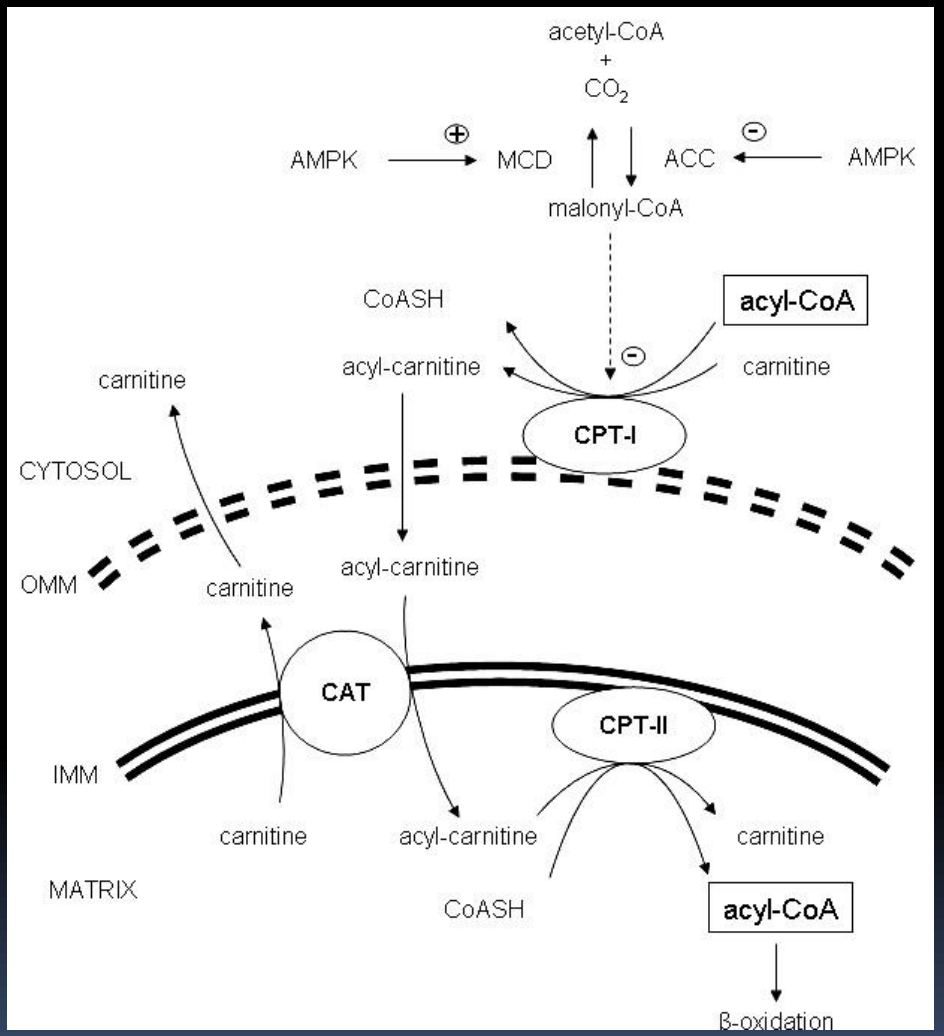
www.annualreviews.org (Bennett MJ, Fatty acid oxidation disorders, 2002)

Carnitine cycle

Long chain free fatty acids are "activated" to acyl-CoA esters in cytosol and are imported to mitochondria via carnitine cycle

Medium- and shortchain fatty acids are imported to mitochondria directly and are activate to CoA esters in mitochondrial matrix

CPT1 - carnitine palmitoyl transferase I
CPT2 - carnitine palmitoyl transferase II
CACT – carnitine /acylcarnitine translocase



Disorders of mitochondrial-beta oxidation of fatty acids

- CPT I – cardiomyopathy, arytmia, liver dysfuntion
- CPT II –*Mild adult form:* attacks of rhabdomyolysis after exercise, fasting old cold. Myoglobinuria.
  Severe neonatal form: coma, cardiomyopathy, muscle weakness, congenital malformations of brain and kidneys
- **CACT – carnitine /acylcarnitine translocase :** Hypoketotic hypoglycemia after fasting, coma, arrythmias, apnoe, often death in early infancy

Disorders of mitochondrial-beta oxidation of fatty acids

- VLCAD – infant.form – SIDS
  - late onset – rhabdomyolysis, hypotony
- LCHAD a MTP (trifunctional protein) – cardiomyopathy, hepatopathy, retinopathy, rhabdomyolysis; neuropathy, Reye-like epizodes
  - HELLP (Hemolysis, Elevated Liver enzymes, Low Platelets) syndrome in pregnancy
  - dg – OH-acylcarnitine
  - th – diet, frequent meals, avoid fasting

Disorders of mitochondrial-beta oxidation of fatty acids

- MCAD – most common – 1:6000 in Europe
  - prevalent mutation p.K329E
  - newborn screening
  - clinical - Reye like (after fasting, during illness)
    vomiting, lethargy, seizures, cardiac arrest, hypoglycaemia, hyperamonneamia
    no primary muscle involvement
    frequently asymptomatic (p.Y67H)
  - laboratory – C6 and C8acylcarnitine, dicarboxylic aciduria, glycine conjugate
  - therapy – avoidance of fasting

Disorders of mitochondrial-beta oxidation of fatty acids

- SCAD – possible non disease

  mild myopathy

- Glutaric aciduria II type – (ETF) – deficient electron transport from FAD dehydrogenases to respiratory chain

  severe form (Reye syndrome, hypoglycemia, progr.encephalopathy, (cardio)myopathy)

  mild myopatic form

  cystic renal disease

  laboratory – hypoglycemia, metabolic acidosis, elevated lactate

Disorders of mitochondrial-beta oxidation of fatty acids

- Main symptoms - chron. Weakness, hypotony, exercise intolerance, rhabdomyolysis

- Fasting, infection, surgery, other catabolic state – trigger rhabdomyolysis
- Wide clinical spectrum – acute x chronic
  - infantil x late onset
  - isolated (myopathy) x multisystem.impairment (cardiomyopathy, encefalopythy)

Thank you.