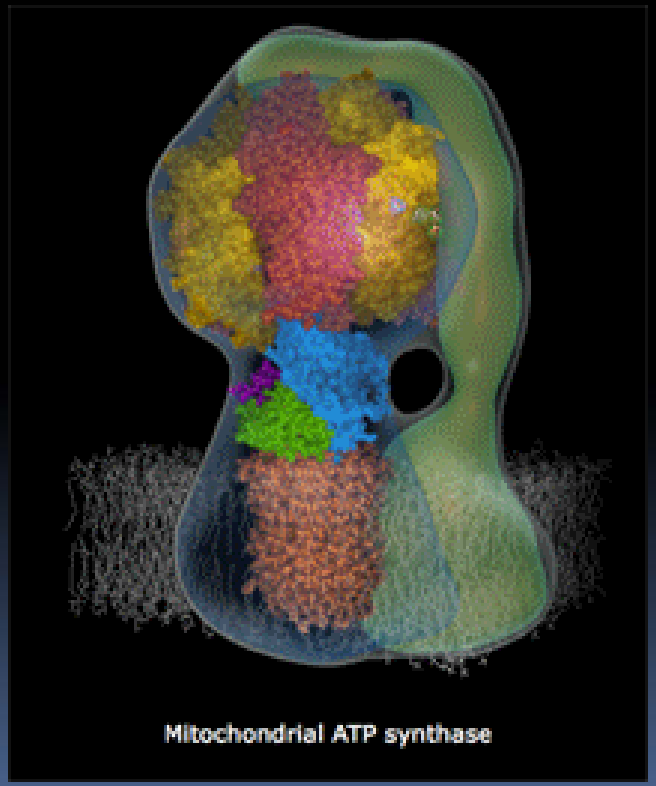
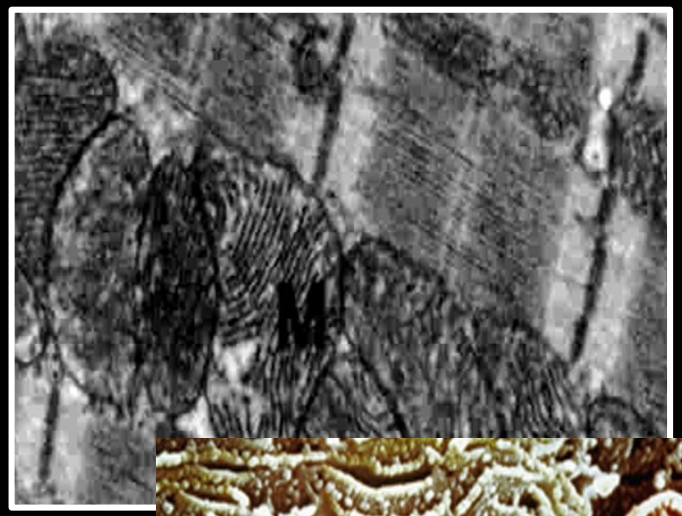
Mitochondrial disorders
Defects of fatty acid metabolism
Pathobiochemistry of fasting

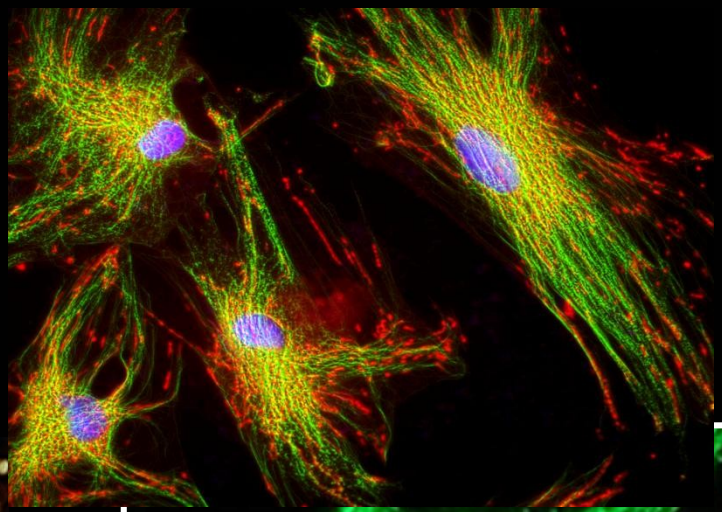
Pavel Ješina

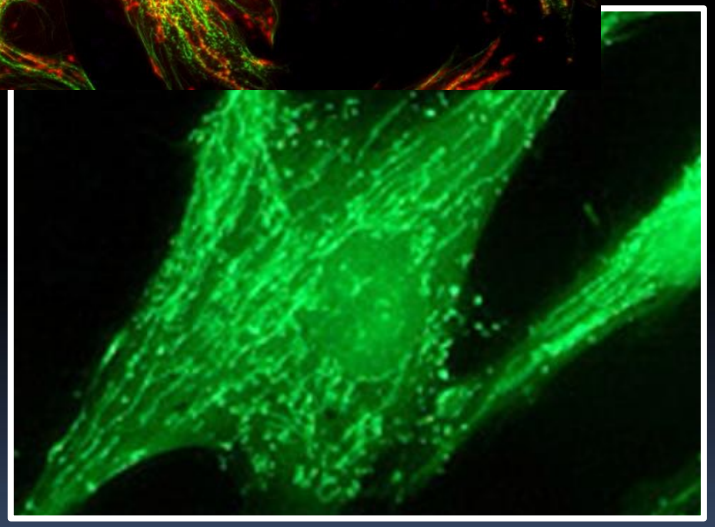
Institute of Inherited Metabolic Disorders
1.LF



Mitochondria - structure

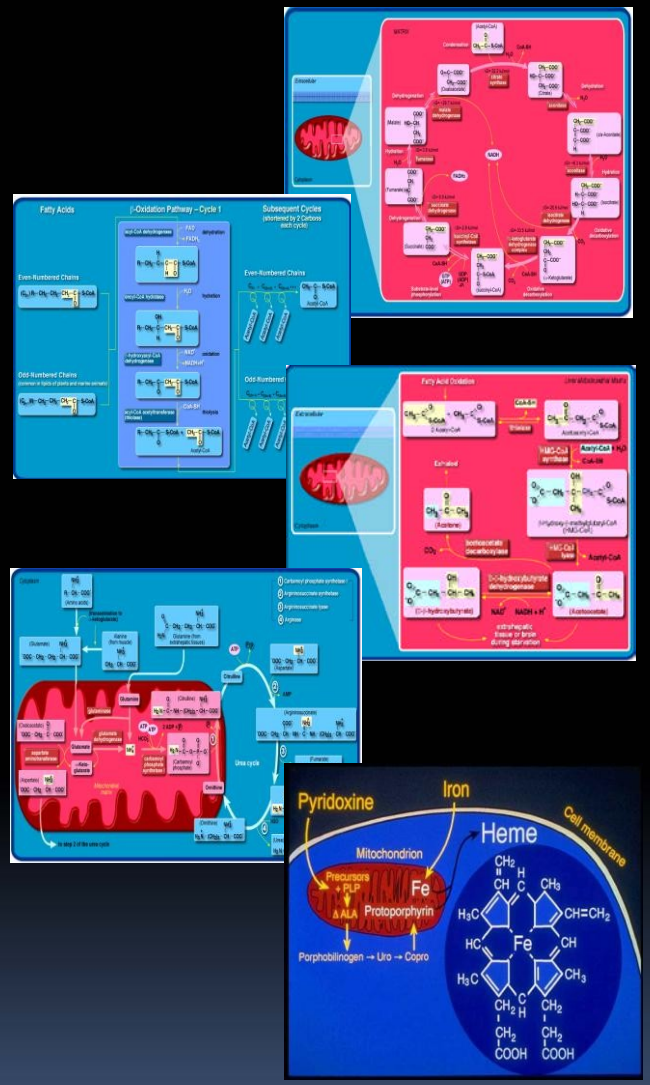






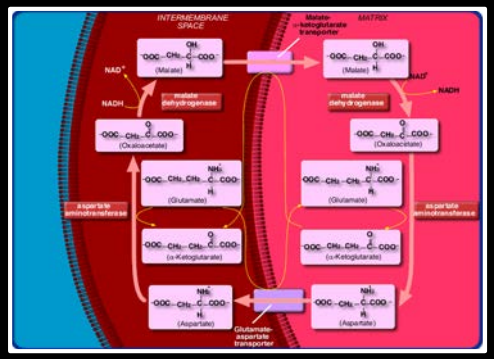
Metabolic pathways in mitochondria

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

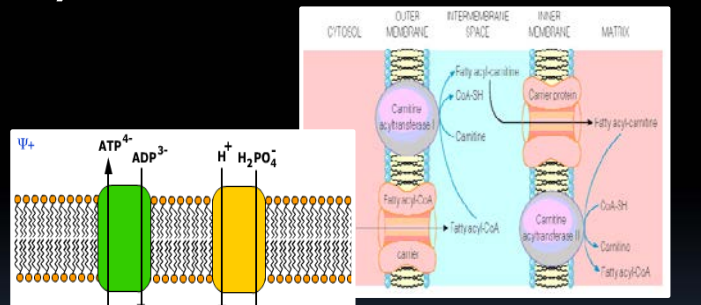


Mitochondriální transportní systém

- cykly pro přenos transport $NADH.H^+$
  - malát-aspartátový cyklus
  - glycerolfosfátový cyklus

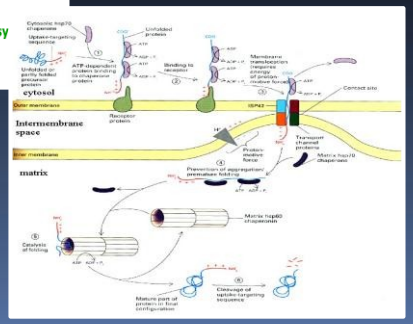


- přenašeče pro substráty a produkty metab. drah probíhajících v mitochondriích



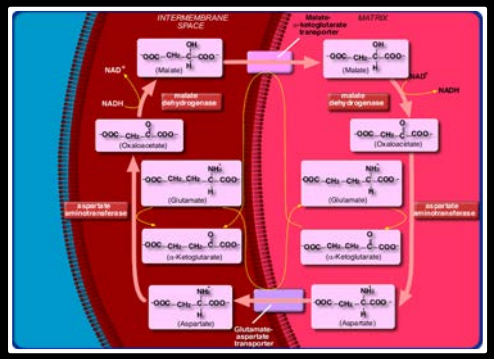
- ADP-ATP transokátor

- import proteinů

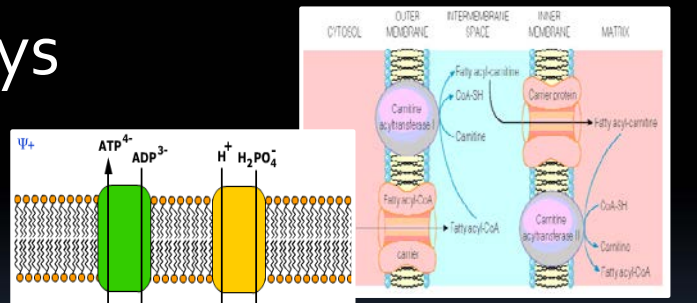


Mitochondrial transport system

- Shuttles for NADH.H⁺ transport
  - malate-aspartate shuttle
  - glycerolphosphate shuttle

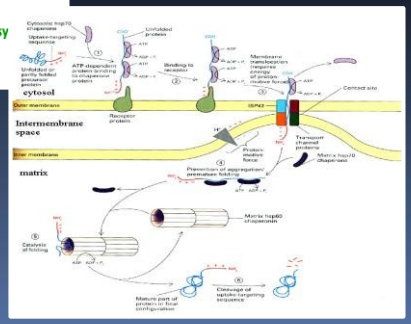


- Transport for substrates and products of mitochondrial metabolic pathways

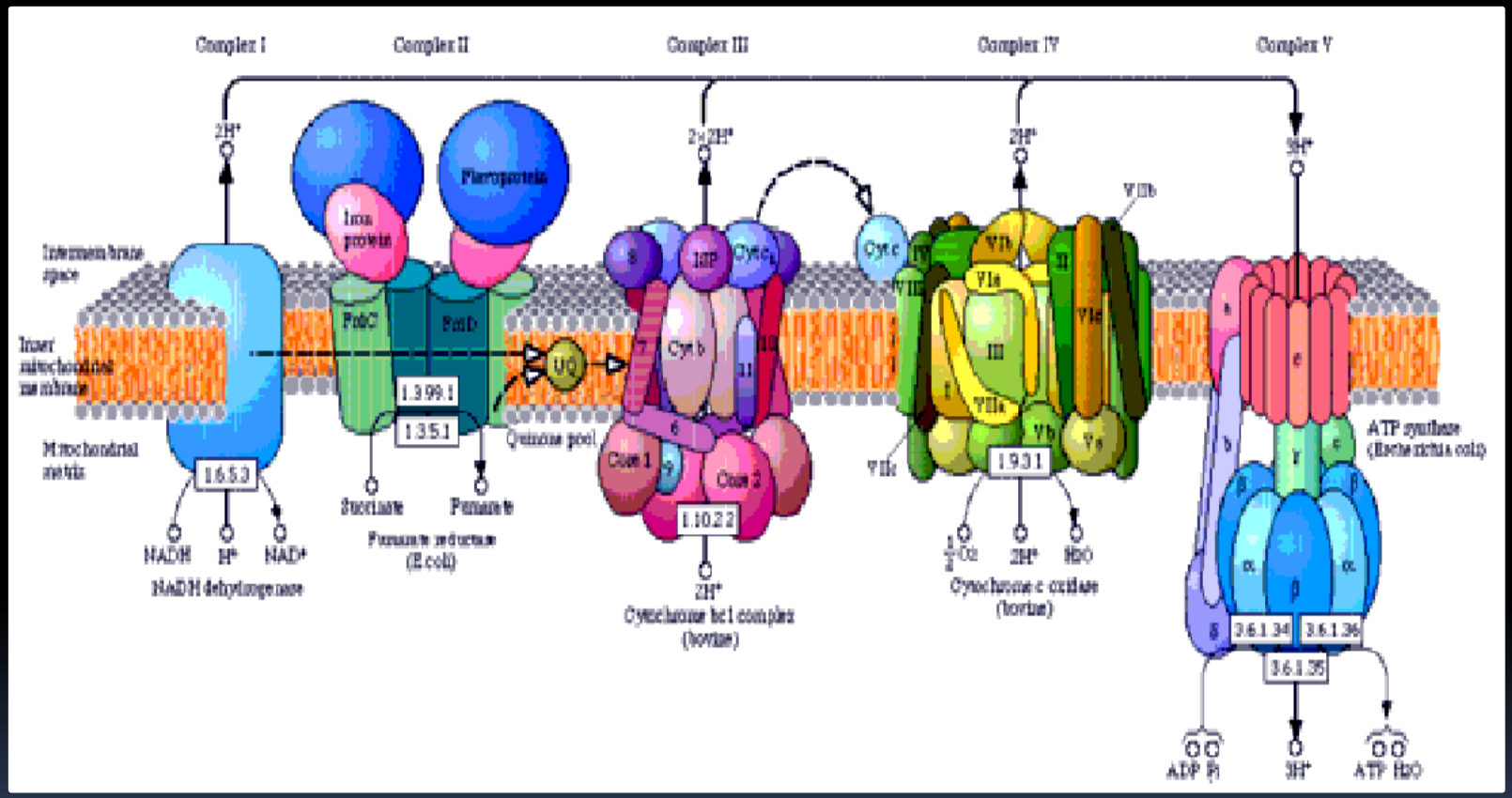


- ADP-ATP translocator

- Protein import



Oxidative phosphorylation



> 43 4 11 13 16 subunits

Mitochondrial Energetics

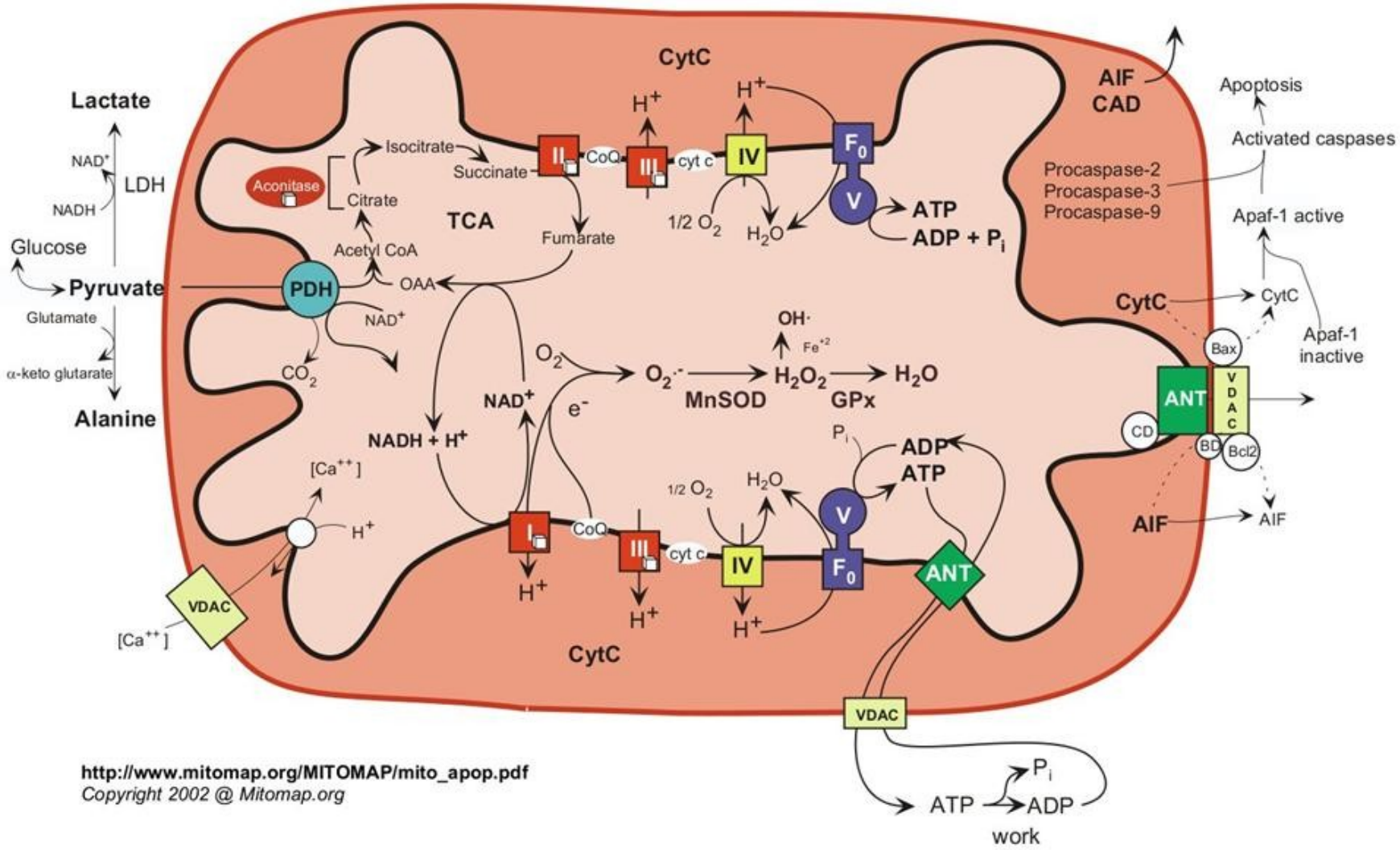


Diagram of the mammalian mitochondrion showing the relationship between energy production, ROS generation, and regulation of apoptosis.

Mitochondrial disorders

- Defects of mitochondrial energy metabolism - OXPHOS
  - Nuclear genes
  - Mitochondrial genes
- Defects of Krebs cycle and pyruvate metabolismu
- Defects of mitochondrial fussion and fission
- Defects of mitochondrial membrane stability

…

Incidence – 1:4000

Genetic of mitochondrial disorders

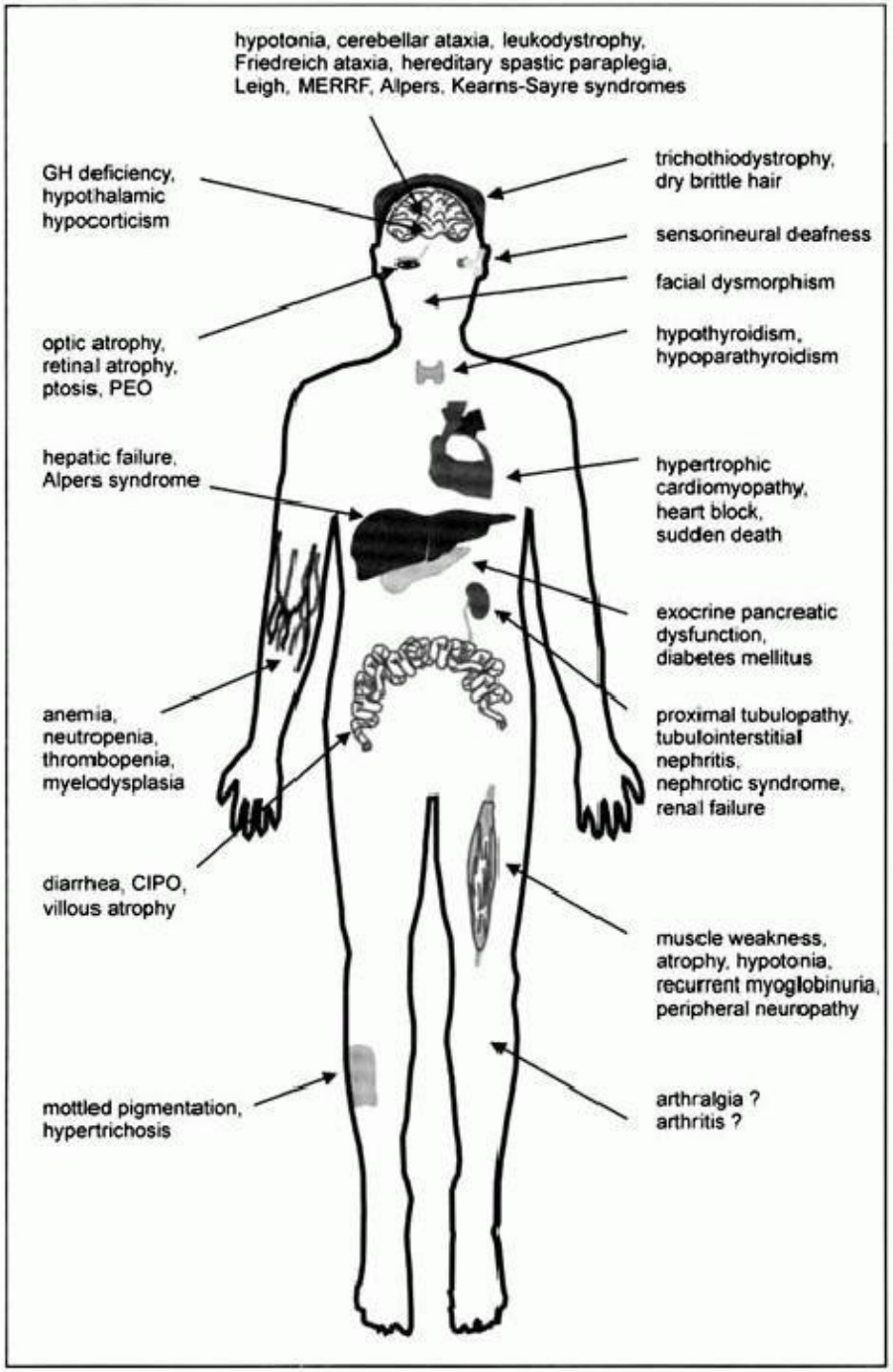
mtDNA

- OXPHOS subunits
- rRNA
- tRNA
- Amount of mtDNA

nDNA

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

OXPHOS defects

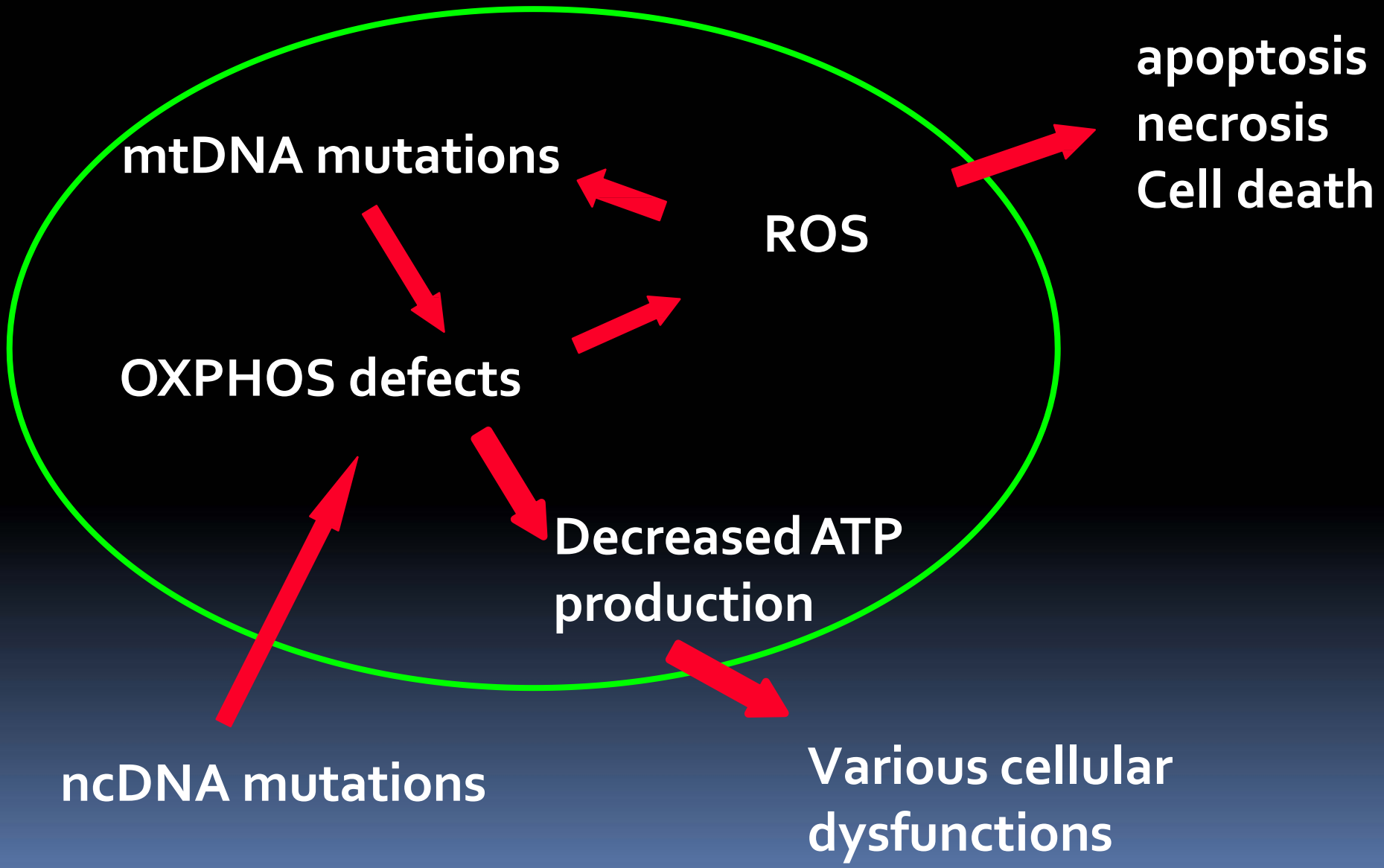


*"**Any symptom, in any organ, at any age**, and with any mode of inheritance"*

Energy dependend tissues – nervous systém, heart, muscle

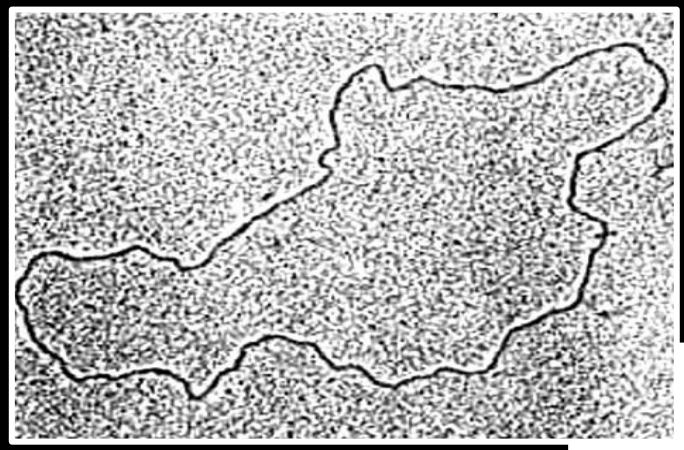
Munnich *et al*, OMMBID, Ch 99

Patogenetic mechanisms



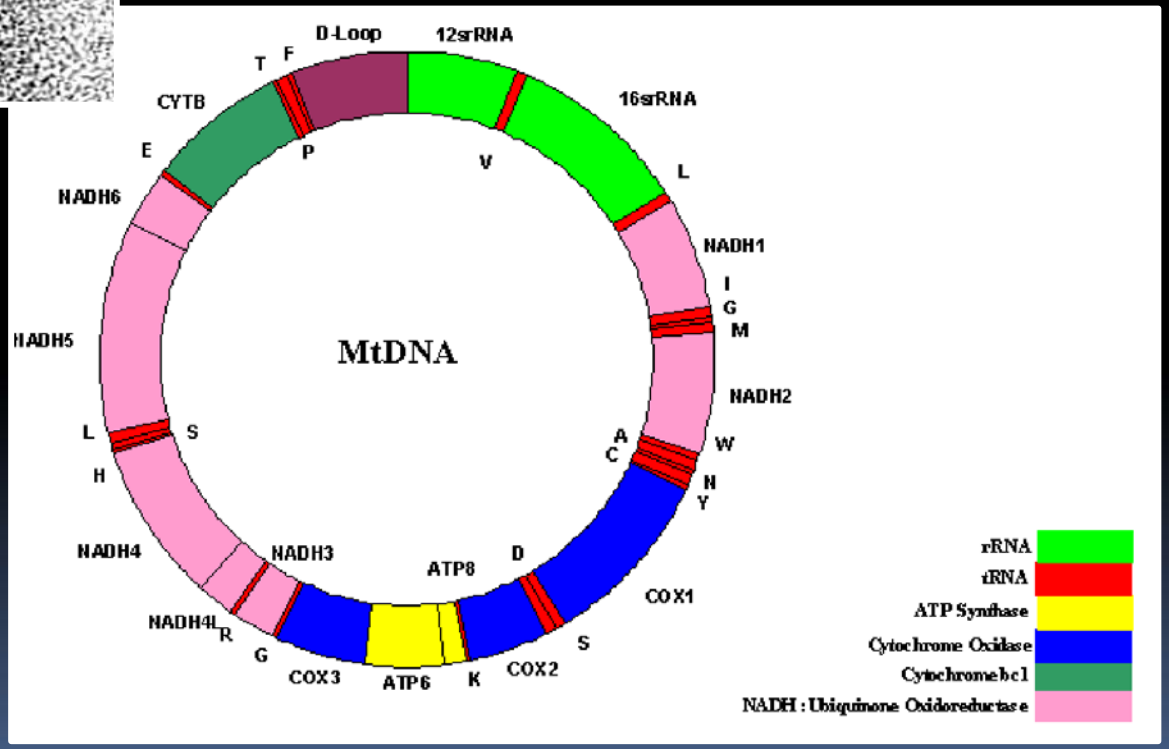
Mitochondrial disorders caused by mtDNA mutations

Mitochondrial DNA



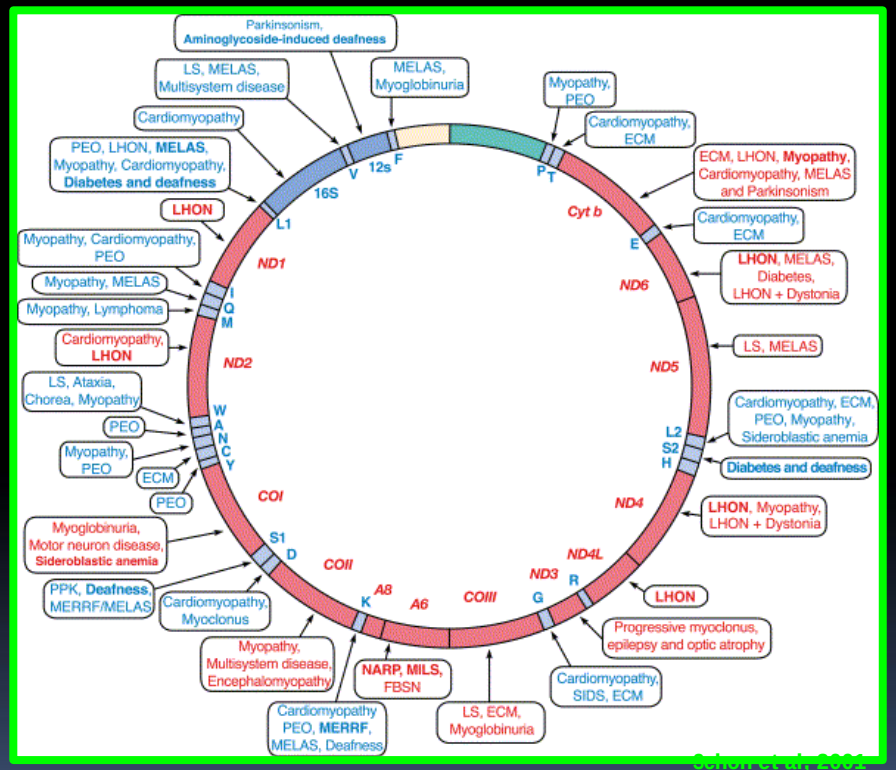
- 16 569 base pairs
- 13 mRNA, 2 rRNA; 22 tRNA

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



Mitochondrial disorders

- Mutation in mtDNA



Schon et al, 2001

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

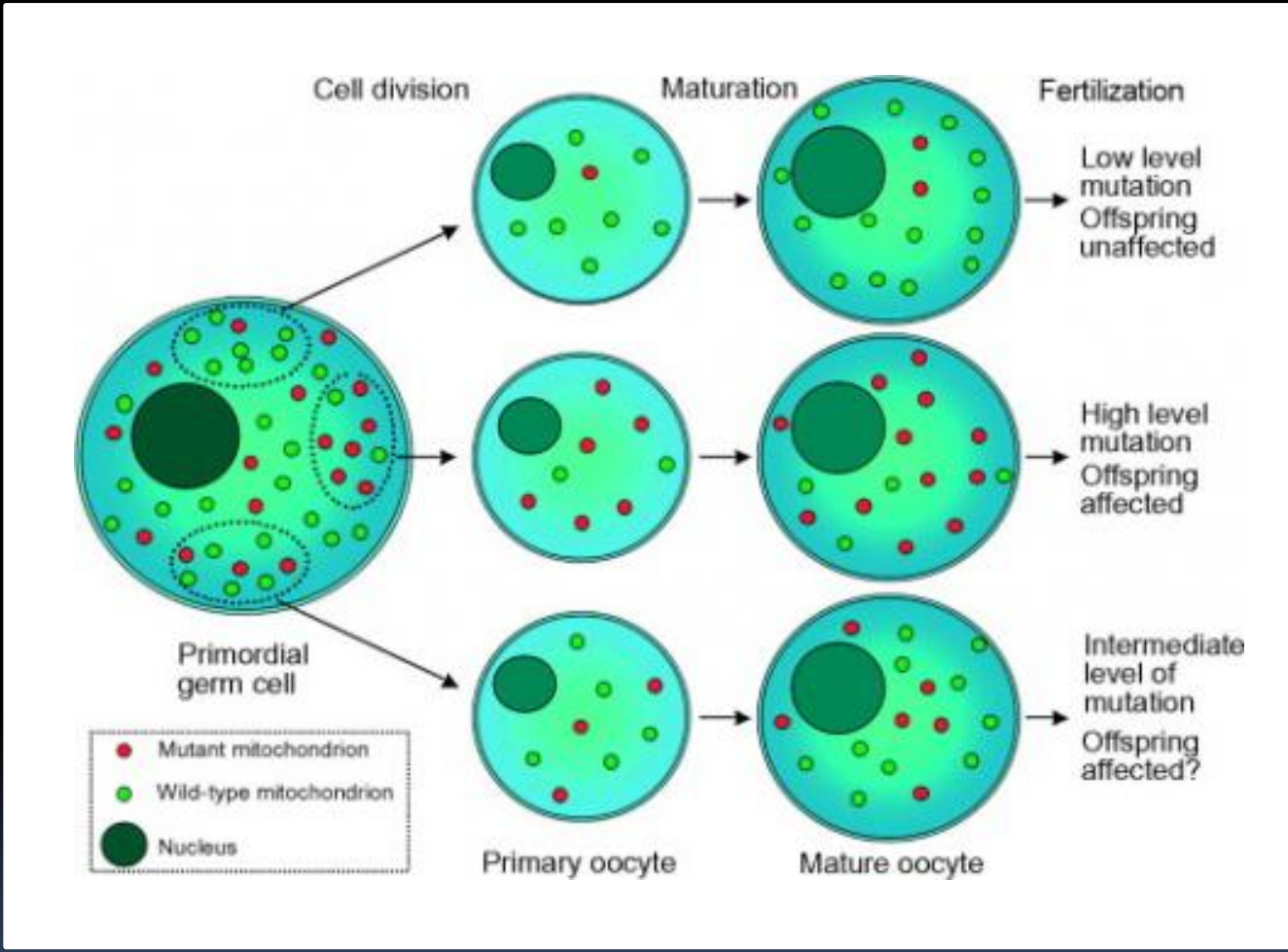
Hereditary of mtDNA mutation

- Sporadic mtDNA defects
  single deletion/duplication

- Maternally inherited mtDNA mutation
  point mutations
  single deletion/duplication

- Autosomal inherited mtDNA mutations
  mulriple deletions
  depletions

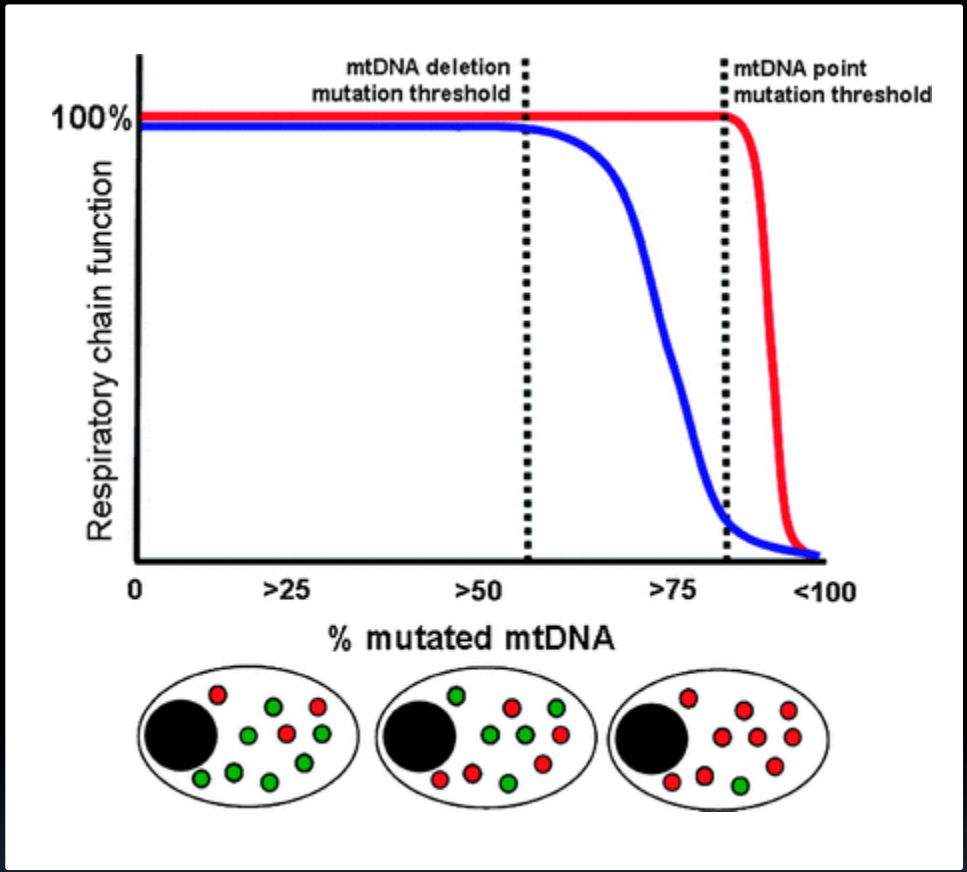
Heteroplasmy



Segregation of mtDNA

Mitochondrial genetic bottleneck

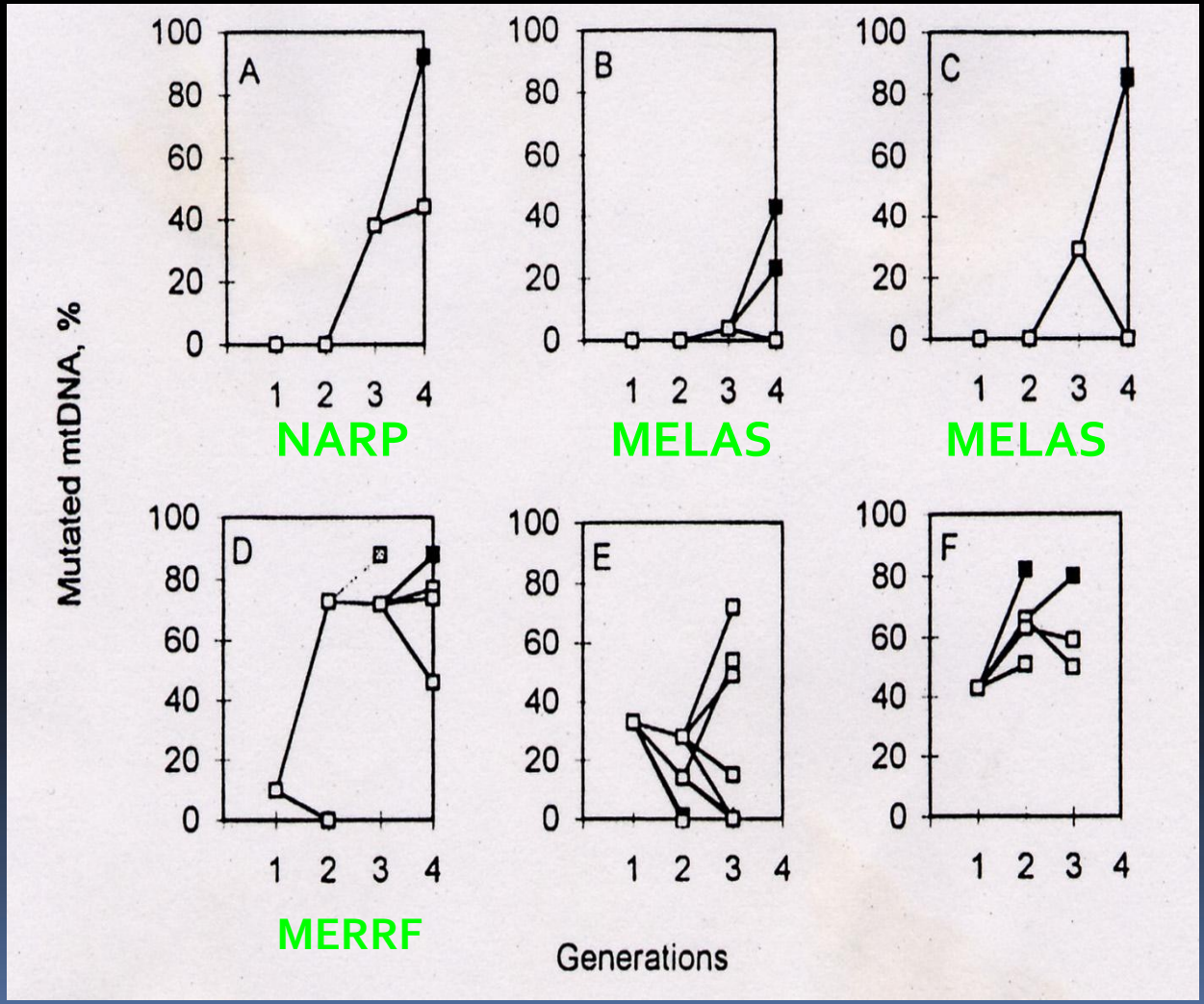
Threshold effect



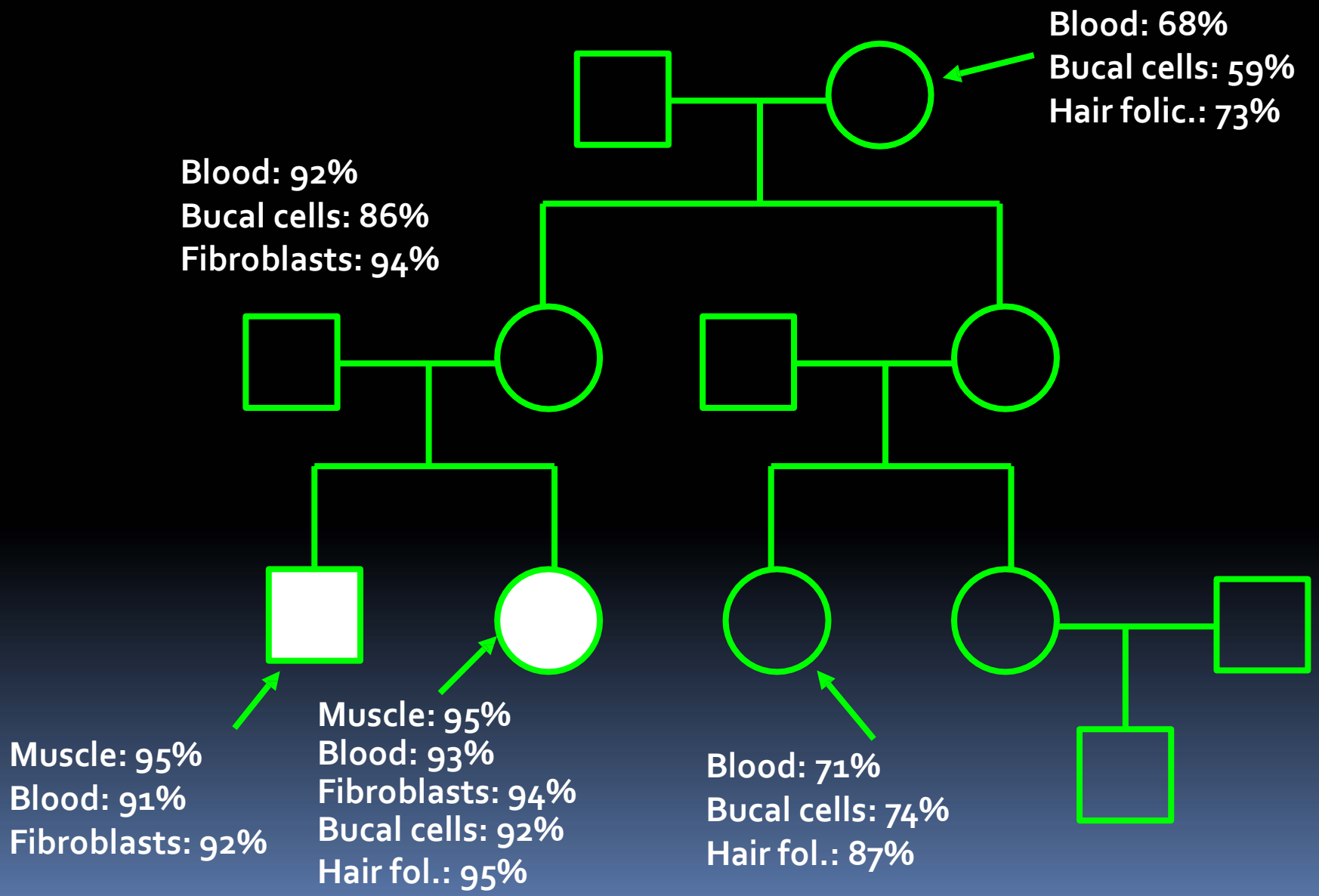
Non-linear relation between heteroplasmy and dysfunction/amount

Tissues heteroplasmy

mtDNA segregation in failies with NARP (8993), MELAS (3243) a MERRF (8344)



7512T>C mutation in mtDNA



Mitochondrial disorders caused by mtDNA mutations

NARP/Leigh syndrome – neuropathy, ataxy, retinitis pigmentosa
- 8993 T>G and others
- psychomotor retardation, lactic acidosis, basal ganglia necrosis

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

-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

.....

Threshold effect – NARP - 8993 T>G

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

Leigh syndrome

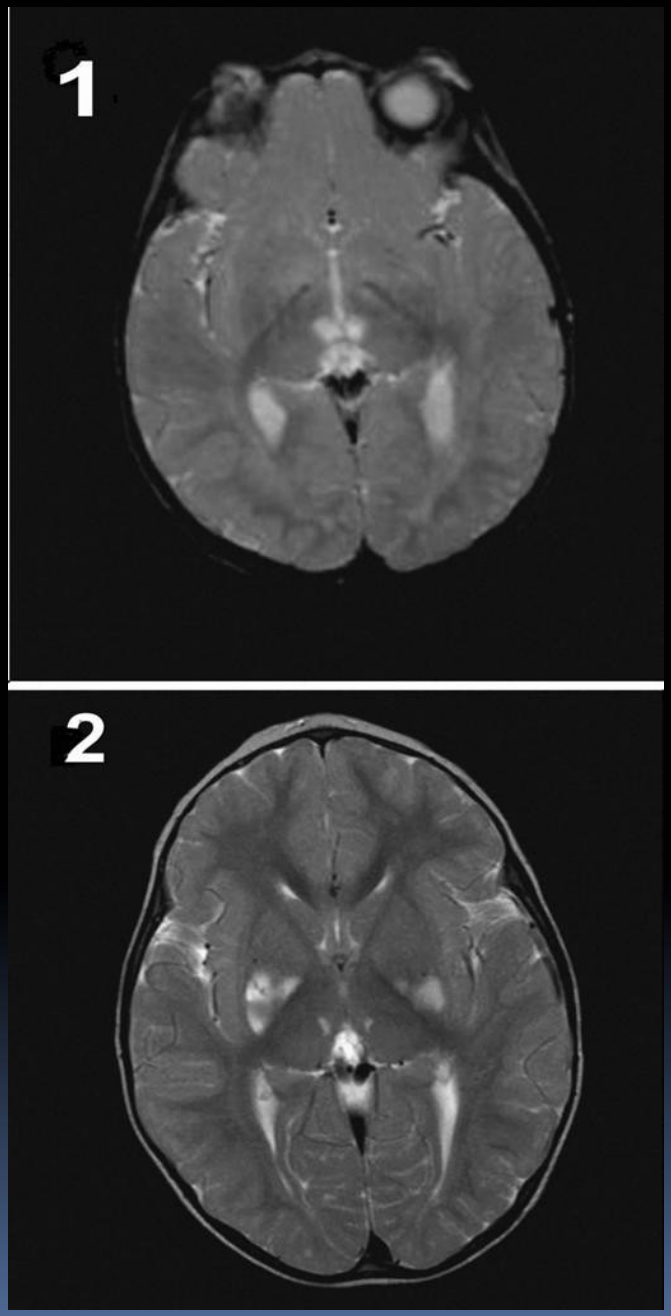
Neurodegenerative disorder usually starting before 1 year of age and leading to death within months or years.

„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:
• ATP synthase – 8993T>G and others
• 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
•O ther complexes of respiratory chain



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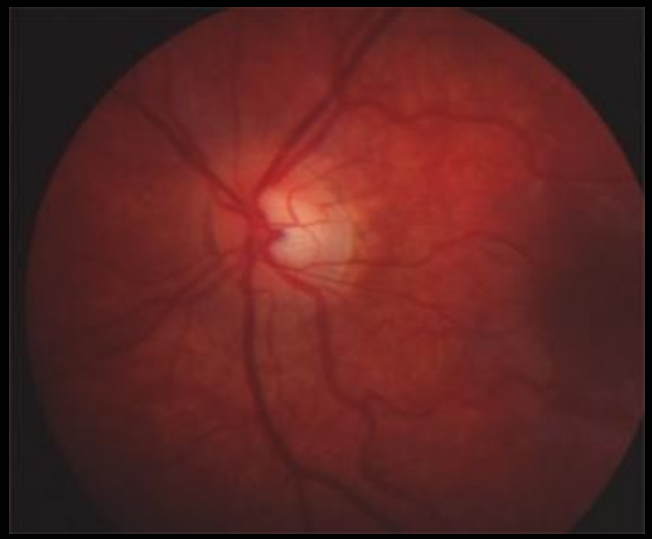
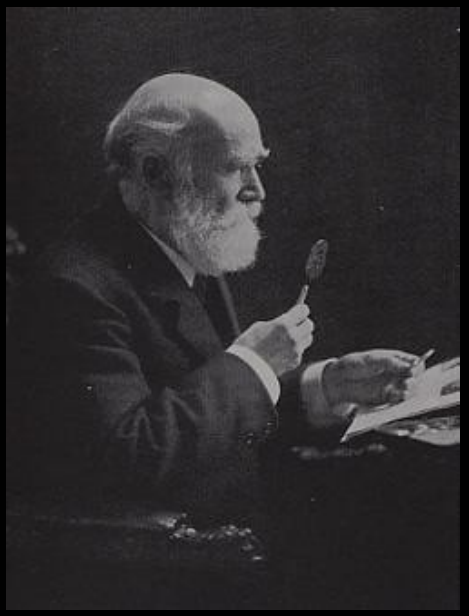
Leber heteditary optic neuropathy - LHON



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

Theodore Leber

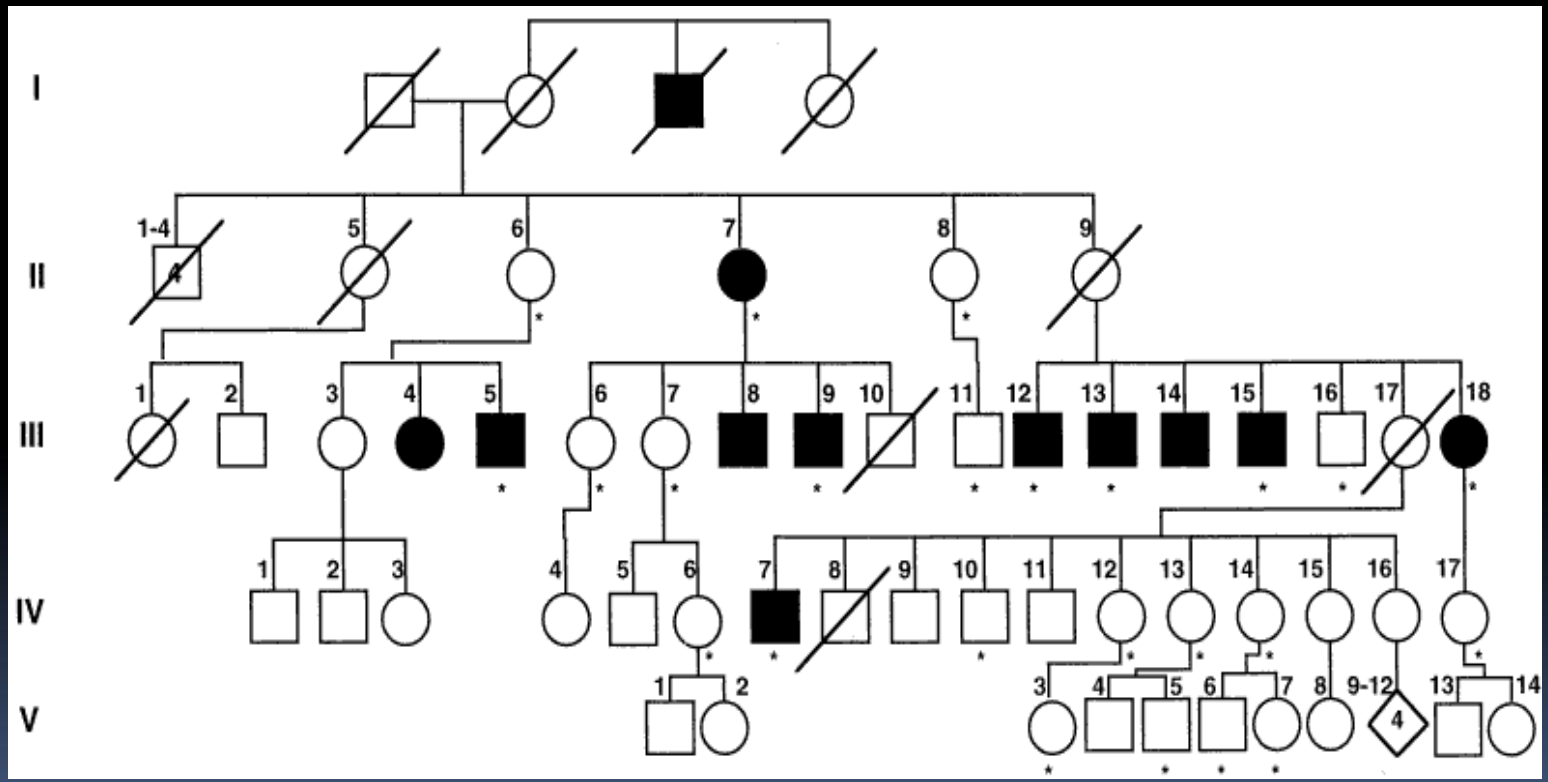


LHON is a **maternally inherited, late-onset, acute, optic atrophy.** In some families also there is also optic neuritis.
Incomplete penetrance (40% males, 10% females develop symptoms)
Caused by homoplasmic missense mutations in mtDNA (complex I).

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



Mitochondrial disorders caused by mtDNA mutations

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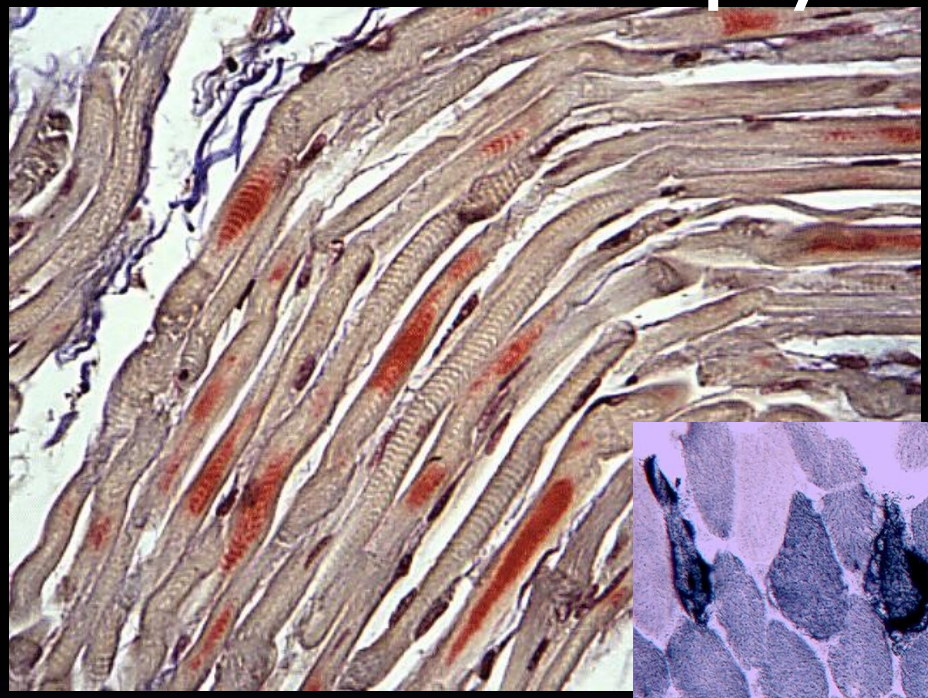
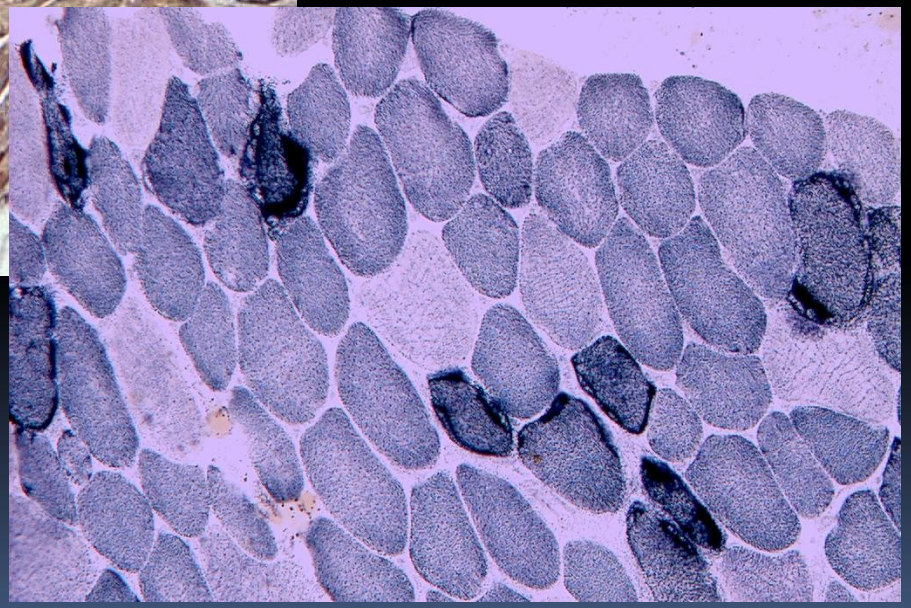
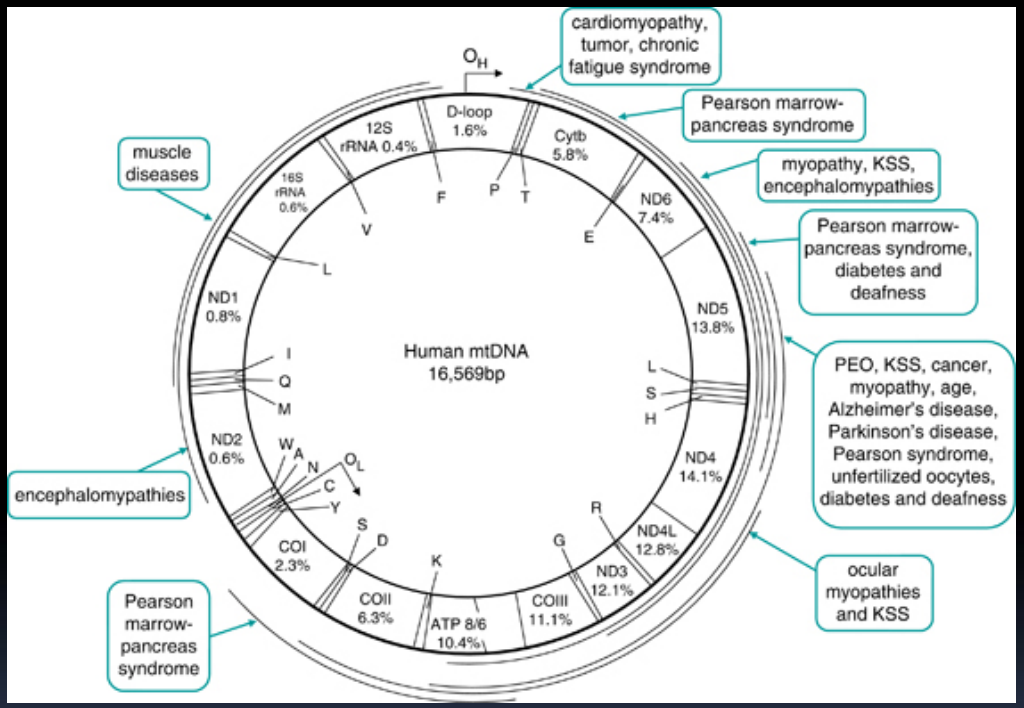
MtDNA defects– MERRF
Muscle biopsy

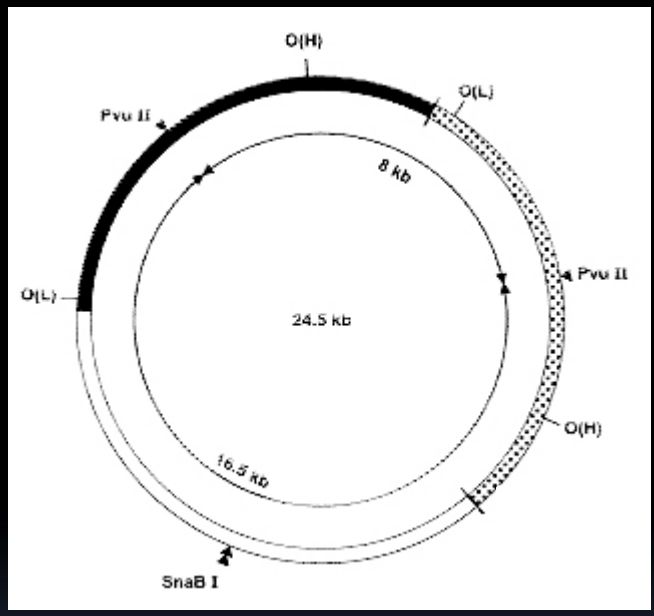




Ragged red fibres

Mitochondrial disorders caused by mtDNA deletions/duplications/depletions

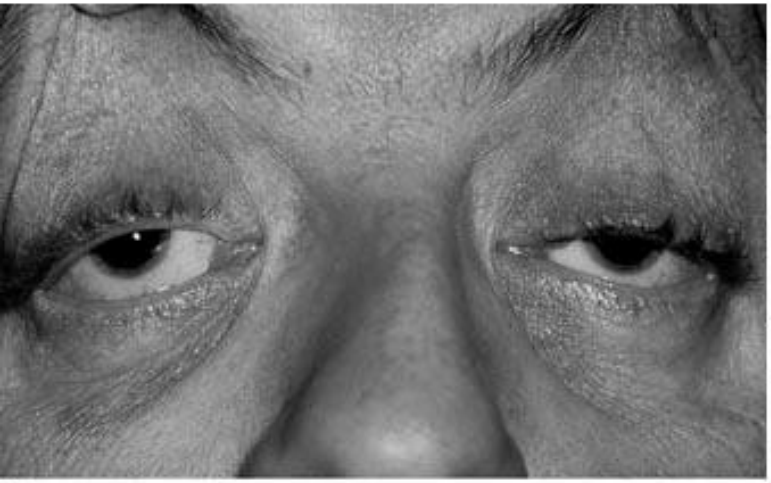




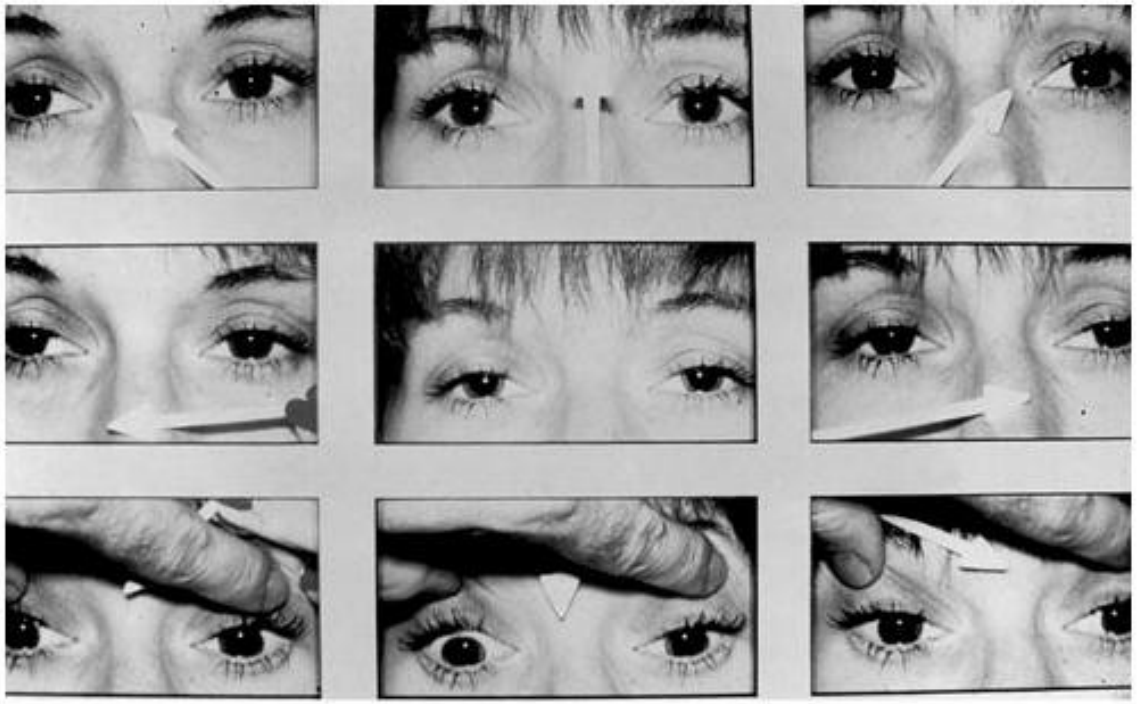
http://www.nature.com

Progressive external ophftalmoplegia
Pearson syndrome
Kearns-Sayre syndrome

Mitochondrial disorders caused by mtDNA deletions/duplications/depletions



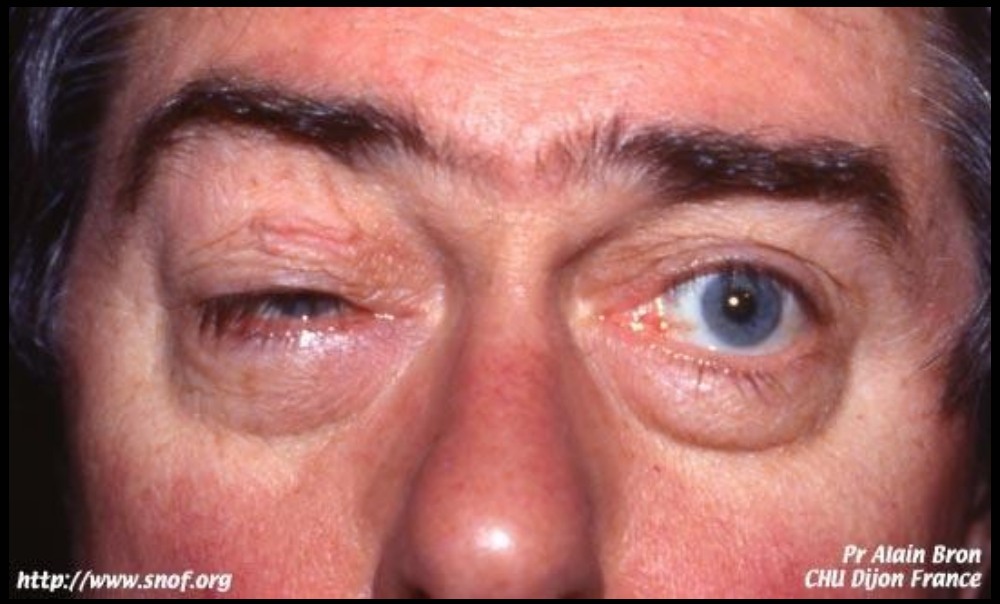
A



B

PEO – progressive external oftalmoplegia

Mitochondrial disorders caused by mtDNA deletions/duplications/depletions



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

Kearns-Sayre syndrome

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

Pearson syndrome – anaemia/pancytopenia, pancreas and liver dysfunction in infancy
- survivors may later go on to develop Kearns-Sayre syndrome

Mitochondrial disorders caused by nuclear DNA mutations

Nuclear defects of the mtDNA

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

- defects in genes for OXPHOS structural subunits
  Leigh syndrome, cardiomyopathy, ataxia,
  encefalomyopathy, myoclonic epilepsy,
  optical atrophy, heredital paraganglioneuromas

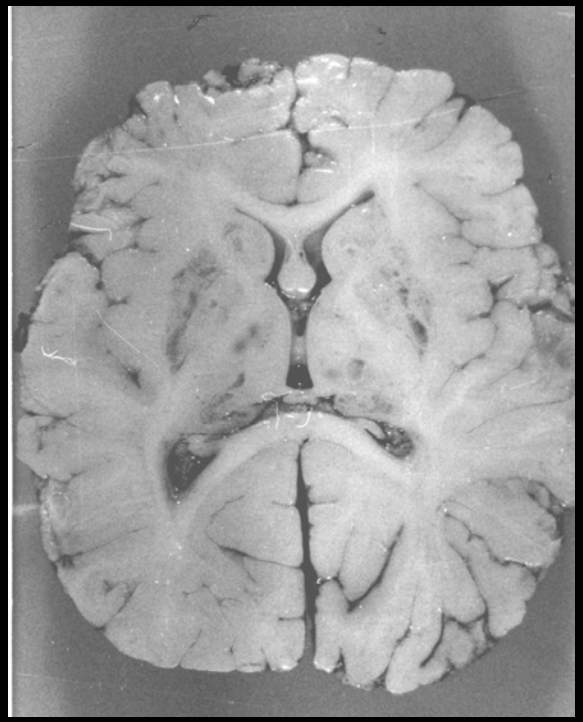
- defects in assembly proteins
  COX – SURF-1, SCO2, SCO1, COX10, COX15, …
  ATPase – ATP12, TMEM70

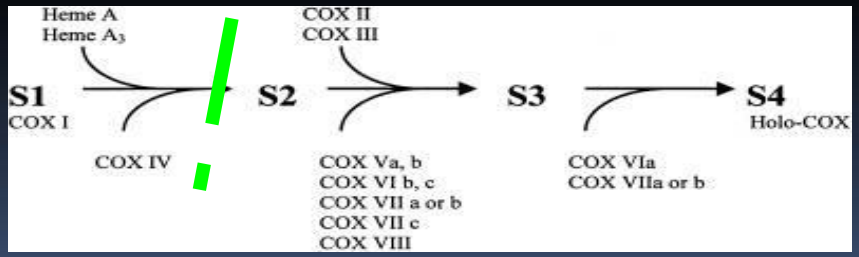
Defects of nuclear DNA

- Integrity and replication of mtDNA – thymidin fosforylase ANT1, twinkle, polymerase, POLG
- Protein transport systém
- Fission / fuse of mitochondria – OPA1
- Stability of mitochondrial membrane – Barth syndrome
- ……

Leigh syndrome

- several cautions
- defect in SURF1
- severe neurodegenerative disease
- neonates and infancy
- basal ganglia necrosis
- fatal prognosis





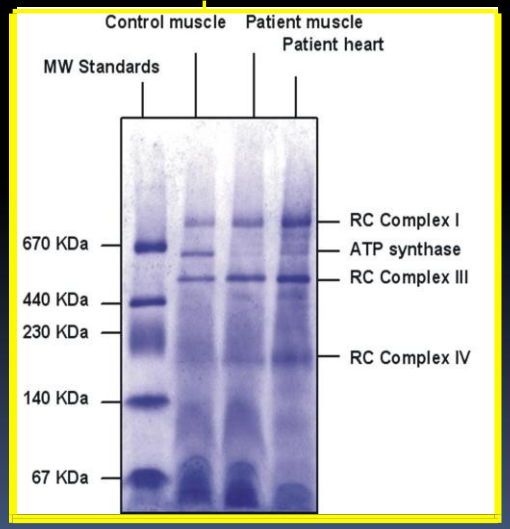
Non-complet froms of COX

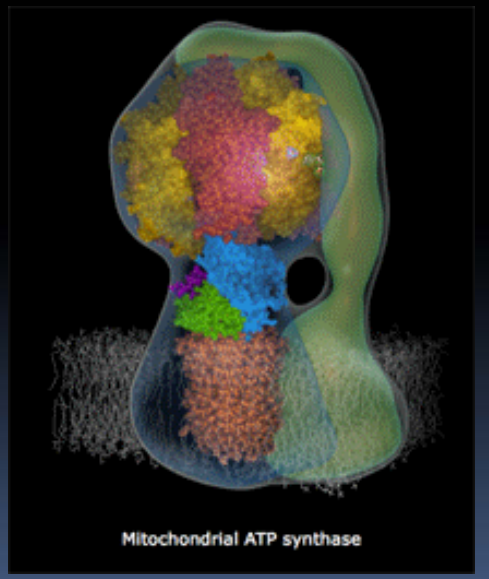
- absence of regulating nc-encoded subunits
- Subcomplex lability
- H+transport defect

Defects of ATP synthase due to nuclear origin

TMEM70

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

Krebs cycle and pyruvate metabolism defects

Pyruvate carboxylase defect
Phosphoenolcarboxykinase
Fumarase defects
Pyruvate transport defect
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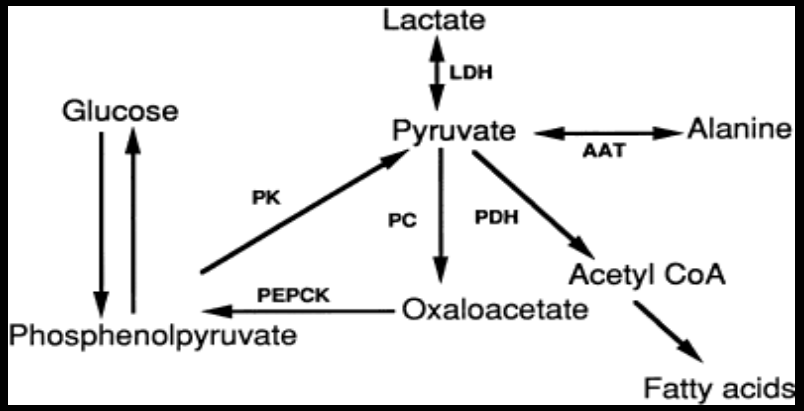


Pyruvate dehydrogenase defect (PDH)

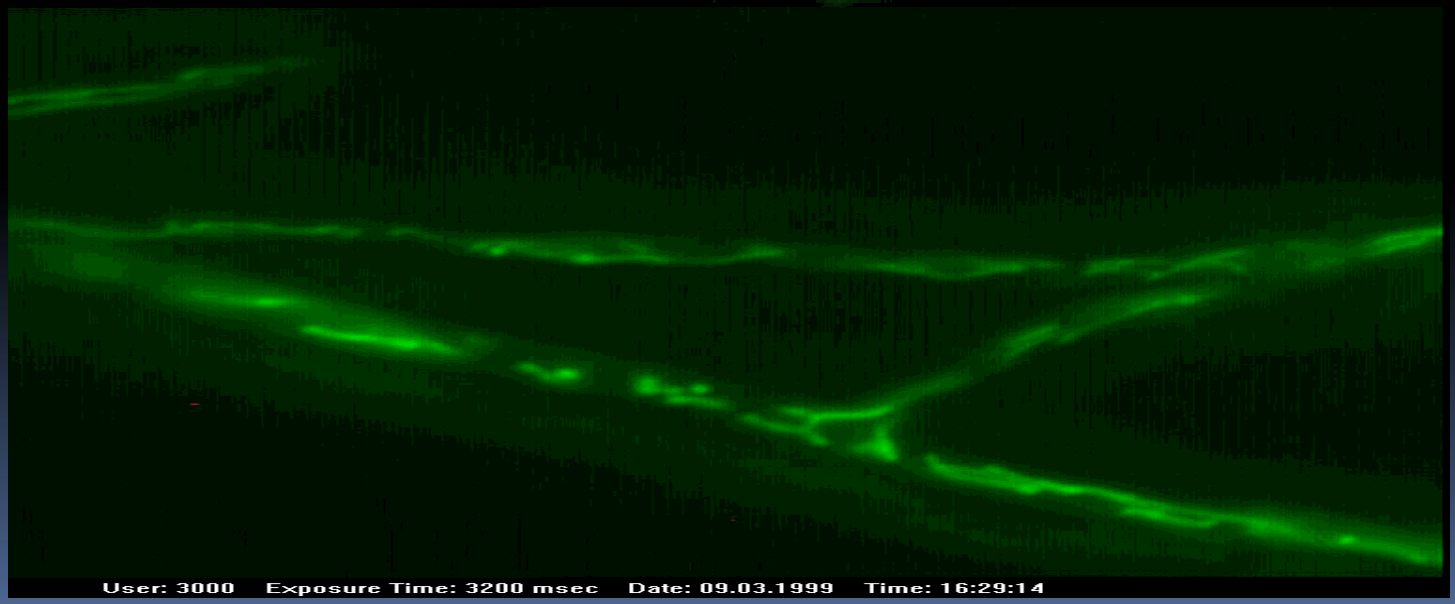
E1 subunit (α) X-linked hereditary

Symptoms and phenotype: neonat lactic acidosis,
Leigh syndrome, encephalopathy, ataxia, muscle weakness, psychomotor delay, basal ganglia degeneration
Females: facial dysmorphy, seizures, subscortical and cortical atrophy,
Deficiencies of other subunits are rare – E3 subunit (AR)

Treatment : ketogenic diet, thiamin

Unfavourable prognosis

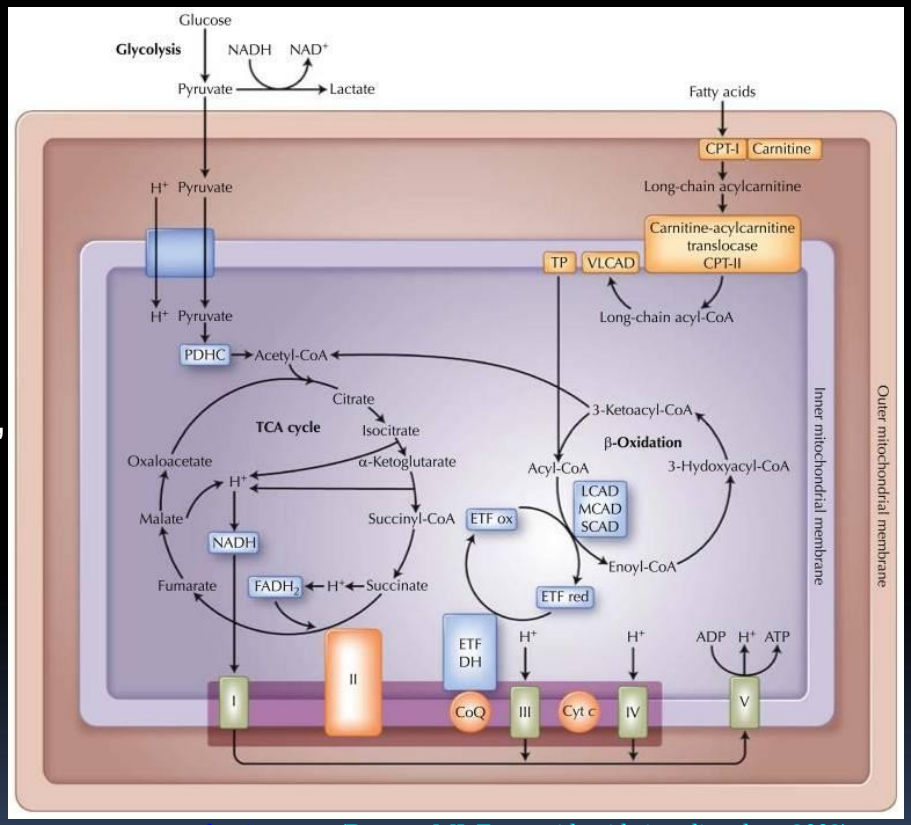
Mitochondria structure



Defects of fatty acid metabolism

Disorders of fatty acid metabolism

- Transport deficiency
  (carnitine cycle)
- Disorders of FA β-oxidation



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

Symptoms often develop after fasting, exercise, during catabolism

Hypoglycaemia

Low ketone bodies in blood and urine

In some disorders muscle weakness, rhabdomyolysis, cardiomyopathy.

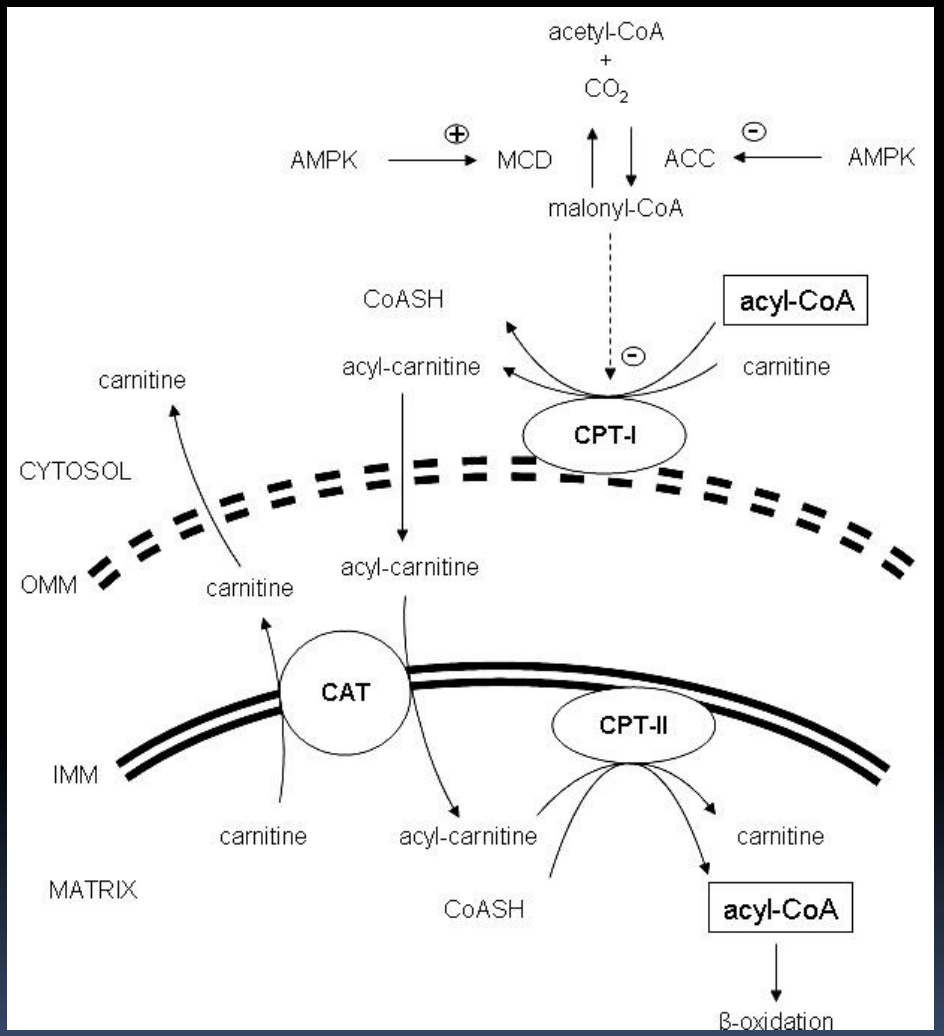
Carnitine shuttle

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

<u>CPT I and CACT</u> – rare
CPT I – hepatopathy, RTA
CACT - cardiomyopathy, arrhythmias, liver disease
lethal in infancy

<u>CPT II</u> – early onset – similar to CACT

- late onset – luscle weakness, rhabdomyolysis, exercise intolerance



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Fatty acid β-oxidation diseases

- VLCAD – early onset – SIDS
  - late onset – rhabdomyolysis, exercise intolerance
- LCHAD a MTP (trifunctional protein) – cardiomyopathy, exercise intolerance, hepatopathy, retinopathy, neuropathy, rhabdomyolysis, Reye-like episodes (hypoglycaemia, hyperammonaemia, hepatopathy, liver steatosis)
  - HELLP syndrome in pregnancy
  - dg – OH-acylcarnitine, U-OK
  - th – low fat diet, frequent meals, avoid fasting, MCT oil

Fatty acid β-oxidation diseases

- MCAD – most common – 1:6000 in Europe
  - prevalent mutation p.K329E
  - symptoms – no primary myopathy
    - liver disease – after fasting -
      hypoglycaemia, vomiting, coma, seizures, SCID
    - asymptomatic period before crisis!!!
  - dg – newborn screening, carnitine profile
  - th – avoidance of fasting

- SCAD – very wide clinical spectrum

  asymptomatic … failure to thrive … metabolic crisis with hypoglycaemia, coma, seizures

Fatty acid β-oxidation diseases

- Main symptoms - wide symptoms spectrum
  muscle impairment - muscle weakness, hypotony, exercise intolerance, rhabdomyolysis
  liver disease – Reye-like syndrome
  heart disease – cardiomyopathy, arrhythmias

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

Pathobiochemistry of fasting/starvation

Pathobiochemistry of fasting/starvation

Fasting – after nutrient reabsorbation to starvation

↑ glucosis in blood -> ↑ insuline and ↓ glucagon ->

glycolysis, lipogenesis, glycogenesis, proteosynthesis

Starvation - 3 phasis –

phase early gluconeogenetic

phase of adaptation

terminal phase – catabolism, critical

Pathobiochemistry of fasting/starvation

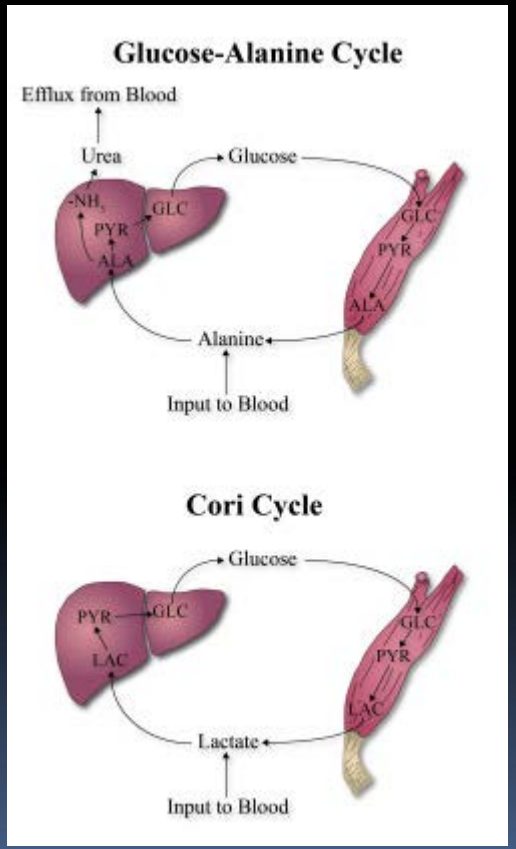
Early gluconeogenetic phase – normoglycaemia

glycogenolysis in liver

gluconeogenesis in liver

Cori cycle; Alanine cycle

BCAA in muscle and Gln (GIT, kidney)



Pathobiochemistry of fasting/starvation

Phase of adaptation – hours/days
glycogen pool is limited – no glycogen degradation
decreased gluconeogenesis (glucose from glycerol)

increased lipolysis - hormone sensitive lipase (↑ glucagon, adrenalin; ↓ insuline)

glycerol in liver
β oxidation of FFA in muscle, myocard, liver and kidney
ketogenesis (in liver) **and ketolysis** (in brain, muscle, myocard)

protein sparing phase

Pathobiochemistry of fasting/starvation

Terminal phase – days/weeks

no fat pool; no glycogen pool

Activation of proteocatabolism –

- o muscle loss – weight loss
- o imunite reaction inhibition
- o coagulapathy
- o decreased tissue reparation
- o enzyme and transport dysfuntion (↓ synthesis, ↓ ATP)
- o….
- odeath

IMD and fasting/starvation

Glykogenosis – ketotic hypoglycaemia, symptom „second wind“

FA β-oxidace deisorders – non-ketotic hypoglycaemia

Disorders of ketogenesis – non-ketotic hypoglycaemia
Disorders of ketolysis - hyperketotic hypoglycaemia

Organic acidaemia (MMA, PA, Leucinosis, IVA, …), urea cycle disorders -

amino acides catabolism prevention

ketotic hypoglycaemia, hyperammonaemia

ketoacidosis, lactate acidosis

Thank you very much