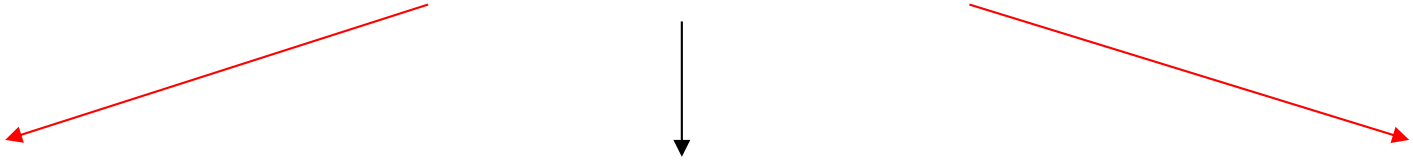
Mitochondria pathology

Genetic pathologies caused by mutations of mitochondrial proteins



Degenerative diseases Cancer **Aging**

1962. The first discovered pathology: Luft Disease loss of uncoupling of mitochondrial respiration and ATP synthesis in skeletal muscle tissue

1988. Identification of the first mutations of mtDNA in a rare optic myopathy

Today. OX-PHOS alterations are frequent causes of degenerative disorders. A frequency of about 1/10.000 births

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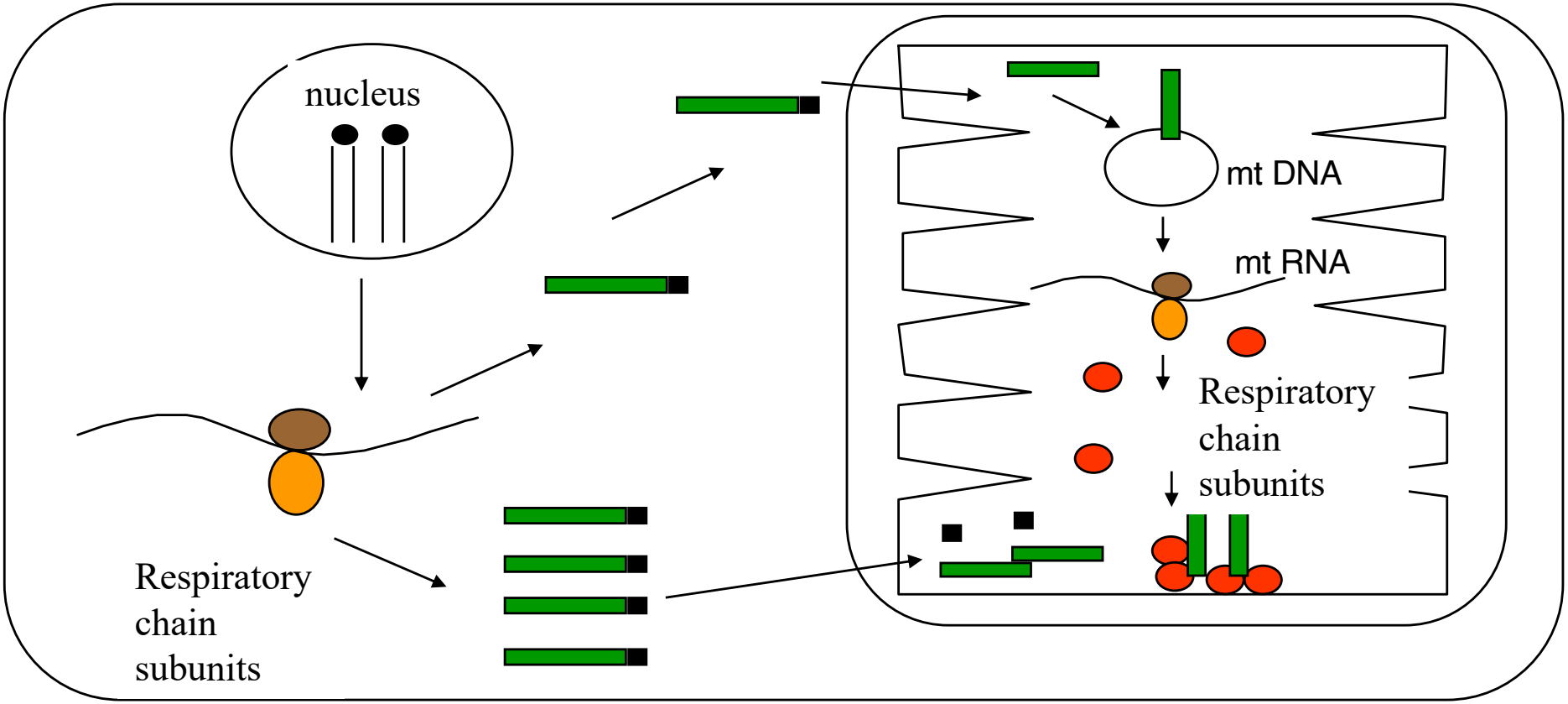
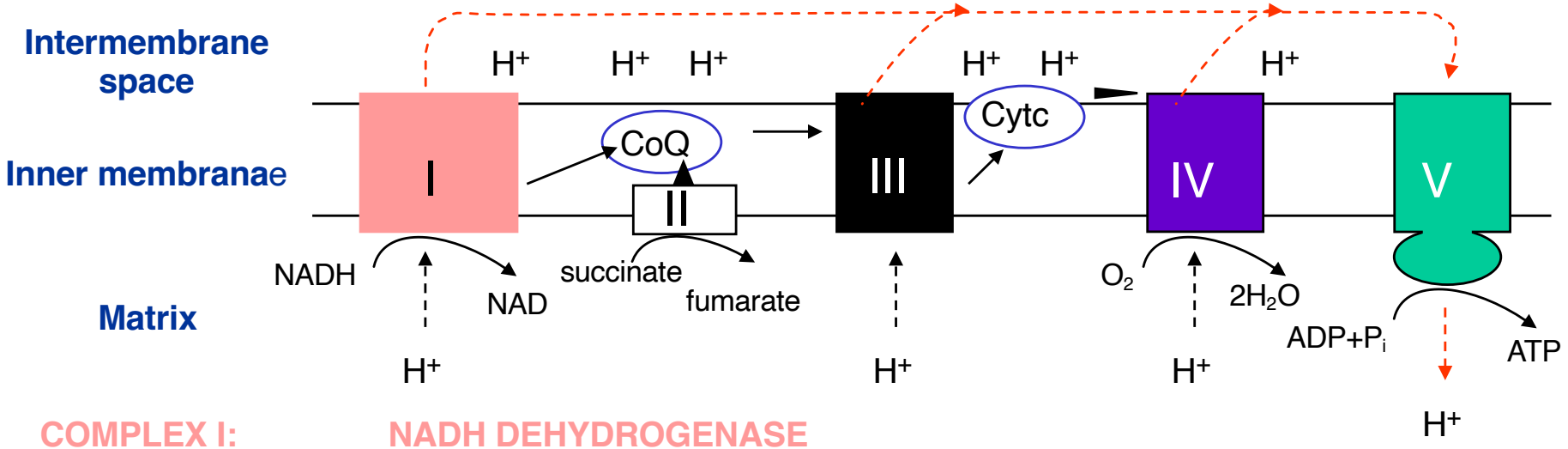
MITOCHONDRIA: structure - function

- Most of the mitochondrial proteins are coded by nuclear DNA (nDNA).
- Transduced in cytoplasm and through the "leader peptide" trasported in mitochondria



Mitochondria are endosymbiotic prokaryotes with their own DNA (mtDNA)

MITOCHONDRION: oxidative phosphorylation (OXPHOS)



COMPLEX I: **NADH DEHYDROGENASE**
30 polypeptides
7 mitochondrial polypeptides

COMPLEX II: **SUCCINATE DEHYDROGENASE**
4 nuclear polypeptides

COMPLEX III: **CYTOCHROME C REDUCTASE**
10 polypeptides
1 mitochondrial polypeptide (cytochrome b)

COMPLEX IV: **CYTOCHROME C OXIDASE**
13 polypeptides
3 mitochondrial polypeptides

COMPLEX V: **ATP SYNTHASE**
12 polypeptides
2 mitochondrial polypeptides (ATPase 6 - ATPase 8)

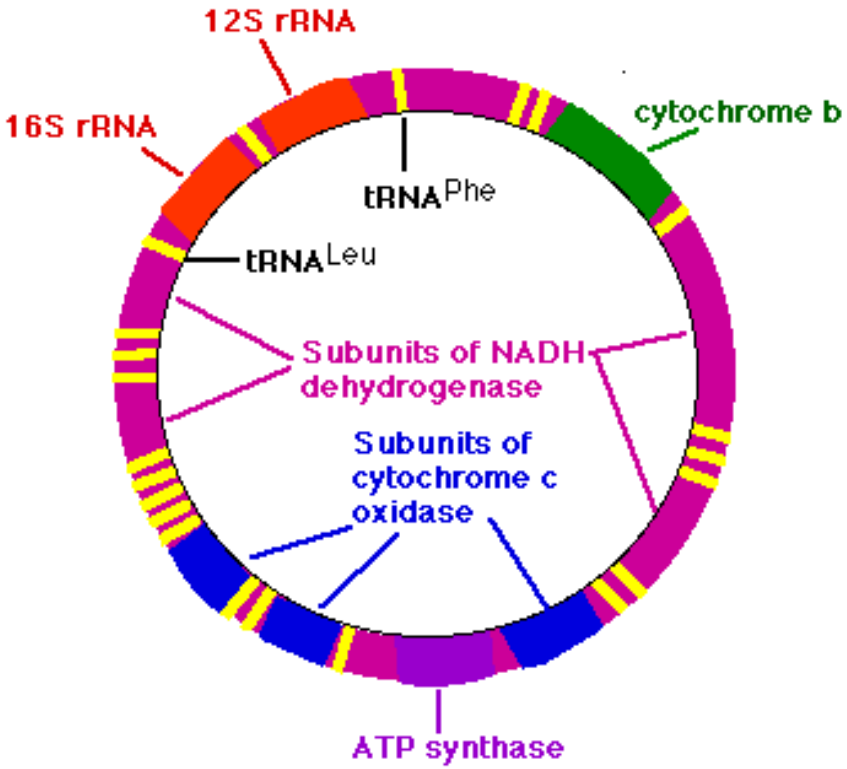
mtDNA Features

Circular Double strand DNA of 16.569 bp

2/10 molecules of mtDNA/mitochondrion $10^3 10^4$ molecules mtDNA/cell

- 37 genes: 24 RNA for protein synthesis
- 22 tRNA
- 2 rRNA (12S e 16S)
- 13 proteins,
- enzyme subunits of the respiratory chain regulation of the oxidative phosphorylation

- Polymerases coded by nDNA



mtDNA Features

Both mtDNA strands are transcribed to generate long POLYCISTRONS processed to release :

tRNA
rRNA
mRNA

Percentage of mutations 10 > NUCLEAR DNA:

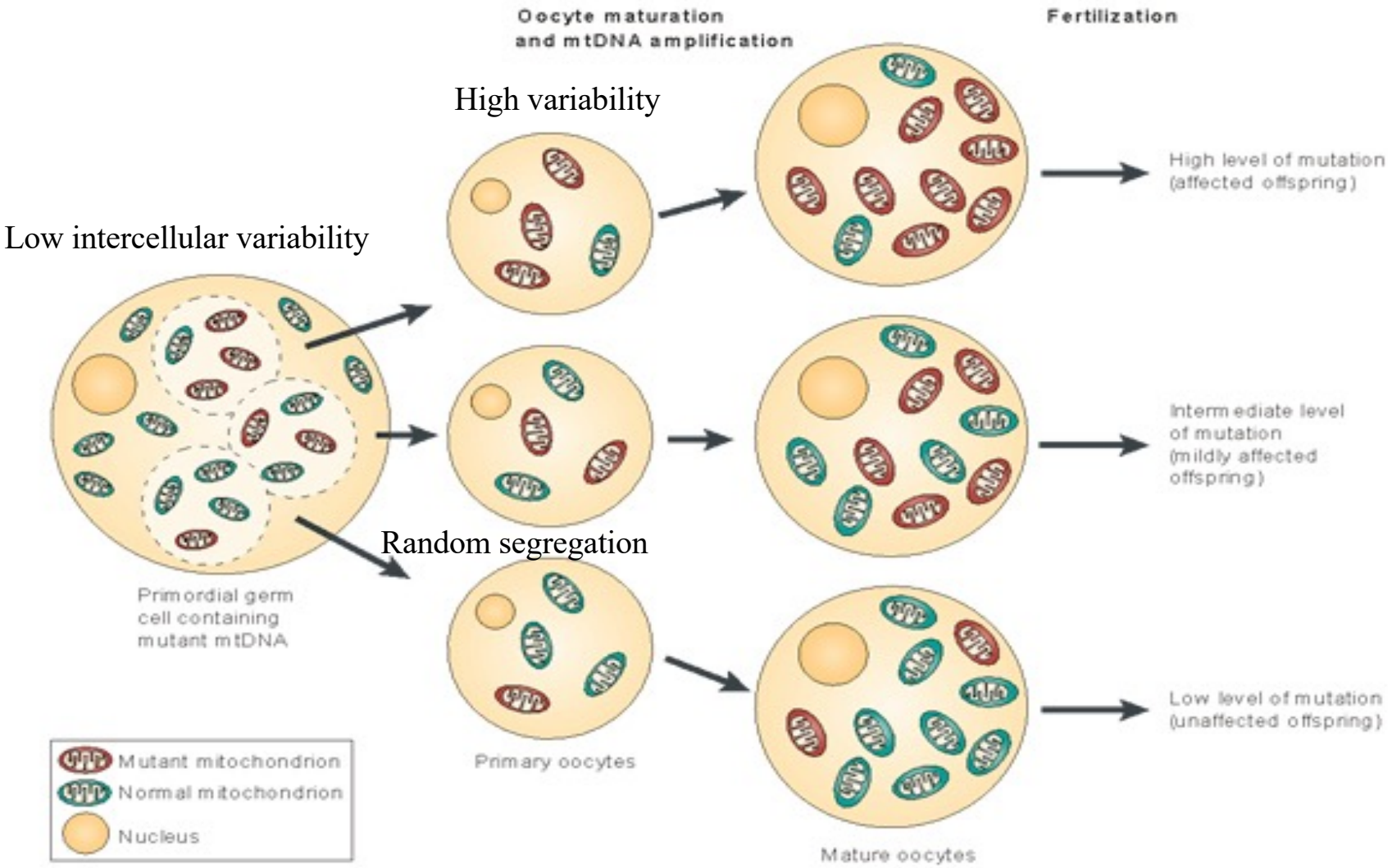
- lack of HISTONES
- Lack of DNA repair systems
- Damage by free radicals

- **Asynchronous and independent replication mtDNA /nDNA**
- **Randon distribution in terms of numbers of mtDNA molecules in daughter cells**
- **No ricombination between single mtDNA molecules**
- **New alleles --> spontaneous mutations**

- **Exclusively MATERNAL inheritance**
  (mainly transmitted through oocytes cytoplasm)
- **Variabiliy in clinical manifestations in affected relatives**
- **HETEROPLASMY: presence of mutated and normal mtDNA in the same cell**
- **Replicative segregation ---> HOMOPLASMY**
  only one kind of mtDNA

Mitochondrial Genetics

- **Maternal inheritance exclusively**
- **Prone to accumulate somatic mutations with age**



Genetic mitochondrial pathology

ALTERATIONS --> wide range of molecular mitochondrial pathology

GENETIC CAUSES { nuclear DNA mutations (mendelian trait)

mtDNA mutations (maternal inheritance – sporadic f.)

mtDNA MUTATIONS

A) REARRANGEMENTS { Deletions

Duplications

→ Defective protein synthesis
OXPHOS defects

B) POINT MUTATIONS

tRNA

→ Defective protein synthesis

rRNA

C) POINT MUTATIONS of polypeptides of OXPHOS

Spontaneus Mutations :
Germinal (maternal inheritance)
Somatic (sporadic diseases)

nDNA MUTATIONS

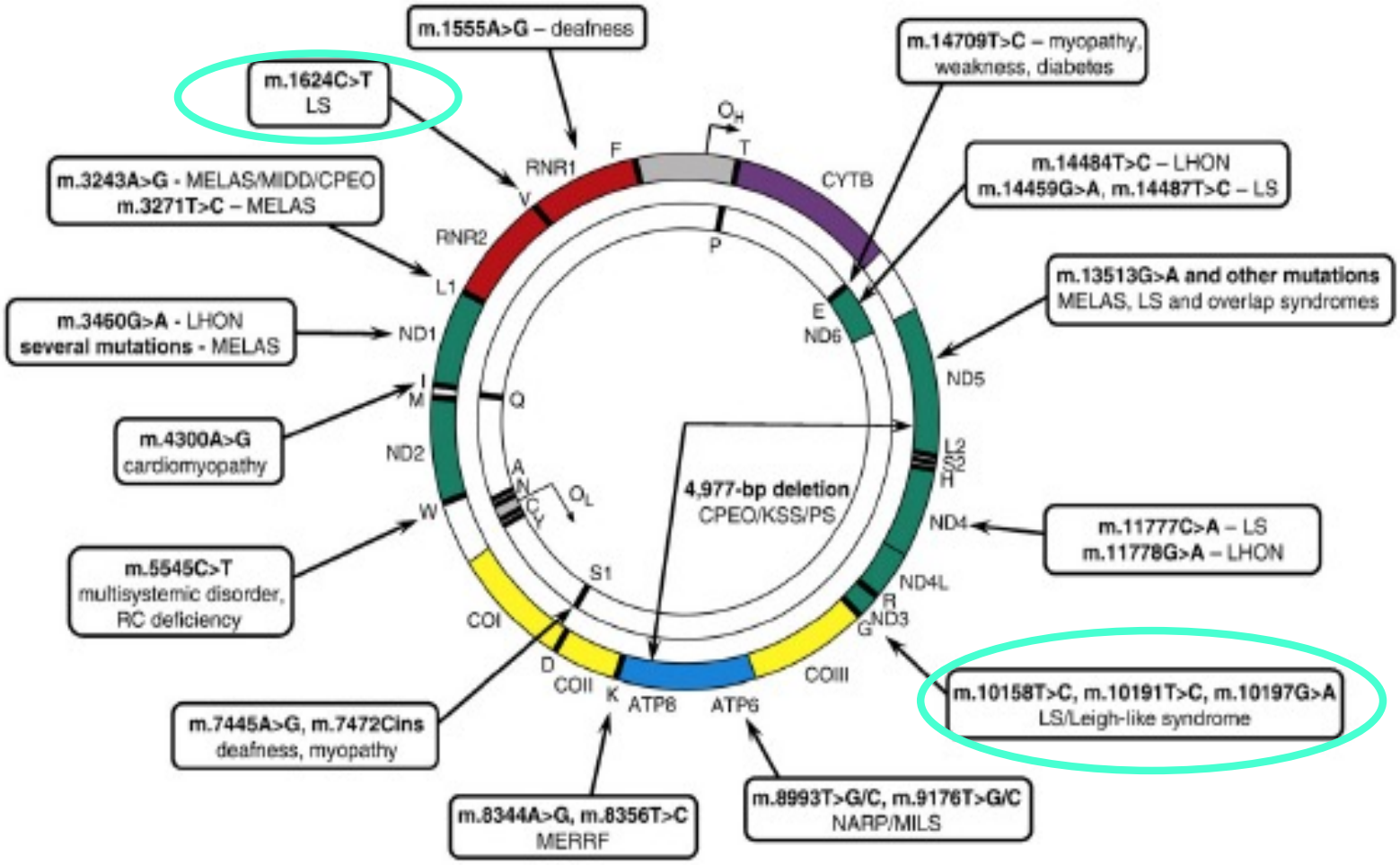
A) Missense / deletion MUTATIONS of OXPHOS complex genes

B) Missense / deletion MUTATIONS of other mt protein genes

C) DESTABILIZATION of mt DNA

Mitochondrial diseases with onset early in infancy/childhood

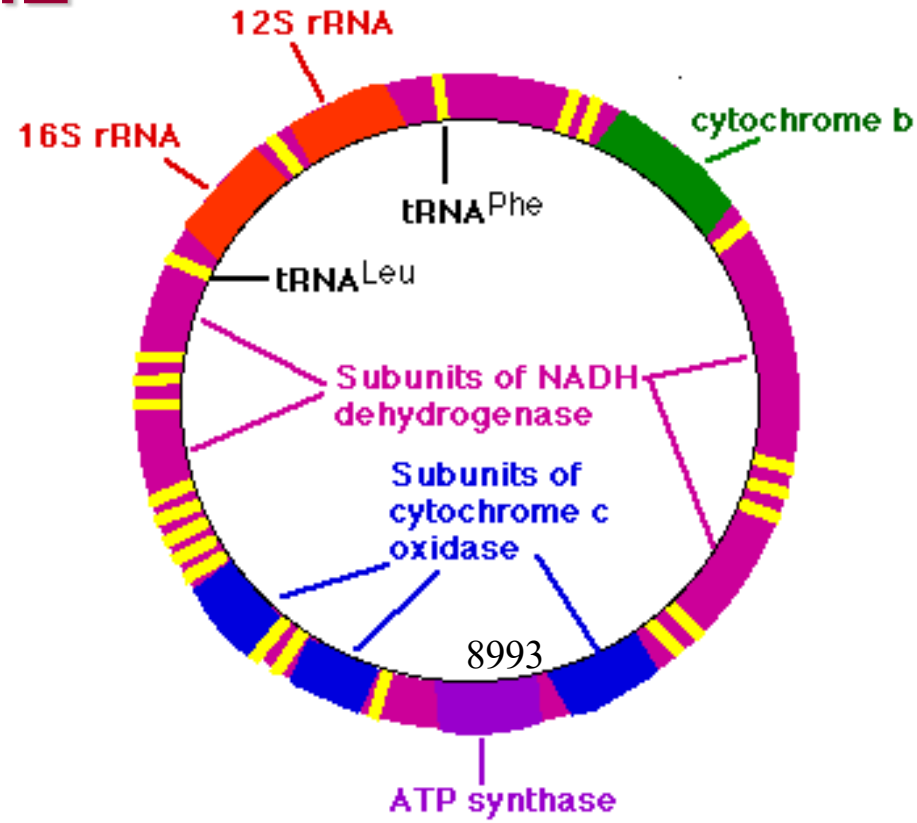
Severe psychomotor delay, generalized hypotonia, lactic acidosis, cardiorespiratory failure



LEIGH, Rearrangements of mtDNA: Kearns-Sayre, Pearson

LEIGH and NARP SYNDROME

(Neurogenic muscle weakness, Ataxia, Retinitis Pigmentosa)



Alteration of ATP synthase

Point mutations of mtDNA

Most frequent:

MTATP6*NARP - T8993---> Leu 156 ---> Arg

MTATP6*FBSN - 9176 ---> Leu 217 ---> Pro

block of protons translocation- channel complex V

Maternal Inheritance with constant HETEROPLASMY:

correlation between mutated mtDNA and morbidity

High levels >95%: severe Leigh S. infantile onset

Low levels <75% NARP delayed onset

Kearn-Sayre SYNDROME (KSS) and CPEO

(Chronic progressive external ophthalmoplegia)

Mainly sporadic pathologies

Chronic progressive ophthalmoplegia
Mitochondrial Myopathy (RRF)

CPEO
After age 20

Retinitis pigmentosa
Lactic Acidosis
Neurosensorial loss od hearing
Ataxia
Epileptic attack
Dementia
Hypertrophic and dilatative cardiomiopathy
defects of conductance

Kearn-Sayre Syndrome before age 20

- **83 % di KSS and 47% CPEO ---> mtDNA rearrengements**
- **Prevalent deletions (with or withouth duplications) ripetitive sequences are involved**
- **Blood cells characteristically have no mutations in CPEO.**
- **Positive Segregation of mutated forms**
- **Mostly are new cases of sporadic and spontaneous mutations occurring early in development (oocyte??)**
- **Costant HETEROPLASMY between normal mtDNA and mutated mtDNA**

Kearn-Sayre Syndrome (KSS) and CPEO

Deletions: **ranging from 9 to 50% of mtDNA is removed**

Frequent Deletions ***1,3 - 7,6 Kb mtDNA***
nt 7194 - nt 14595 = 7,4 Kb

↓

Deletion of tRNA genes and OXPHOS proteins

↓

Reduced proteins sythesis

↘

Reduction of OXPHOS

Few case reports of KSS/CPEO are mitochondrial tRNA mutations

Pearson Syndrome

Mainly sporadic

multisystemic disorder characterized by:

- **Bone marrow pancytopenia**
- **Pancreatic fybrosis ---> diabetes**
- **Spleen atrophy**
- **Hepatic and renal failure**

- Child are affected. It is oftenLethal
- Survivors progress to → KSS phenotype

Molecular causes: defective function of OXPHOS due to reduced COMPLEX I activity

Several Deletions: 2461 bp 10367 - 12828
flanked by GCC sequences

Deletion/duplication of mtDNA is HOMOPLASMIC in blood cells------> pancytopenia

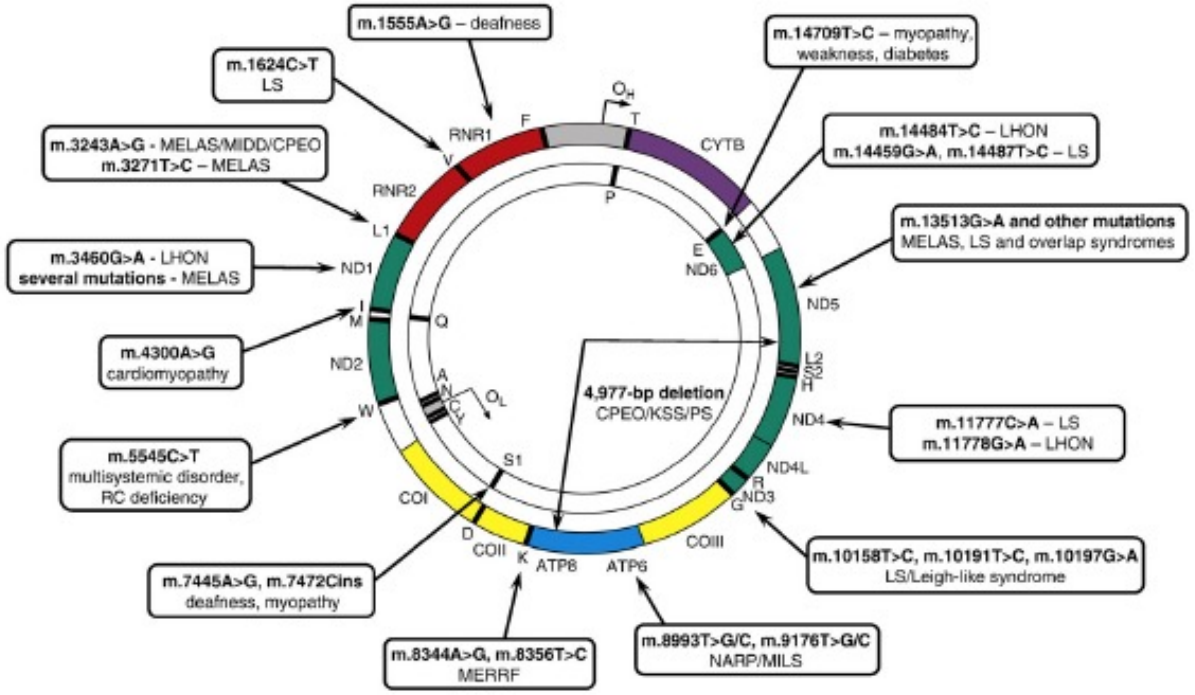
Defects of subunits. ND3, ND4L, ND4, ND5 of complex I

Defects of tRNA (Arg His Ser Leu)

Mitochondrial diseases with onset in late childhood or adult life

Myopathy, variable involvement of CNS.

Muscle weakness, wasting with exercise intolerance.



LOHN, MELAS, NARP, and CPEO

Optic Neuropathy of Leber (LHON)

Molecular basis and Pathophysiology

- 18 *point mutations of mtDNA* coding proteins of <u>COMPLEX I</u> (NADH dehydrogenase) (90% of affected)

- Low efficiency of oxydative phosphorylation (OXPHOS)

- ATP deficiency leading neurons of optic nerve to death

- More ROS

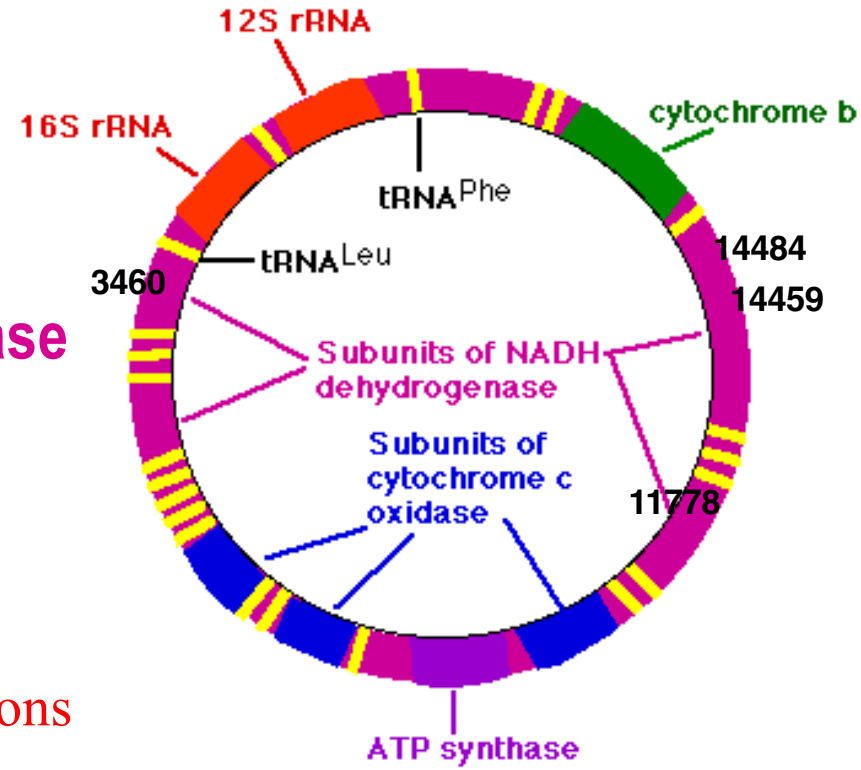
- **channel opening mtPTP ---> apoptosis**
- **inhibited release of NO --> vasocostriction and vasculopathy**

- Found new "primary" mutations of mtDNA of Complex IV subunit III: cytochrome C oxidase

- SECONDARY MUTATIONS: associated to primary mutations: synergistic action

OPTIC NEUROPATHY of Leber (LHON)

Alterations of NADH dehydrogenase



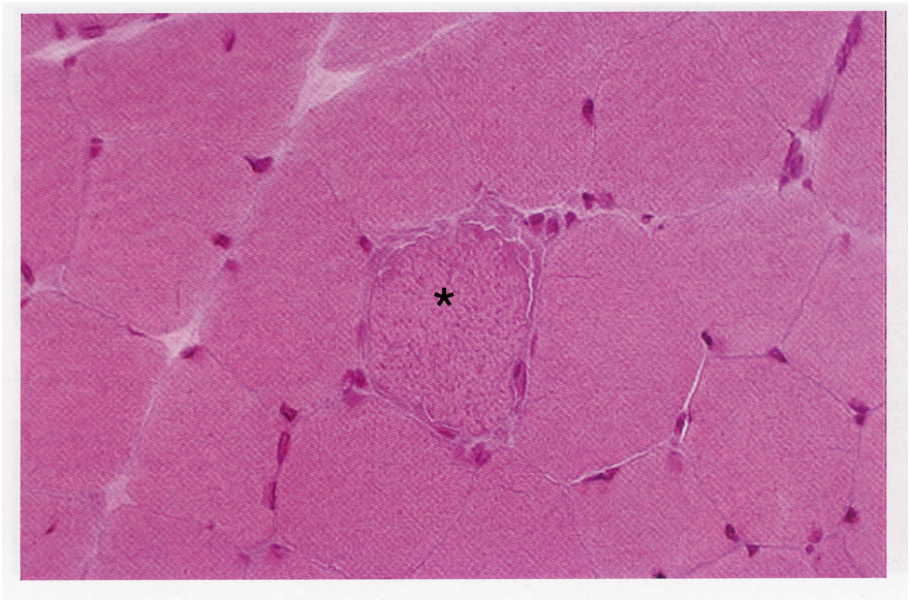
Most frequent Mutations

- 1. MTND4*LHON - G11778A ---> Arg 340 ---> Hys (subunit ND4)
- most frequent variable penetrance and some normal phenotypes
- 2. MTND1*LHON - G3460A ---> Ala 52 --> Thr (subunità ND1)
- second in pathogenicity
- 3. MTND6*LHON - A14484C ---> Met 64 --> Val
- (highly conserved domain of ND6)

more benign sometimes vision recovery

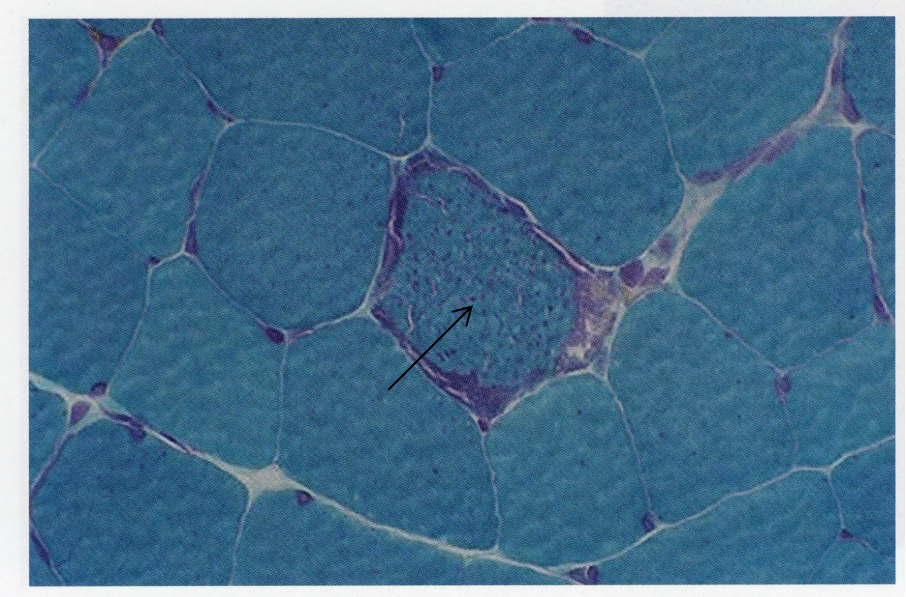
- 4. MTND6*LDYT- A14459G ---> Ala 72 --> Val (subunit ND6)
- most frequently heteroplasmic more pathogenic, associated to dystonia

A) “Ragged-Red-Fibers”



* Granular red areas

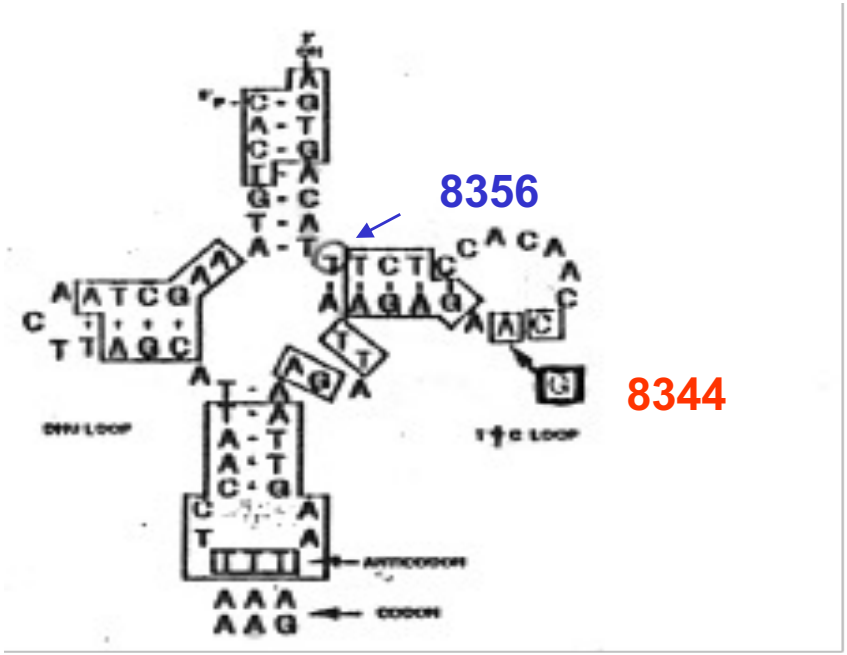
B)



Abnormal mitochondrial deposits

MERRF (Myoclonic Epilepsy and Ragged Red Fiber)

Molecular basis-MERRF SYNDROME

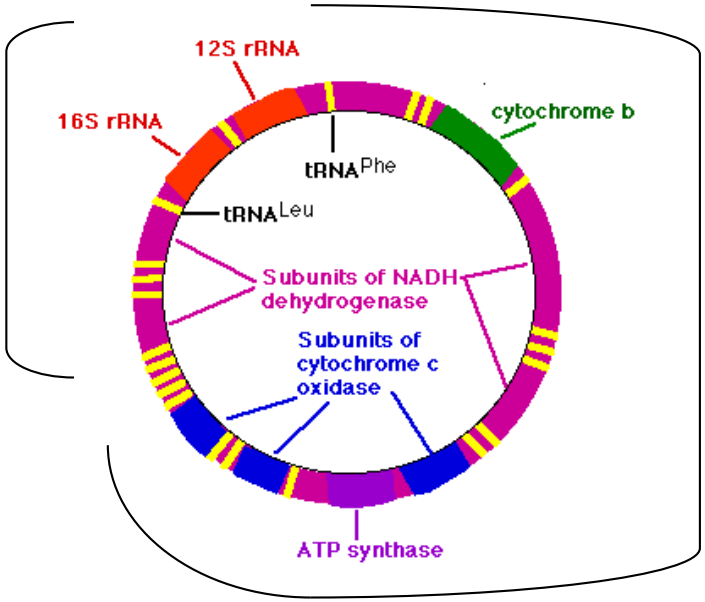


tRNA^{Lys} 8344A --> G in TΨ loop: highly conserved nucleotide
mut. 80-100% present
functionally recessive
affected interaction with ribosome
protein synthesis
function of complexes I and IV

tRNA^{Lys} 8356T --> C stem TΨ loop: - highly conserved nucleotide
mut. less frequent
functionally recessive
protein synthesis

Rearrangements of mtDNA

10%



90%

Most frequent deletion is 4997 bp
In between 13 bases

•Deletions / duplications Range from 2 - 8 Kb. Flanking ripetitive sequences
•Affected ADULTS have:
- **- deleted mtDNA mainly in muscle and brain**
- **- Development of progressive external ophtalmoplegia (PEO)**
- **-KEARNS-SAYRE SYNDROME (the most severe)**

Affected Children have:
- **- severe multysistemic disorders (anemia, exocrine pancreatic failure , diabetes mellitus, nephropathy, hepatopathy, eczema, other endocrine disorders)**

•Diseases associated with deletions progress with age because the ratio mutated mtDNA / normal mtDNA increases.

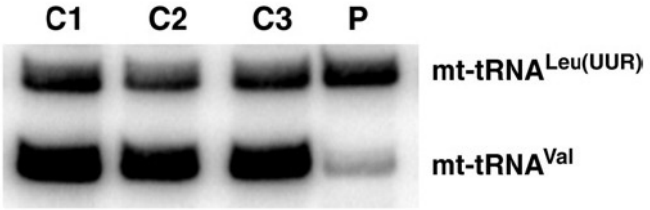
MELAS
(Mitochondrial encephalomyopathy lactic acidosis stroke)

Molecular basis

Point mutations of tRNA of mtDNA: Heteroplasmic mutations

$tRNA^{Leu(UUR)}$ 3243 A --> G loop of dihydrouridine:

- **↓ Alterations in tertiary structures**
- **↓ tRNA stability**
- **↓ ability to interact with aa LEU**
- **↓ assembly ability tRNA with ribosome**



Muscle total RNA

REDUCED MITOCHONDRIAL PROTEIN SYNTHESIS

> 85% mtDNA ---> MELAS
5 - 35% mtDNA -→associated to diabetes mellitus type II maternally inherited and deafness

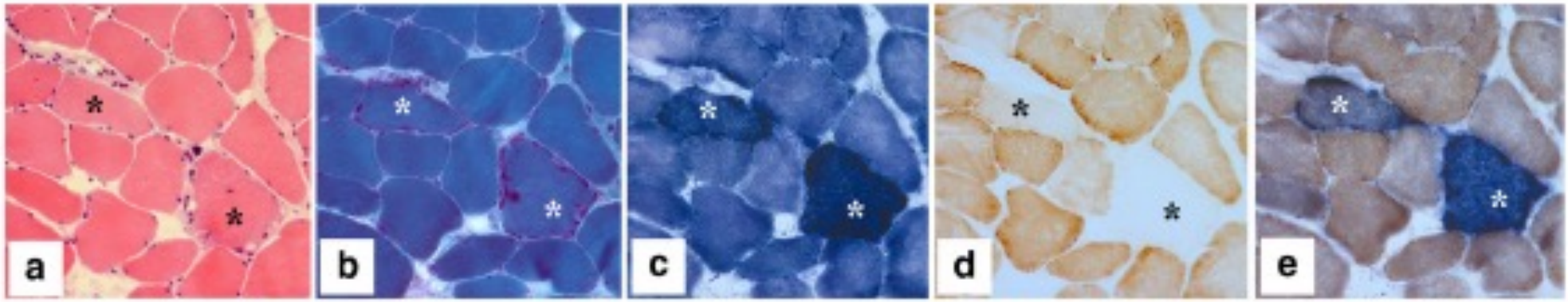
$tRNA^{Leu(UUR)}$ 3271T --> C terminal nucleotide of anticodon:

$tRNA^{Leu(UUR)}$ 3251A --> G loop di dihydrouiridine

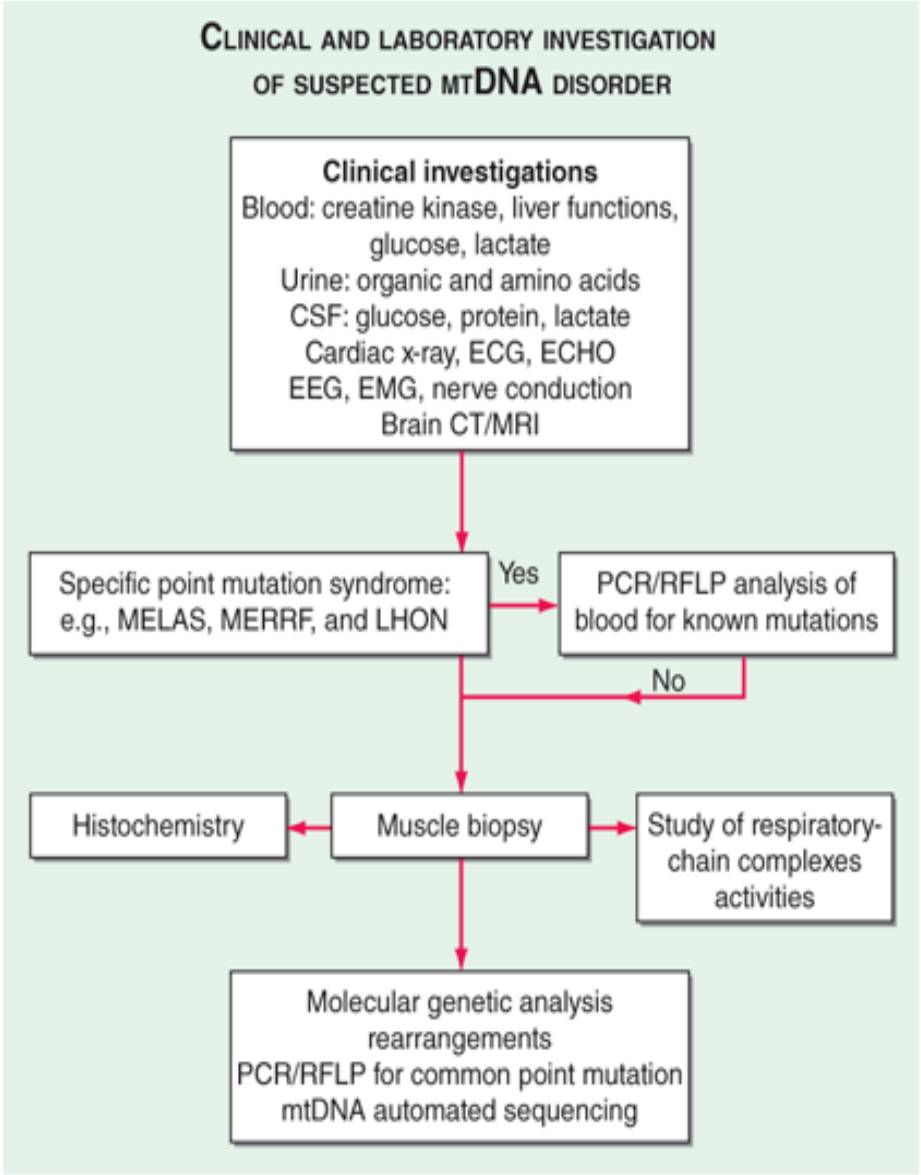
Other non characterized mutations of mtDNA
Mutations of nuclear DNA

Diagnosis of mtDNA disease

- Clinical testing
- Histochemical and biochemical assays
- Molecular genetic testing



H&E Gomori trichrome SDH COX COX-SDH



LOHN and MERRF

Source: D. L. Kasper, A. S. Fauci, S. L. Hauser, D. L. Longo, J. L. Jameson, J. Loscalzo: Harrison's Principles of Internal Medicine, 19th Edition
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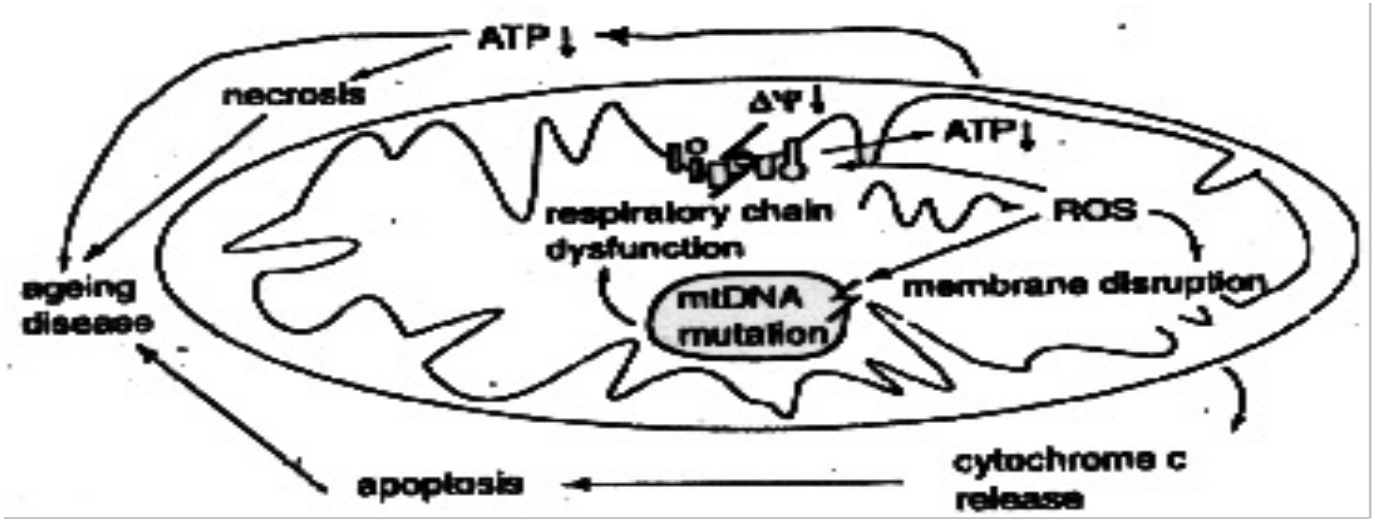
Other disease associated to mitochondrial DNA abnormalities

mtDNA anomalies cause more severe forms with late onset and related to age. Neurodegenerative diseases, diabetes mellitus, cardiomyopathy, deafness, dementia, Alzheimer

CAUSE: genetic predisposition
decreased OXPHOS age correlated

mtDNA somatic mutations ---> postmitotic tissue with high energy requirement
ATP (muscle and brain)

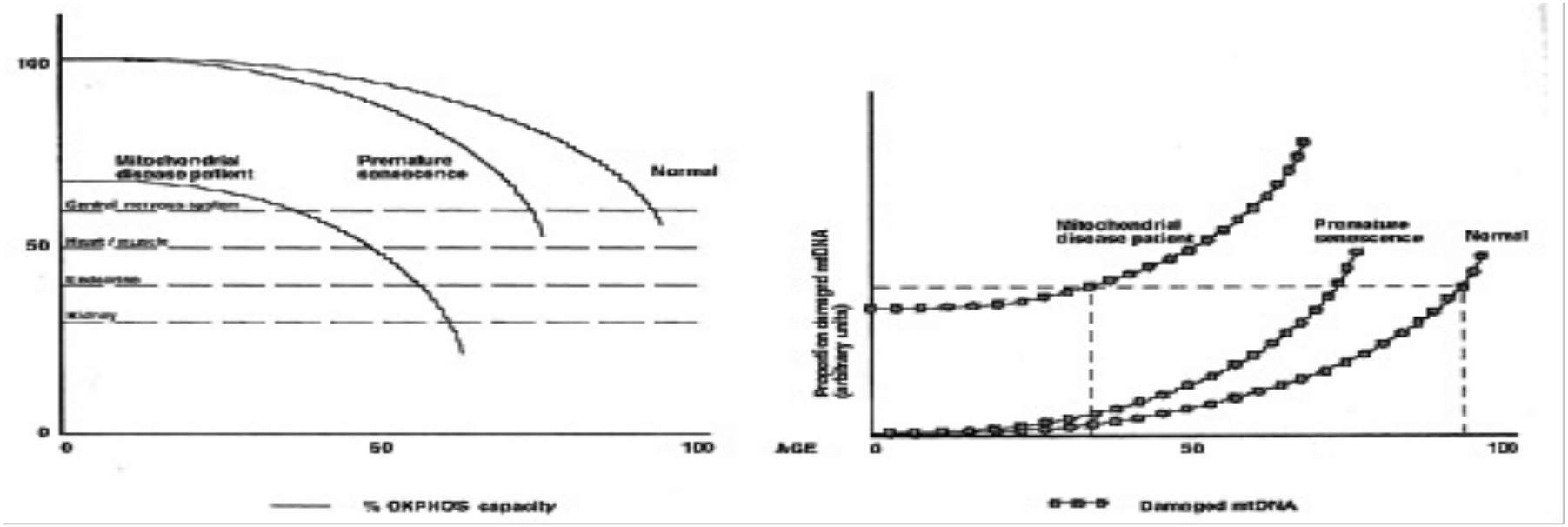


Oxidative Damage

In example: Deletion 4977 (5 Kb) accumulates in basal ganglia and in other cortical regions of brain after age 75.

Threshold hypothesis

OXPHOS Capacity vs. mtDNA Damage



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Genetic mitochondrial pathology

Pthophysiology of mitochondrial genetics

DEFECTS in ENERGETIC METABOLISM

Free radicals production (ROS)

APOPTOSIS

Accumulation of postmitotic mutations:
DEGENERATIVE PATHOLGIES
AGING- COMMON DISESADES

Clinical variability---> HETEROPLASMIA - % mutated mtDNA and energy request by the target tissued

Same Mutations---> DIFFERENT MITOCHONDRIAL SYNDROMS
(multifactorial elements)

Therapeutic options in management of mtDNA disease

- **Exercise therapy** to improve physical capacity and quality of life
- **Substrates, carriers** and **antioxidant**

•**Gene Therapy**: manipulation of heteroplasmy levels to shift the balance of **mutant** to **wt** genomes.

•**Prevention of mtDNA disease transmission**

- Perspectives
- Cataloguing and understanding the mtDNA defects responsible for the development to uncover novel protein targets or molecular pathways that may be exploited for therapy development.

Diseases and symptoms in energy consuming tissues and organs dipendent on mtDNA alterations