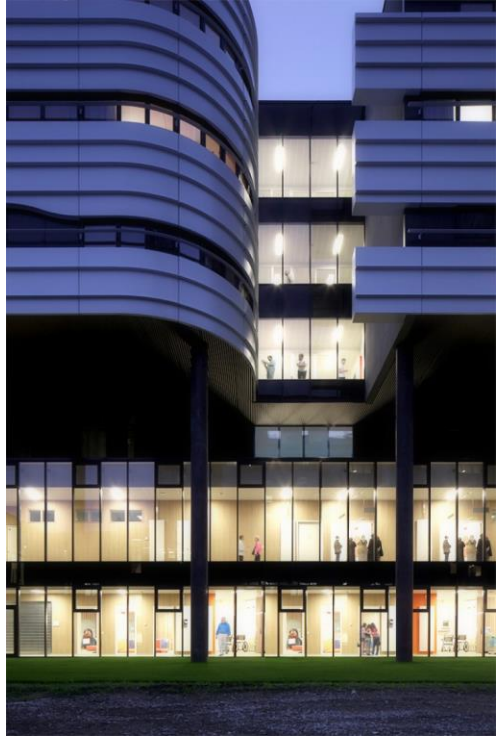

Mitochondrial Diseases

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Division of Pediatric Neurology & Metabolism

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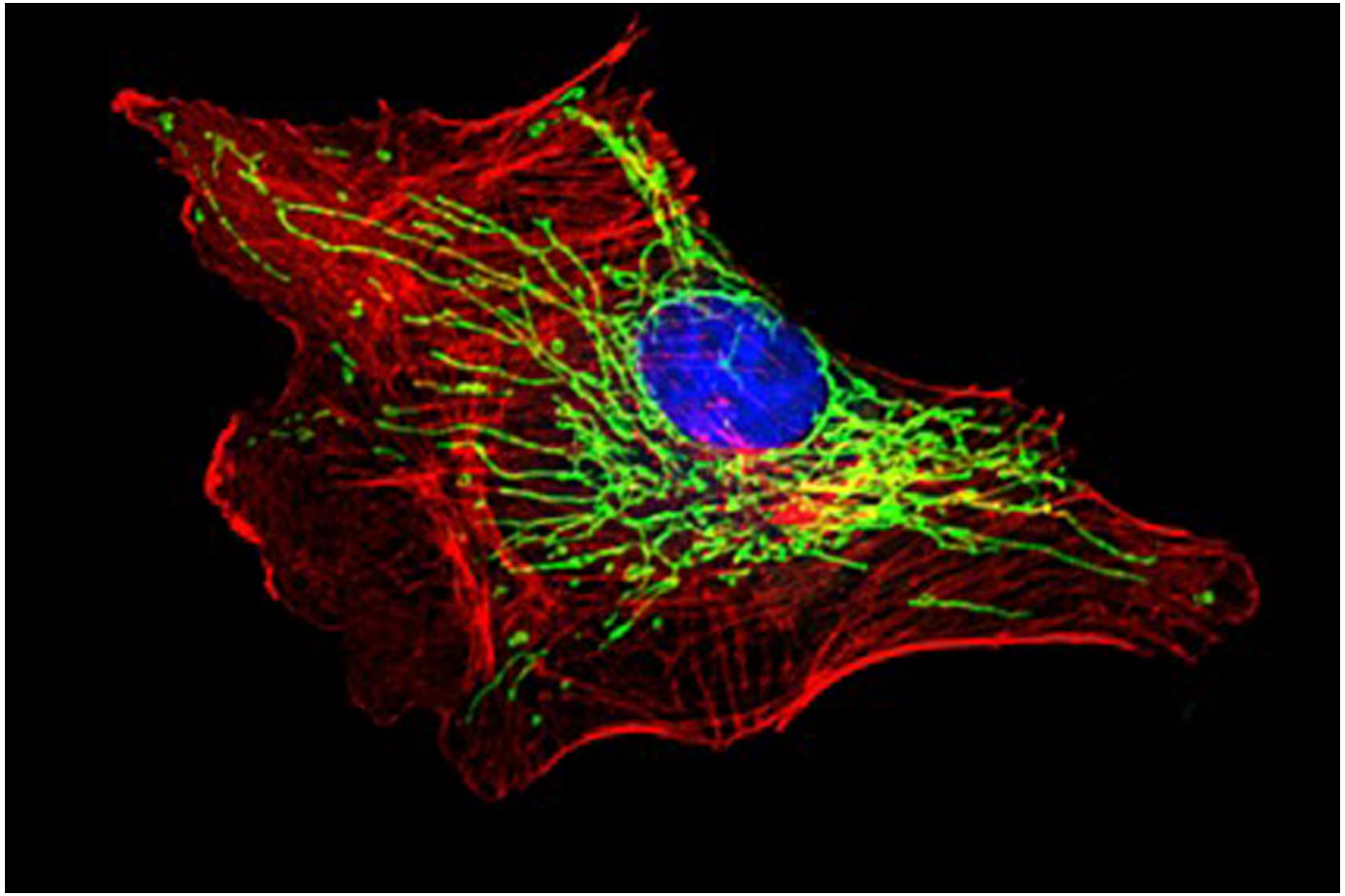
Ghent University Hospital

Mitochondria

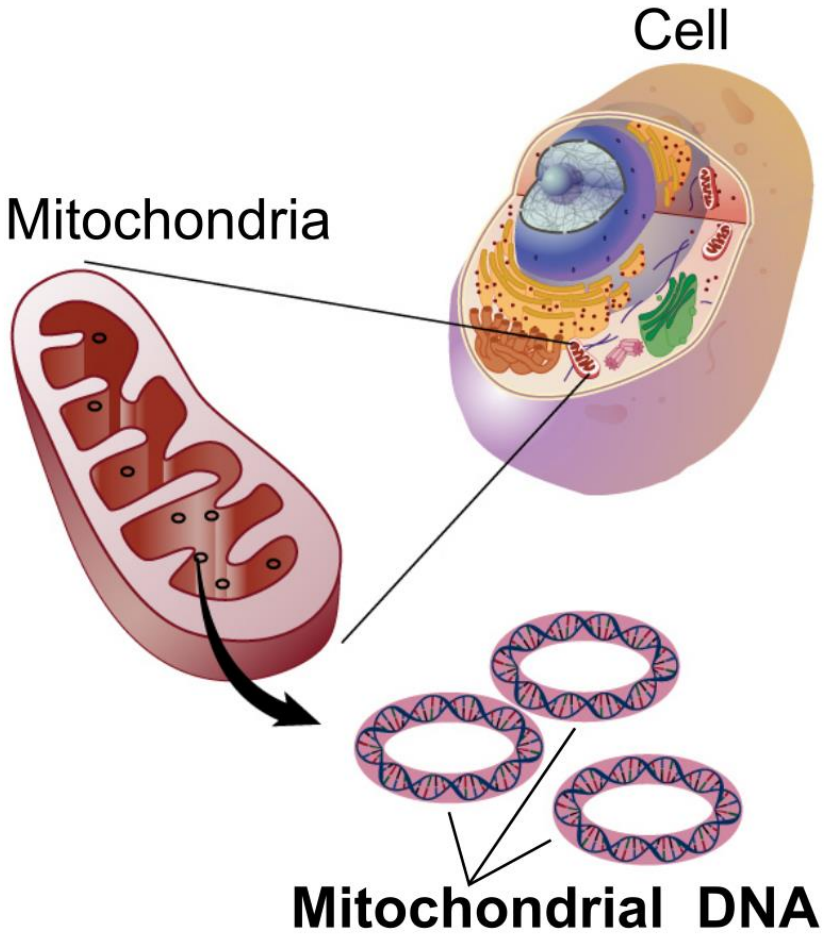
- Present in all cells (except RBC's)
- Multiple copies/cell
- Dynamic network



Mitochondria - functions

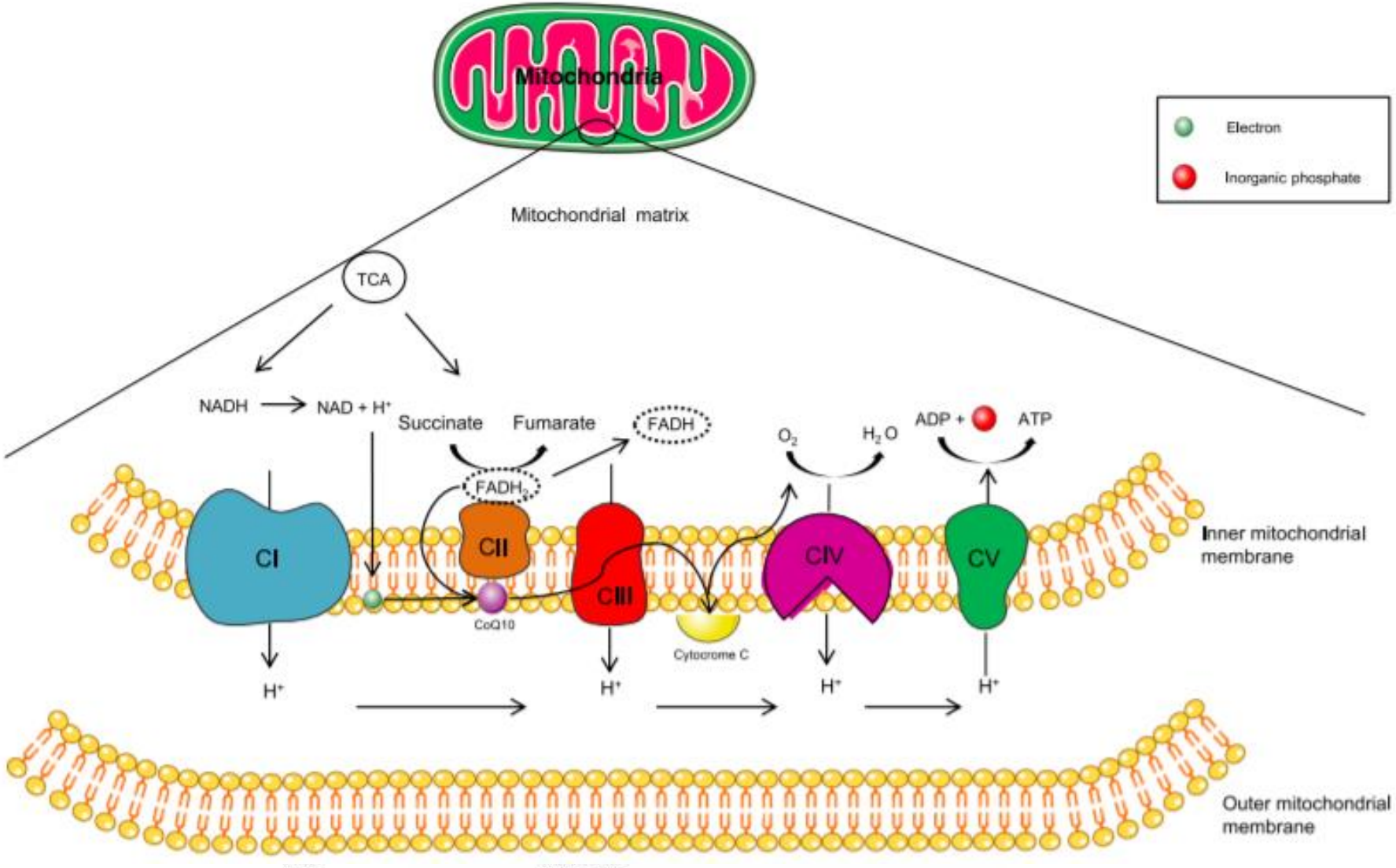
- Calcium homeostatis
- Apoptosis
- Autophagy
- Different enzymatic pathways (e. g. Krebs cycle, urea cycle, heme synthesis)
- ATP-production
- Redox balance

Mitochondria

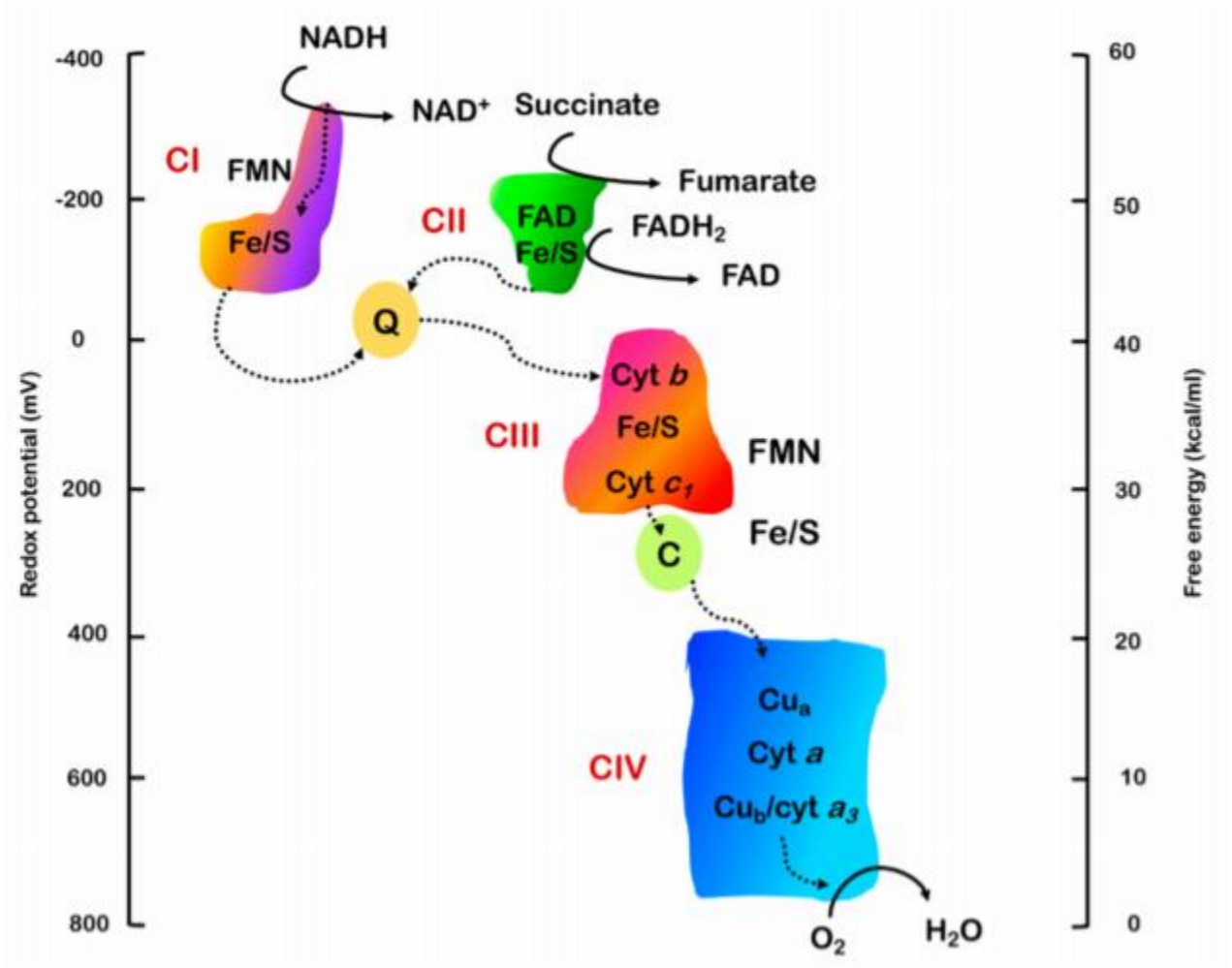


Mitochondria – ATP production

- Oxidative Phosphorylation (OXPHOS)



Electron transportchain

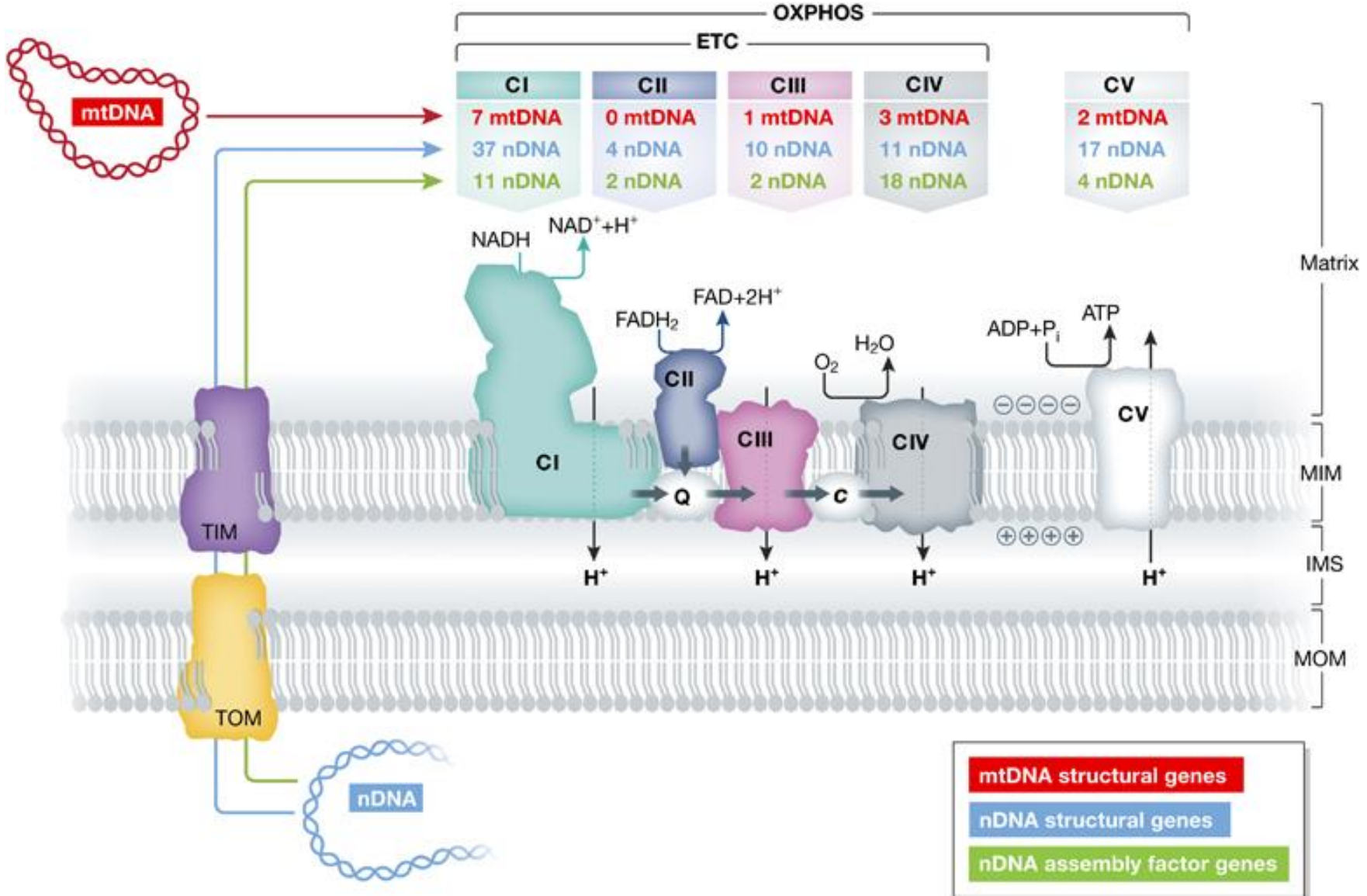


Particularities in mitochondrial disorders

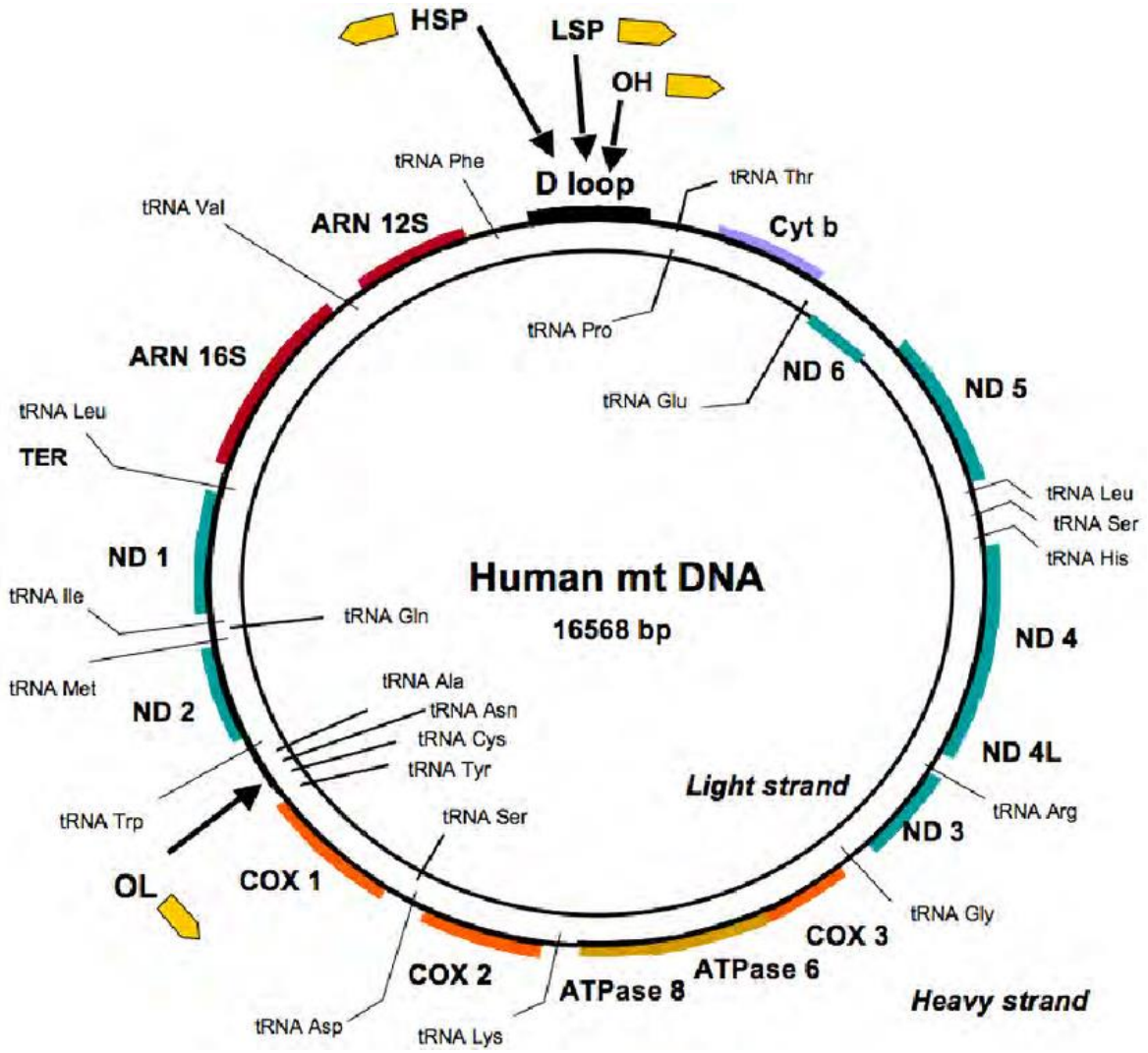
- Dual genomic control: mtDNA en nDNA
- Maternal inheritance VS autosomal recessive/(autosomal dominant)
- Heteroplasmy
- Tissue specific genotype
- Depletion
- Bottleneck phenomenon

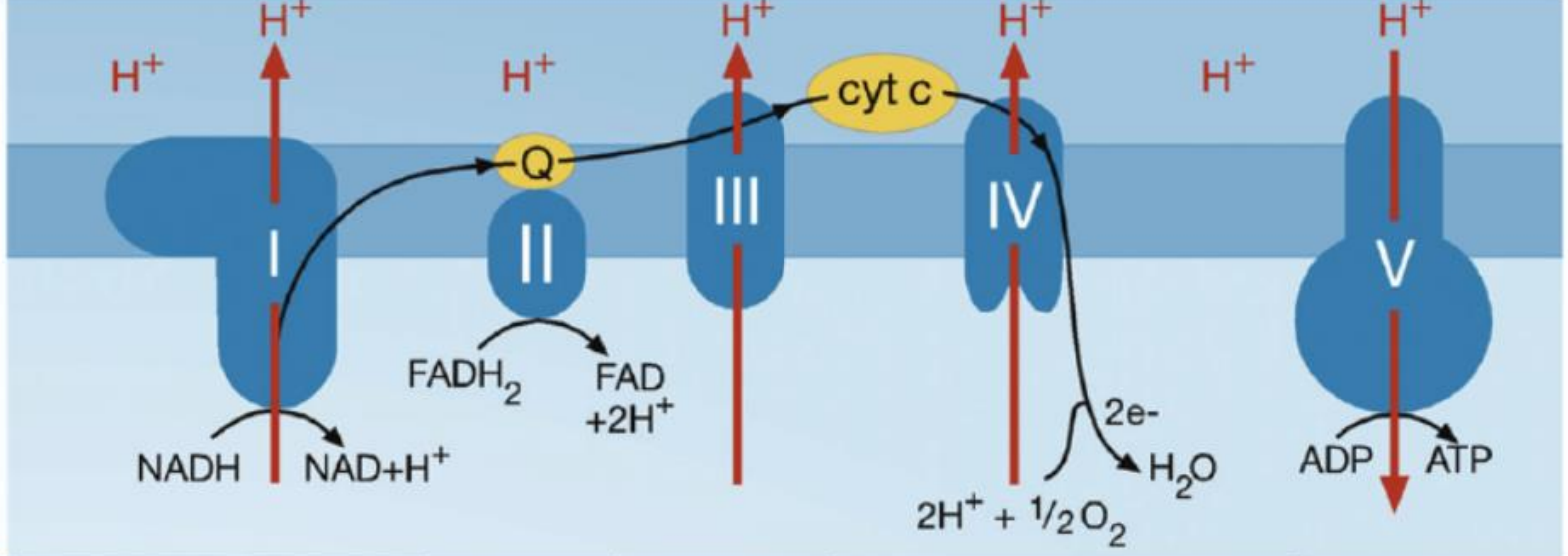
Particularities

- Functions thanks to the concerted action of two genomes



mtDNA


HSP
LSP
OH
D loop
tRNA Phe
tRNA Thr
tRNA Val
ARN 12S
Cyt b
tRNA Pro
ARN 16S
ND 6
tRNA Glu
ND 5
tRNA Leu
TER
tRNA Leu
tRNA Ser
tRNA His
ND 1
Human mt DNA
16568 bp
tRNA Ile
tRNA Gln
ND 4
tRNA Met
tRNA Ala
tRNA Asn
ND 2
tRNA Cys
tRNA Tyr
ND 4L
Light strand
tRNA Arg
tRNA Trp
tRNA Ser
ND 3
OL
COX 1
tRNA Gly
COX 3
ATPase 6
COX 2
ATPase 8
Heavy strand
tRNA Asp
tRNA Lys

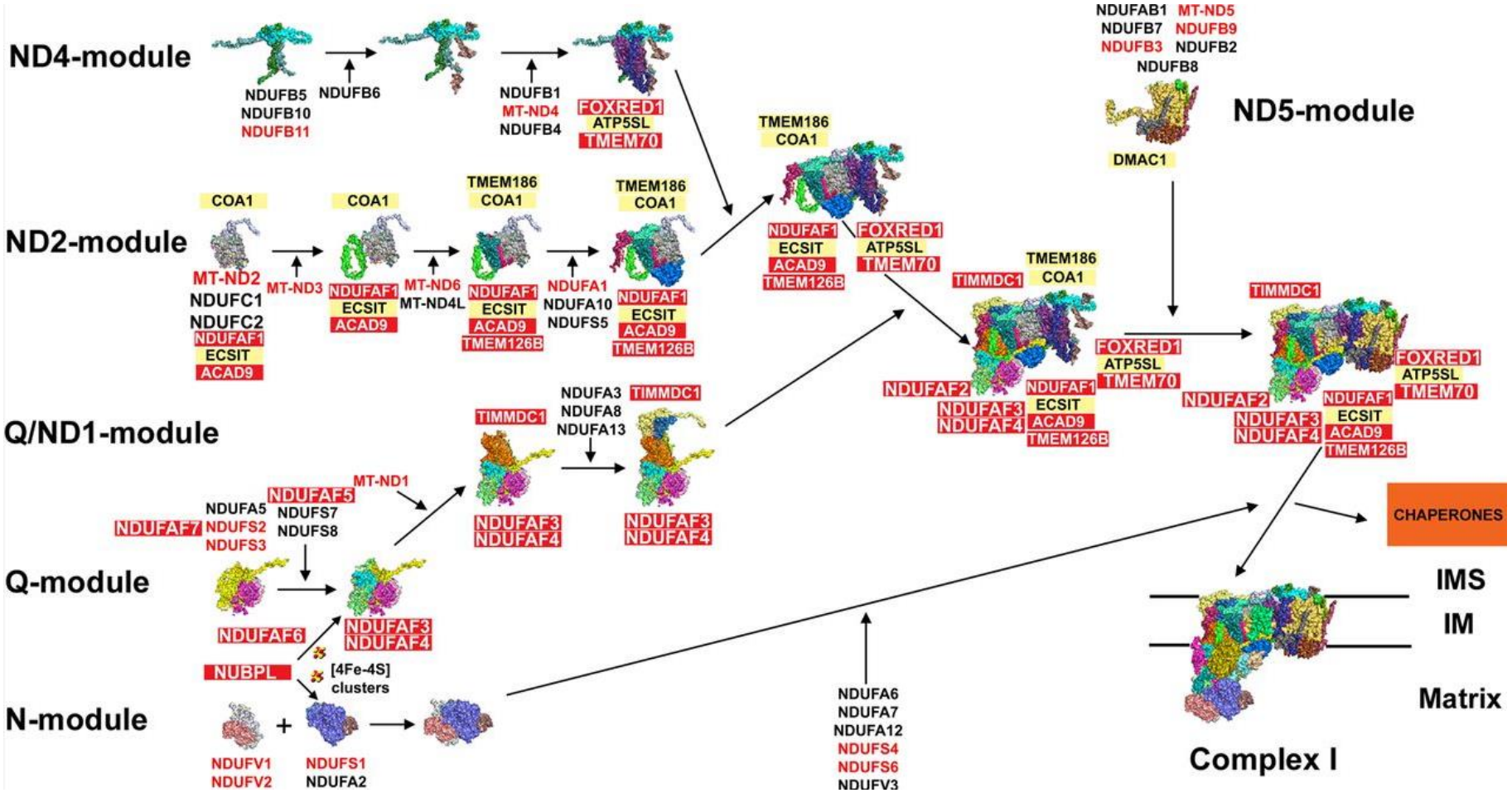


mtDNA

nDNA

OXPHOS Component	Complex I	Complex II	Complex III	Complex IV	Complex V
mtDNA structural subunit genes	***MTND1***[120] ***MTND2***[121] ***MTND3***[122] ***MTND4***[123] ***MTND4L***[124] ***MTND5***[125] ***MTND6***[126]	-	***MTCYB***[127]	***MTCO1***[128] ***MTCO2***[129] ***MTCO3***[130]	***MTATP6***[131] ***MTATP8***[132]
Nuclear structural subunit genes	***NDUFS1***[133] ***NDUFS2***[134] ***NDUFS3***[135] ***NDUFS4***[136] *NDUFS5* ***NDUFS6***[137] ***NDUFS7***[138] ***NDUFS8***[139] ***NDUFA1***[140] ***NDUFA2***[141] *NDUFA3 NDUFA5 NDUFA6 NDUFA7 NDUFA8* ***NDUFA9***[142] ***NDUFA10***[143] ***NDUFA11***[21] ***NDUFA12***[144] ***NDUFA13***[145] *NDUFAB1* ***NDUFV1***[146] ***NDUFV2***[147] ***NDUFV3*** *NDUFB1 NDUFB2* ***NDUFB3***[148] *NDUFB4 NDUFB5 NDUFB6 NDUFB7 NDUFB8* ***NDUFB9***[149] *NDUFB10* ***NDUFB11***[150] *NDUFC1 NDUFC2*	***SDHA***[25] ***SDHB***[151] *SDHC* ***SDHD***[152]	***UQCRB***[153] *UQCRC1* ***CYC1***[156] ***UQCRC2***[154] *UQCRFS1 UQCRH* ***UQCRQ***[155] *UQCR10 UQCR11*	***COX4***[157] *COX5A COX5B* ***COX6A***[57] ***COX6B***[158] *COX6C COX7A* ***COX7B***[159] *COX7C* ***COX8***[160]	***ATP5A1***[76] *ATP5B ATP5C1 ATP5D* ***ATP5E***[161] *ATP5F1 ATP5G1 ATP5G2 ATP5G3* ***ATP5H*** *ATP5I ATP5O ATP5J ATP5J2 ATP5L ATP5L2*
Assembly factor and ancillary protein genes	***NDUFAF1***[162] ***NDUFAF2***[163] ***NDUFAF3***[164] ***NDUFAF4***[165] ***NDUFAF5***[166] ***NDUFAF6***[167] *NDUFAF7* ***FOXRED1***[168] ***ACAD9***[30] *ECSIT* ***NUBPL***[168] ***TMEM126B***[28, 37] *TIMMDC1 C17orf89*	***SDHAF1***[41] *SDHAF2 SDHAF3 SDHAF4*	***BCS1L***[49] ***LYRM7***[169] *UQCC1* ***UQCC2***[170] ***UQCC3***[171] ***TTC19***[172] *PTCD2*	*COA1* ***COA3***[173] *COA4* ***COA5***[174] ***COA6***[175] *COA7* ***COX10***[176] *COX11* ***COX14***[177] ***COX15***[178] *COX16 COX17 COX18 COX19* ***COX20***[179] ***SCO1***[180] ***SCO2***[181] ***SURF1***[182] *PET117* ***LRPPRC***[183] ***PET100***[184] ***CEP89***[185] ***TACO1***[186] *OXA1L* ***APOPT1***[187] ***NDUFA4***[53] ***FASTKD2***[188]	*ATPAF1* ***ATPAF2***[189] ***TMEM70***[58]

Complex I


ND4-module
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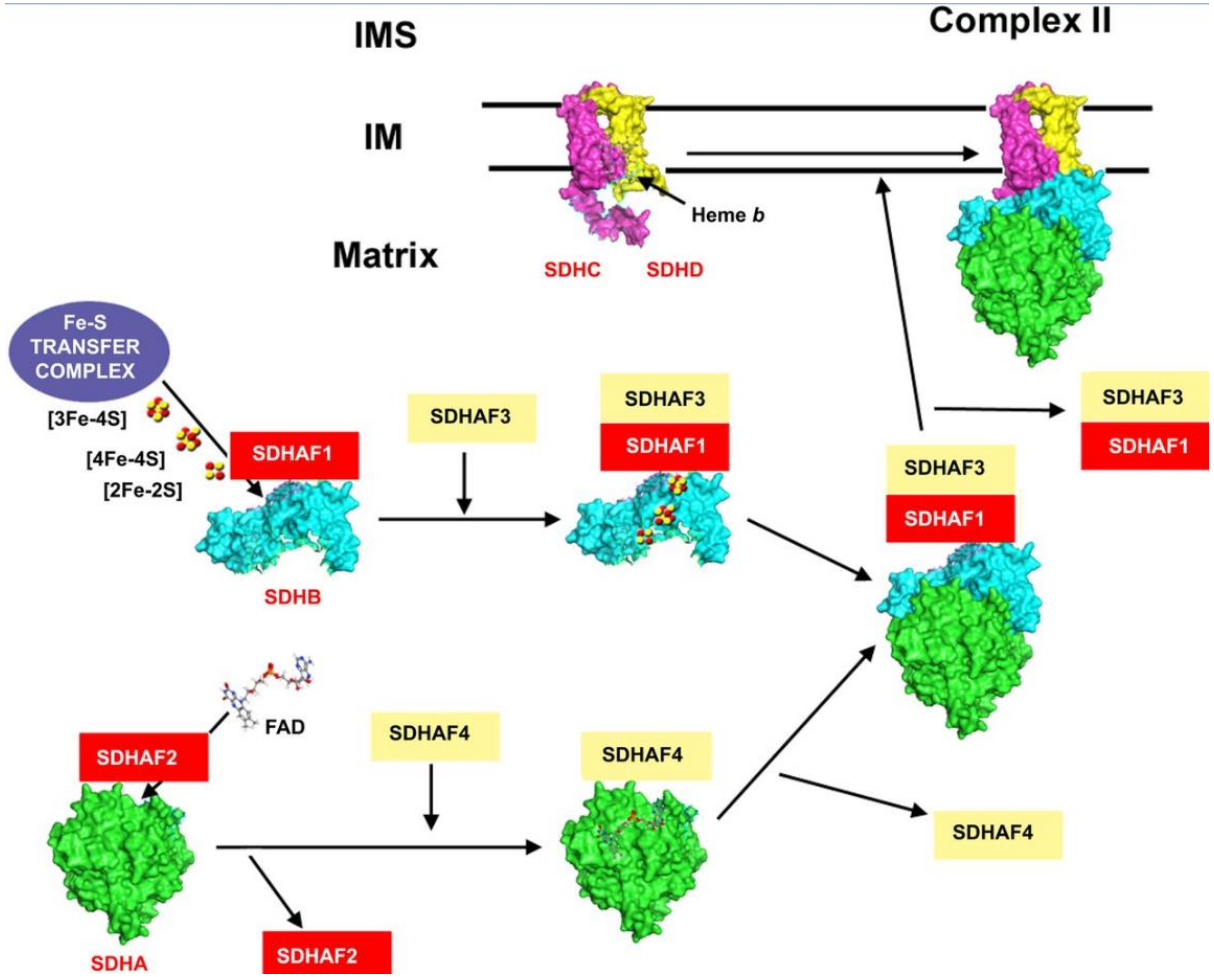
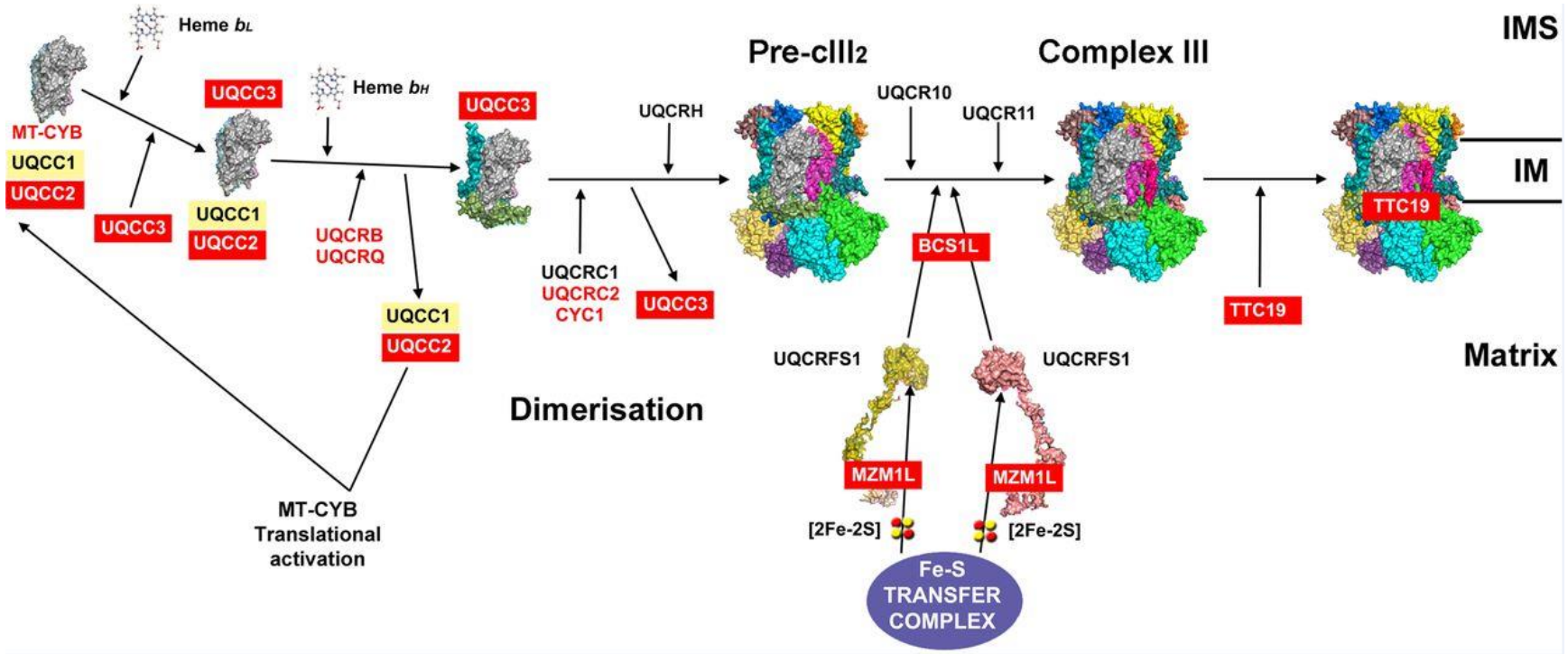
NDUFB10
NDUFB11
NDUFB6
NDUFB1
MT-ND4
NDUFB4
FOXRED1
ATP5SL
TMEM70
ND2-module
COA1
MT-ND2
NDUFC1
NDUFC2
NDUFAF1
ECSIT
ACAD9
MT-ND3
MT-ND6
MT-ND4L
TMEM186
TMEM126B
NDUFA1
NDUFA10
NDUFS5
Q/ND1-module
NDUFA3
NDUFA8
NDUFA13
TIMMDC1
NDUFAF3
NDUFAF4
MT-ND1
NDUFAF5
NDUFA5
NDUFS7
NDUFS8
NDUFAF7
NDUFS2
NDUFS3
Q-module
NDUFAF6
NUBPL
[4Fe-4S]
clusters
N-module
NDUFV1
NDUFV2
NDUFS1
NDUFA2
NDUFA6
NDUFA7
NDUFA12
NDUFS4
NDUFS6
NDUFV3
NDUFAB1
MT-ND5
NDUFB7
NDUFB9
NDUFB3
NDUFB2
NDUFB8
DMAC1
ND5-module
CHAPERONES
IMS
IM
Matrix
Complex I

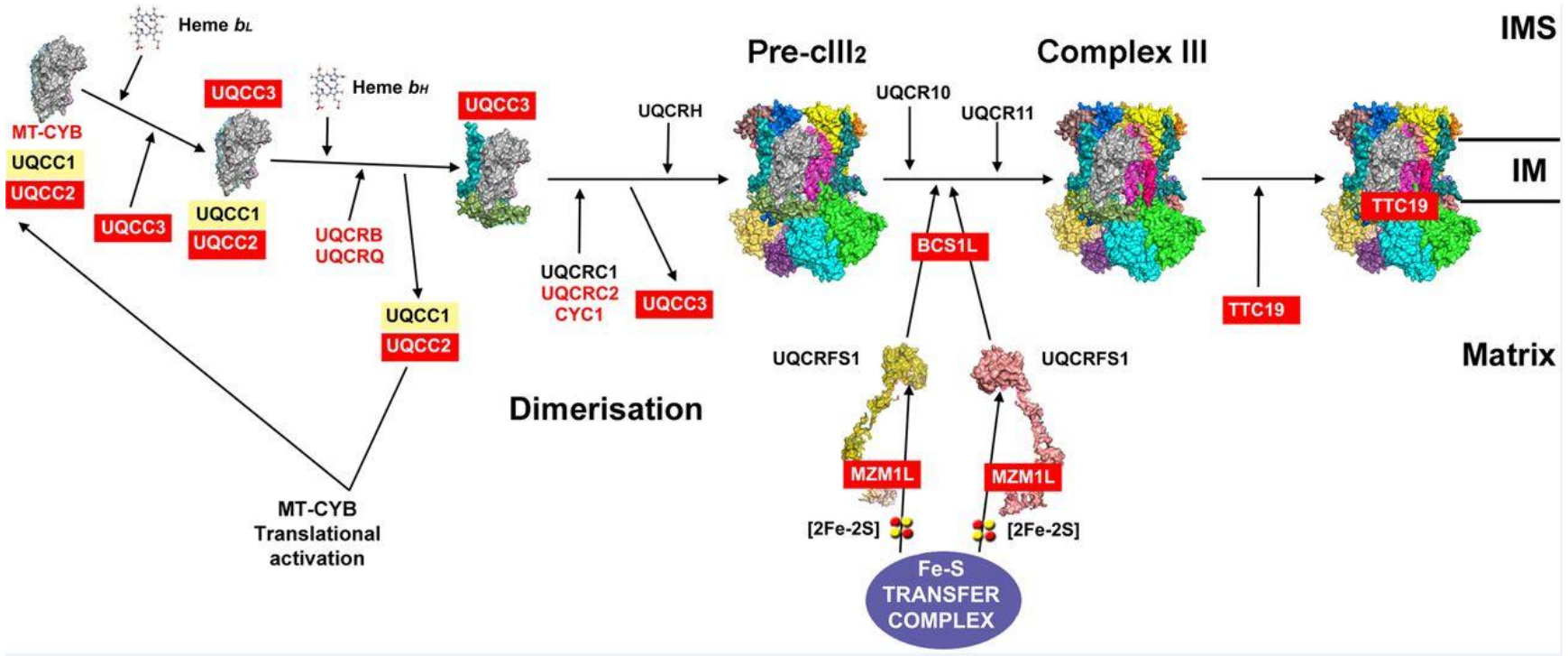
Complex II


IMS
IM
Matrix
Complex II
Heme b
SDHC
SDHD
Fe-S
TRANSFER
COMPLEX
[3Fe-4S]
[4Fe-4S]
[2Fe-2S]
SDHAF1
SDHB
SDHAF3
SDHAF3
SDHAF1
SDHAF3
SDHAF1
SDHAF3
SDHAF1
FAD
SDHAF2
SDHA
SDHAF2
SDHAF4
SDHAF4
SDHAF4

Complex III


Heme b_L
Heme b_H
MT-CYB
UQCC1
UQCC2
UQCC3
UQCC3
UQCC1
UQCC2
UQCRB
UQCRQ
UQCC3
UQCC1
UQCC2
MT-CYB
Translational
activation
UQCRH
UQCRC1
UQCRC2
CYC1
UQCC3
Dimerisation
Pre-cIII2
UQCR10
UQCR11
Complex III
BCS1L
UQCRFS1
UQCRFS1
MZM1L
MZM1L
[2Fe-2S]
[2Fe-2S]
Fe-S
TRANSFER
COMPLEX
TTC19
TTC19
IMS
IM
Matrix

Complex III


Heme b_L
Heme b_H
MT-CYB
UQCC1
UQCC2
UQCC3
UQCC3
UQCC1
UQCC2
UQCRB
UQCRQ
UQCC3
UQCC1
UQCC2
MT-CYB
Translational
activation
UQCRH
UQCRC1
UQCRC2
CYC1
UQCC3
Dimerisation
Pre-cIII2
UQCR10
UQCR11
Complex III
BCS1L
UQCRFS1
UQCRFS1
MZM1L
MZM1L
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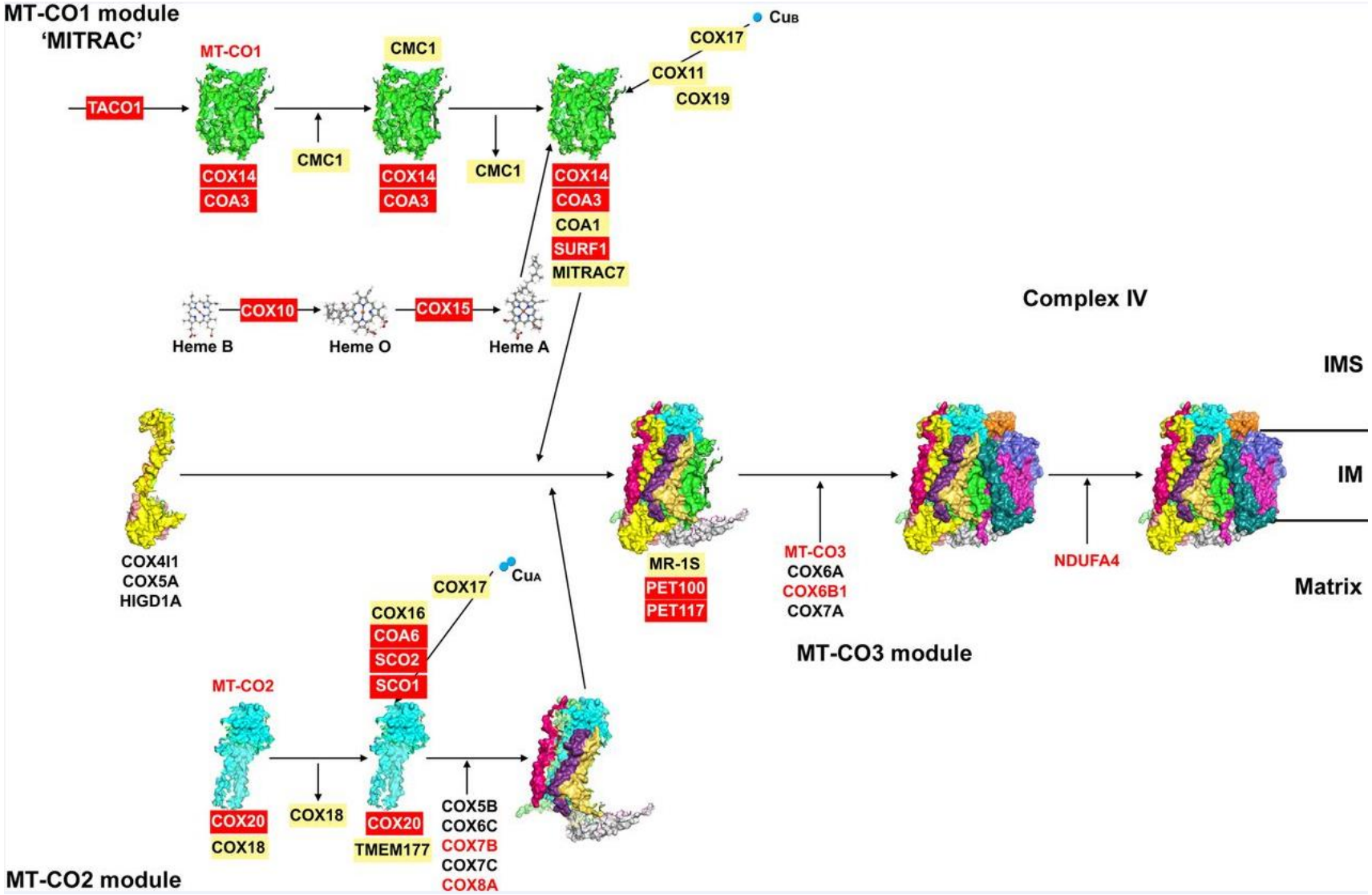
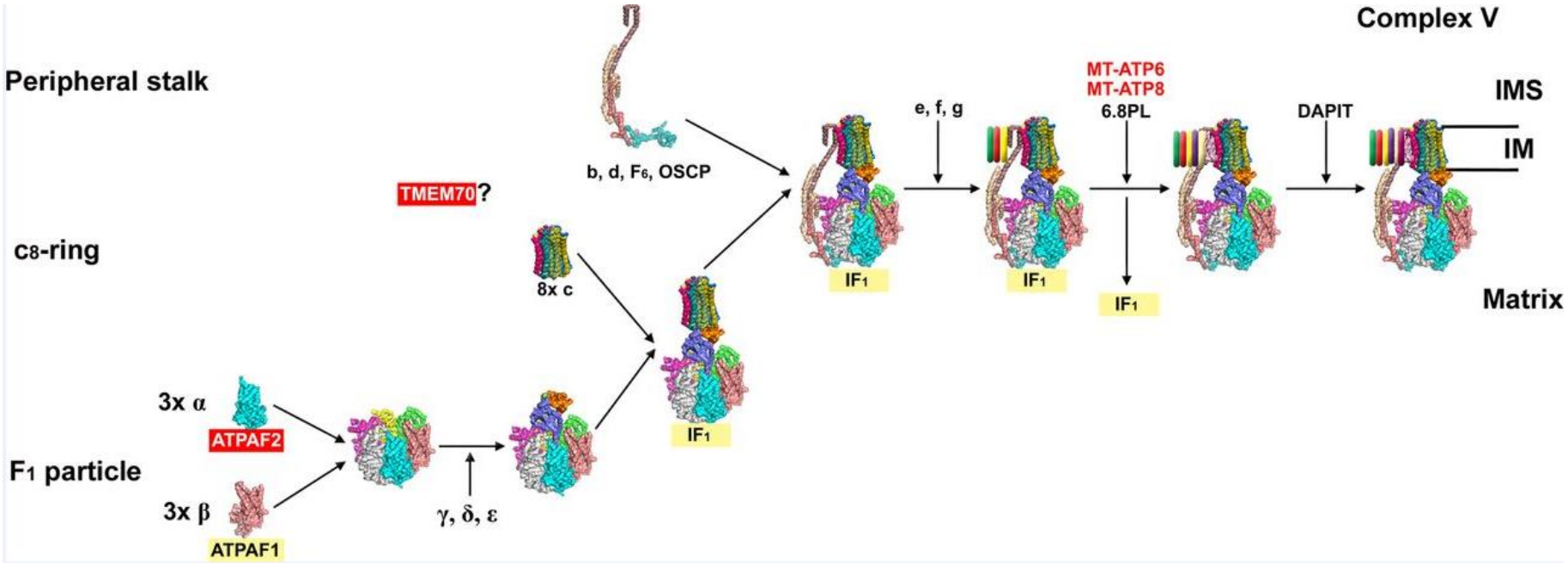
Fe-S
TRANSFER
COMPLEX
TTC19
TTC19
IMS
IM
Matrix

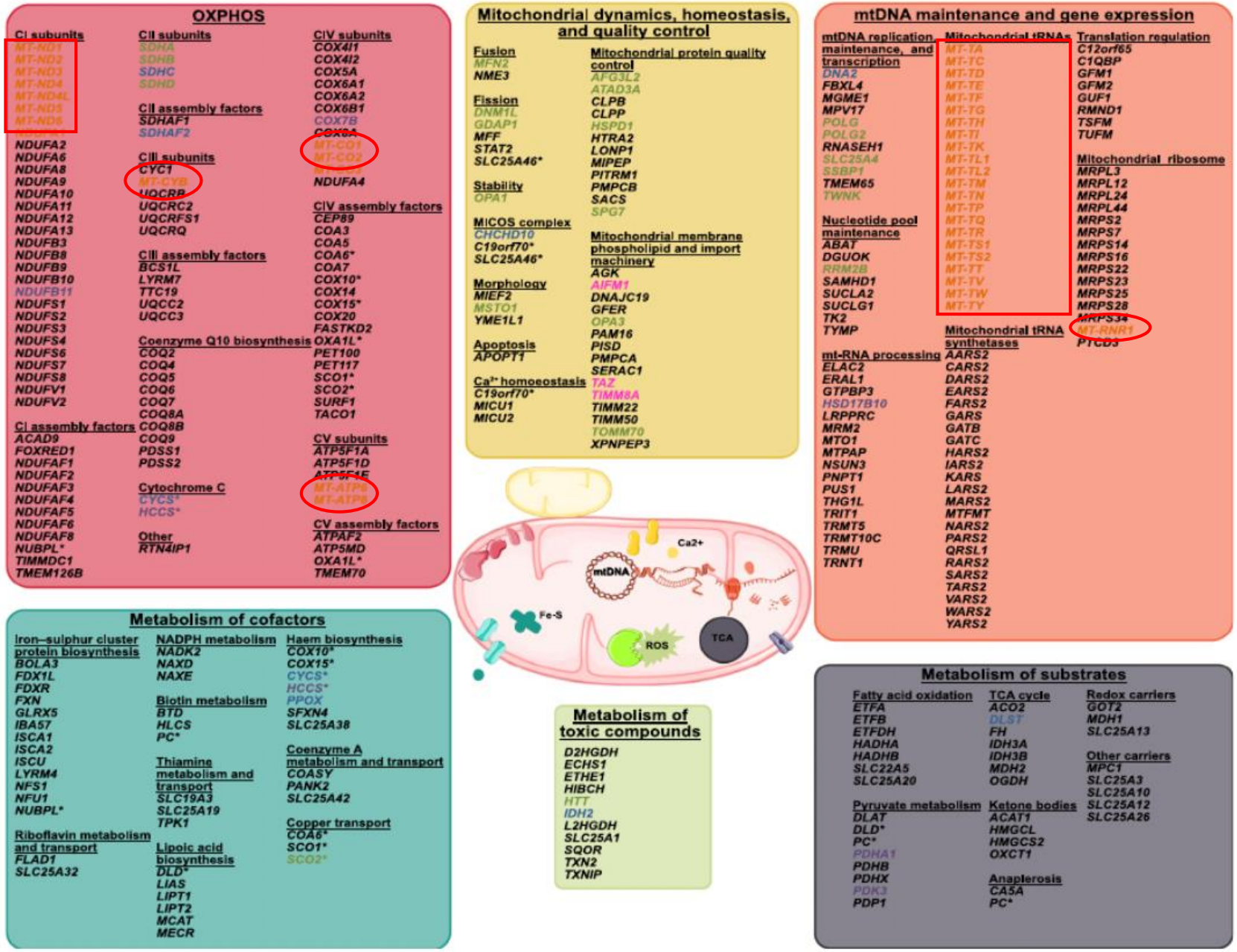
Complex IV


MT-CO1 module
'MITRAC'
MT-CO1
TACO1
CMC1
CMC1
CMC1
COX14
COA3
COX14
COA3
COX14
COA3
COA1
SURF1
MITRAC7
CuB
COX17
COX11
COX19
COX10
COX15
Heme B
Heme O
Heme A
Complex IV
IMS
IM
Matrix
COX4I1
COX5A
HIGD1A
MR-1S
PET100
PET117
MT-CO3
COX6A
COX6B1
COX7A
NDUFA4
MT-CO3 module
COX17
CuA
COX16
COA6
SCO2
SCO1
MT-CO2
COX20
COX18
COX18
COX20
TMEM177
COX5B
COX6C
COX7B
COX7C
COX8A
MT-CO2 module

Complex V


Complex V
Peripheral stalk
b, d, F6, OSCP
TMEM70?
c8-ring
8x c
3x α
ATPAF2
F1 particle
3x β
ATPAF1
γ, δ, ε
IF1
IF1
e, f, g
IF1
MT-ATP6
MT-ATP8
6.8PL
DAPIT
IMS
IM
Matrix

Mitochondrial diseases

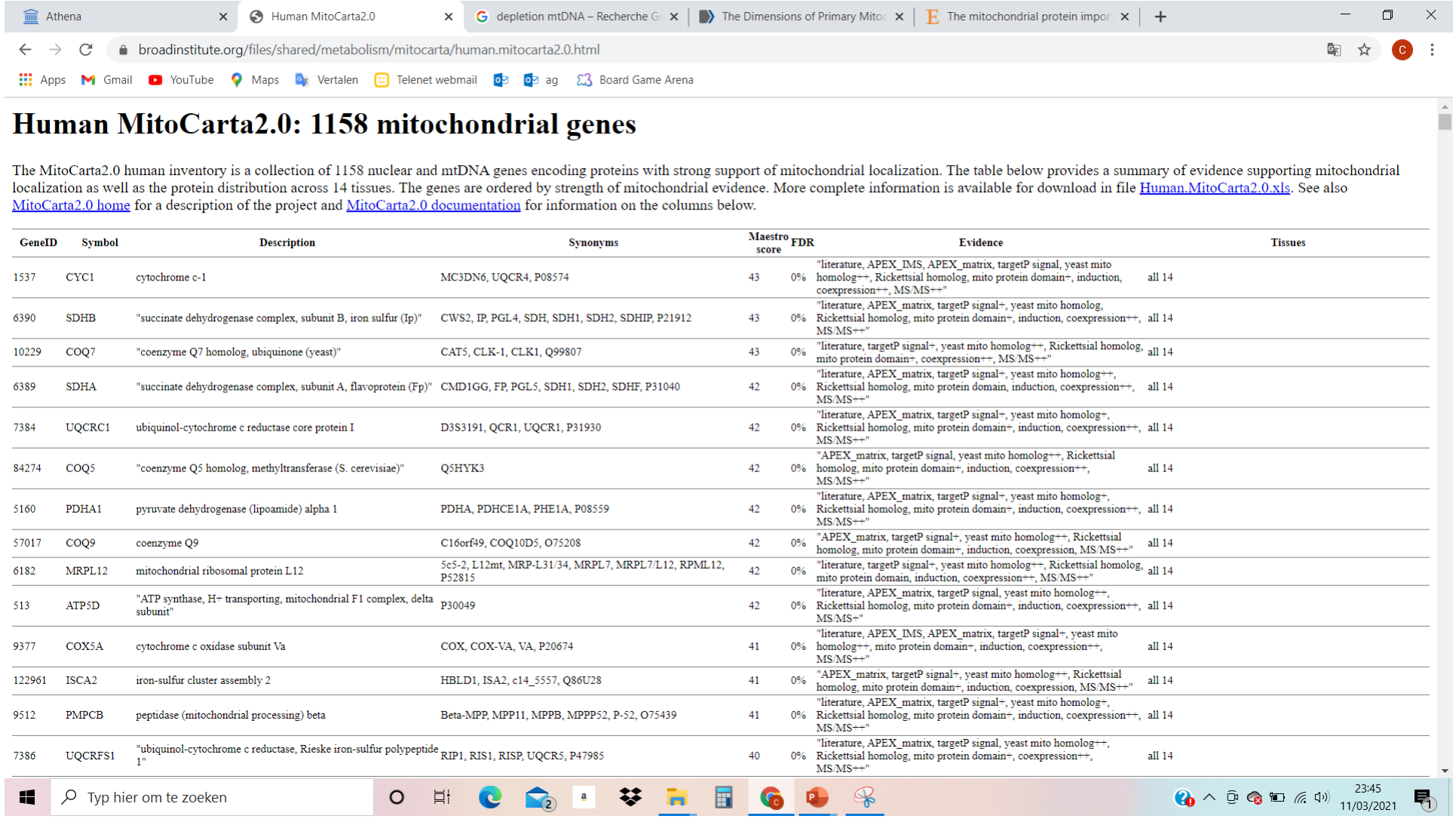


343 disease associated genes

36 mtDNA encoded

Gusic and Prokisch, 2021

Nuclear genes involved in mitochondrial functioning: Mitocarta 2.0



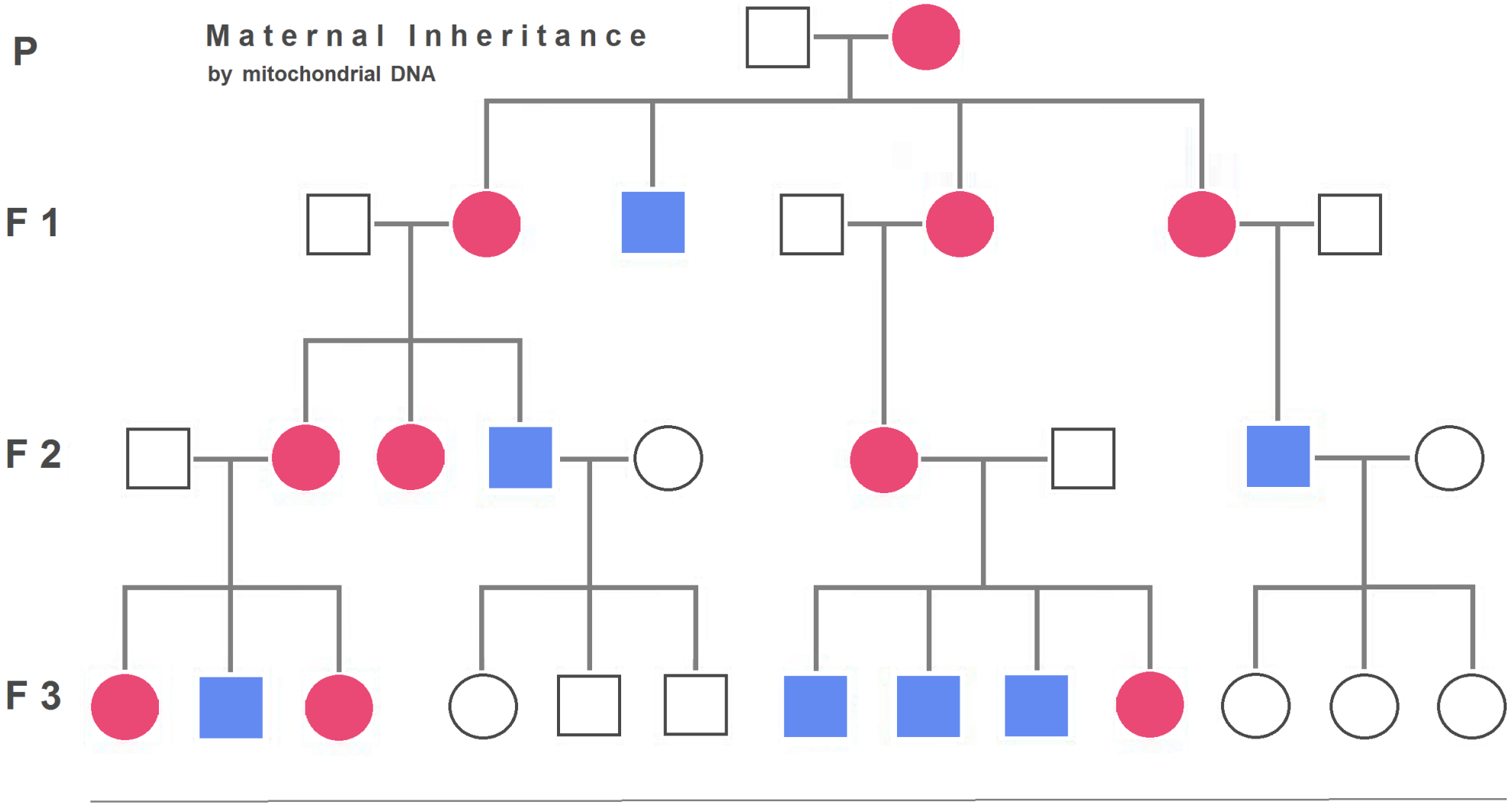
Human MitoCarta2.0: 1158 mitochondrial genes

The MitoCarta2.0 human inventory is a collection of 1158 nuclear and mtDNA genes encoding proteins with strong support of mitochondrial localization. The table below provides a summary of evidence supporting mitochondrial localization as well as the protein distribution across 14 tissues. The genes are ordered by strength of mitochondrial evidence. More complete information is available for download in file Human.MitoCarta2.0.xls. See also MitoCarta2.0 home for a description of the project and MitoCarta2.0 documentation for information on the columns below.

GeneID	Symbol	Description	Synonyms	Maestro score	FDR	Evidence	Tissues
1537	CYC1	cytochrome c-1	MC3DN6, UQCR4, P08574	43	0%	"literature, APEX_IMS, APEX_matrix, targetP signal, yeast mito homolog++, Rickettsial homolog, mito protein domain+, induction, coexpression++, MS/MS++"	all 14
6390	SDHB	"succinate dehydrogenase complex, subunit B, iron sulfur (Ip)"	CWS2, IP, PGL4, SDH, SDH1, SDH2, SDHIP, P21912	43	0%	"literature, APEX_matrix, targetP signal+, yeast mito homolog, Rickettsial homolog, mito protein domain+, induction, coexpression++, MS/MS++"	all 14
10229	COQ7	"coenzyme Q7 homolog, ubiquinone (yeast)"	CAT5, CLK-1, CLK1, Q99807	43	0%	"literature, targetP signal+, yeast mito homolog++, Rickettsial homolog, mito protein domain+, coexpression++, MS/MS++"	all 14
6389	SDHA	"succinate dehydrogenase complex, subunit A, flavoprotein (Fp)"	CMD1GG, FP, PGL5, SDH1, SDH2, SDHF, P31040	42	0%	"literature, APEX_matrix, targetP signal+, yeast mito homolog++, Rickettsial homolog, mito protein domain, induction, coexpression++, MS/MS++"	all 14
7384	UQCRC1	ubiquinol-cytochrome c reductase core protein I	D3S3191, QCR1, UQCR1, P31930	42	0%	"literature, APEX_matrix, targetP signal+, yeast mito homolog+, Rickettsial homolog, mito protein domain+, induction, coexpression++, MS/MS++"	all 14
84274	COQ5	"coenzyme Q5 homolog, methyltransferase (S. cerevisiae)"	Q5HYK3	42	0%	"APEX_matrix, targetP signal, yeast mito homolog++, Rickettsial homolog, mito protein domain+, induction, coexpression++, MS/MS++"	all 14
5160	PDHA1	pyruvate dehydrogenase (lipoamide) alpha 1	PDHA, PDHCE1A, PHE1A, P08559	42	0%	"literature, APEX_matrix, targetP signal+, yeast mito homolog+, Rickettsial homolog, mito protein domain+, induction, coexpression++, MS/MS++"	all 14
57017	COQ9	coenzyme Q9	C16orf49, COQ10D5, O75208	42	0%	"APEX_matrix, targetP signal+, yeast mito homolog++, Rickettsial homolog, mito protein domain+, induction, coexpression, MS/MS++"	all 14
6182	MRPL12	mitochondrial ribosomal protein L12	5c5-2, L12mt, MRP-L31/34, MRPL7, MRPL7/L12, RPML12, P52815	42	0%	"literature, targetP signal+, yeast mito homolog++, Rickettsial homolog, mito protein domain, induction, coexpression++, MS/MS++"	all 14
513	ATP5D	"ATP synthase, H+ transporting, mitochondrial F1 complex, delta subunit"	P30049	42	0%	"literature, APEX_matrix, targetP signal, yeast mito homolog++, Rickettsial homolog, mito protein domain+, induction, coexpression++, MS/MS+"	all 14
9377	COX5A	cytochrome c oxidase subunit Va	COX, COX-VA, VA, P20674	41	0%	"literature, APEX_IMS, APEX_matrix, targetP signal+, yeast mito homolog++, mito protein domain+, induction, coexpression++, MS/MS++"	all 14
122961	ISCA2	iron-sulfur cluster assembly 2	HBLD1, ISA2, c14_5557, Q86U28	41	0%	"APEX_matrix, targetP signal+, yeast mito homolog++, Rickettsial homolog, mito protein domain+, induction, coexpression, MS/MS++"	all 14
9512	PMPCB	peptidase (mitochondrial processing) beta	Beta-MPP, MPP11, MPPB, MPPP52, P-52, O75439	41	0%	"literature, APEX_matrix, targetP signal+, yeast mito homolog+, Rickettsial homolog, mito protein domain+, induction, coexpression++, MS/MS++"	all 14
7386	UQCRFS1	"ubiquinol-cytochrome c reductase, Rieske iron-sulfur polypeptide 1"	RIP1, RIS1, RISP, UQCR5, P47985	40	0%	"literature, APEX_matrix, targetP signal, yeast mito homolog++, Rickettsial homolog, mito protein domain+, coexpression++, MS/MS++"	all 14

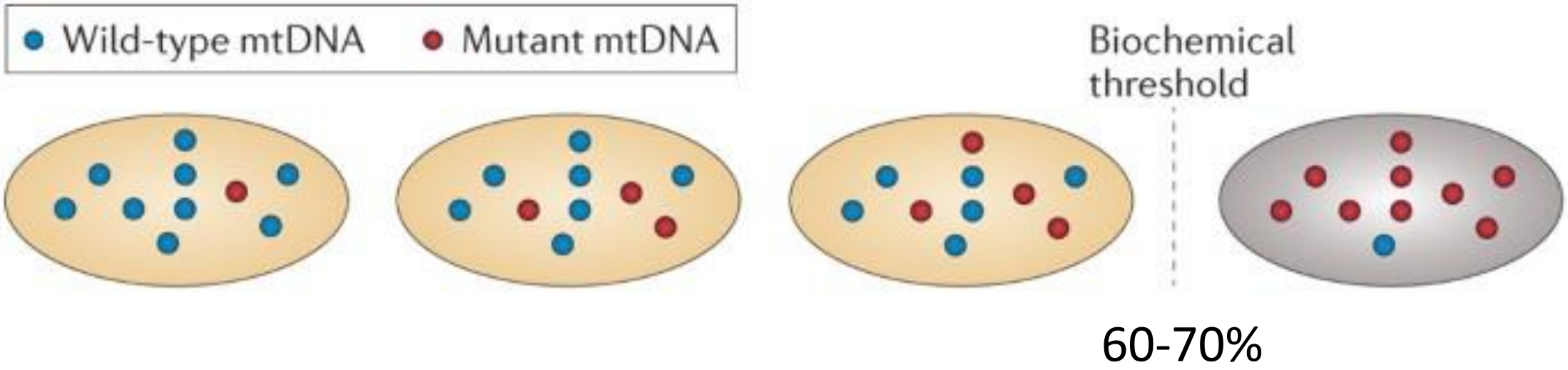
Particularities

- Maternal inheritage for mtDNA

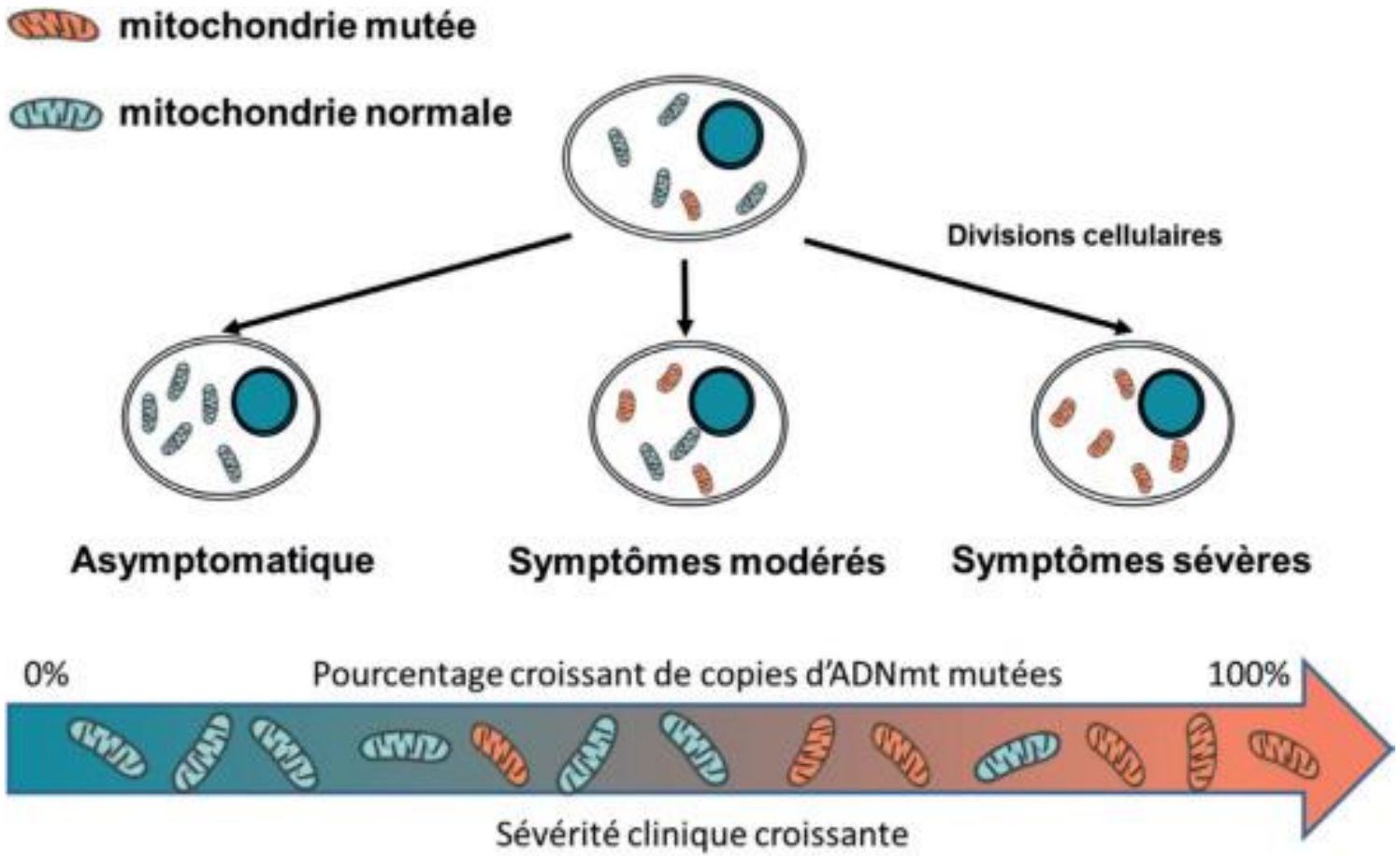


Particularities

- Homoplasmy - heteroplasmy



Heteroplasmy

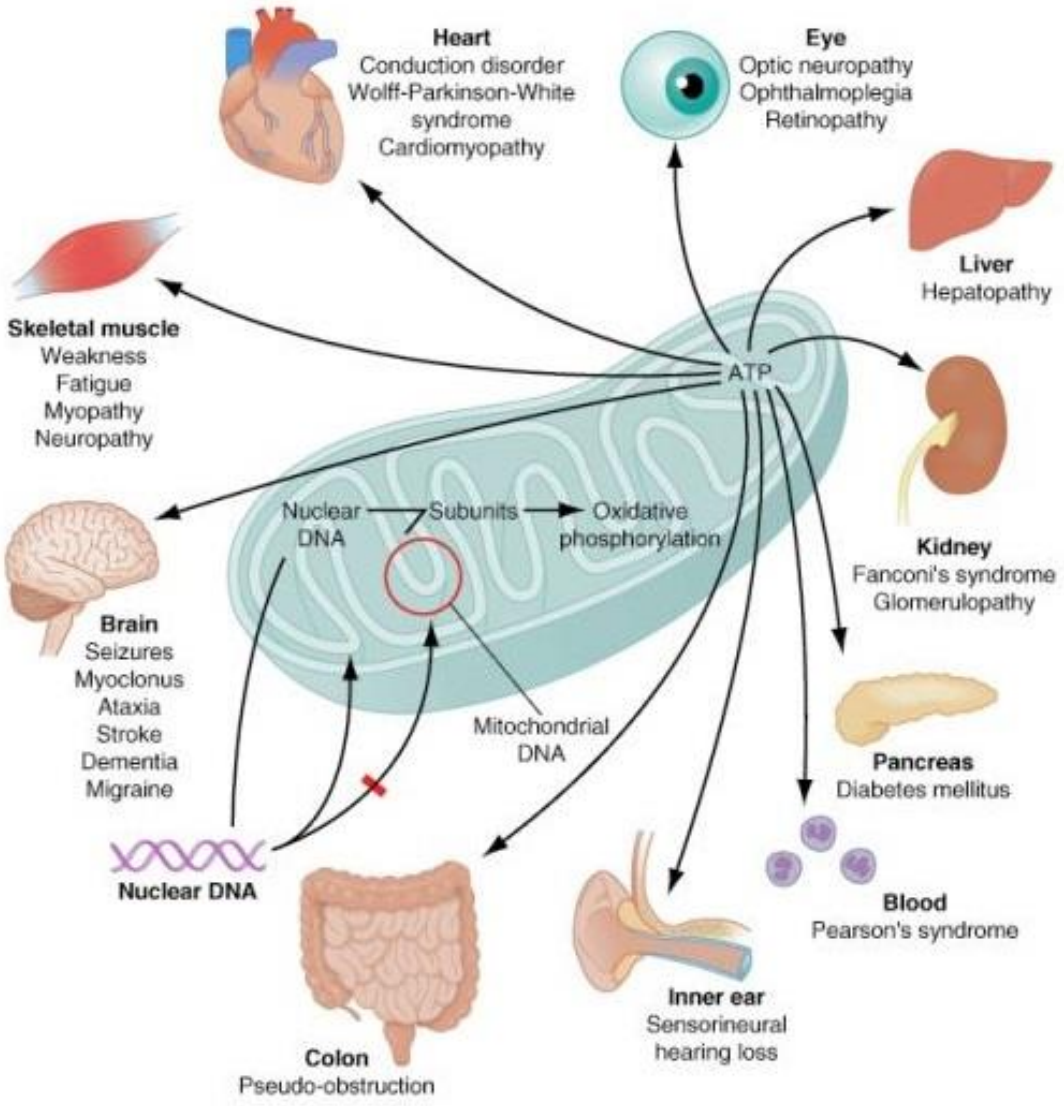


Particularities

Tissue specificity

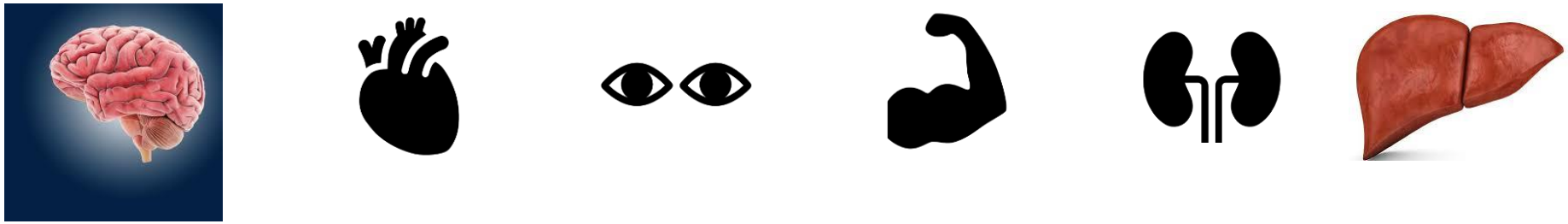
Multisystemic diseases

targeted diagnostics
- biochemical
- molecular



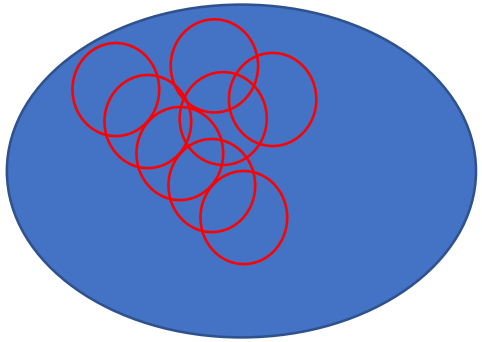
Fauci AS, Kasper DL, et al.: Harrison's Principles of Internet Medicine, 17th Edition

Heteroplasmy – tissue specificity



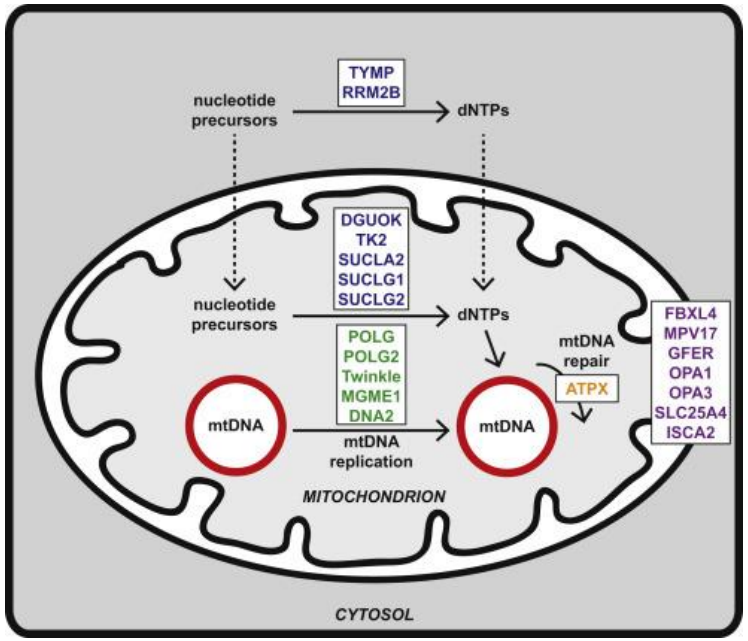
Importance for diagnostics: Think of relevance of mtDNA diagnostics in leukocytes?

Depletion



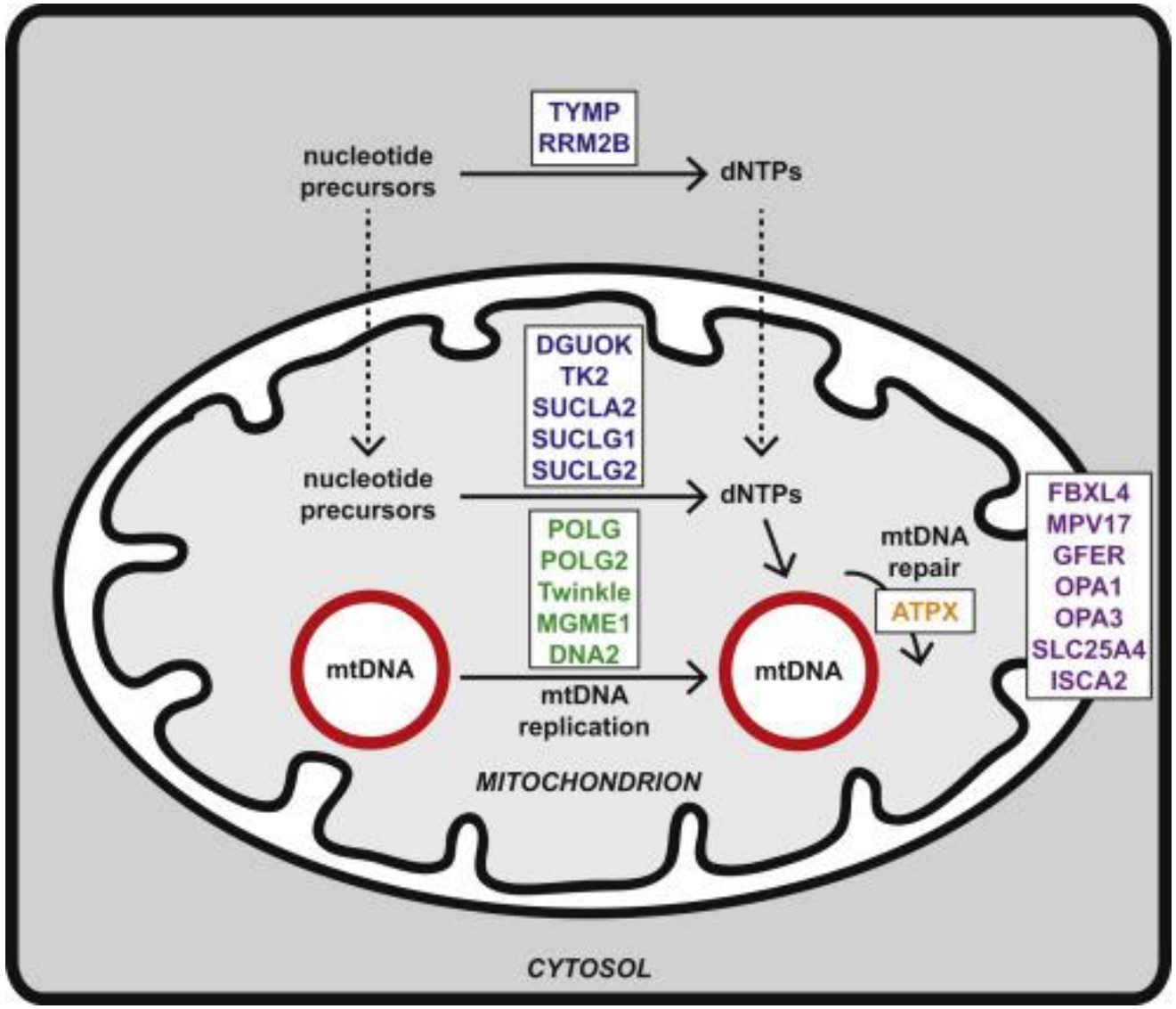
Normal # mtDNA copies

mtDNA depletie



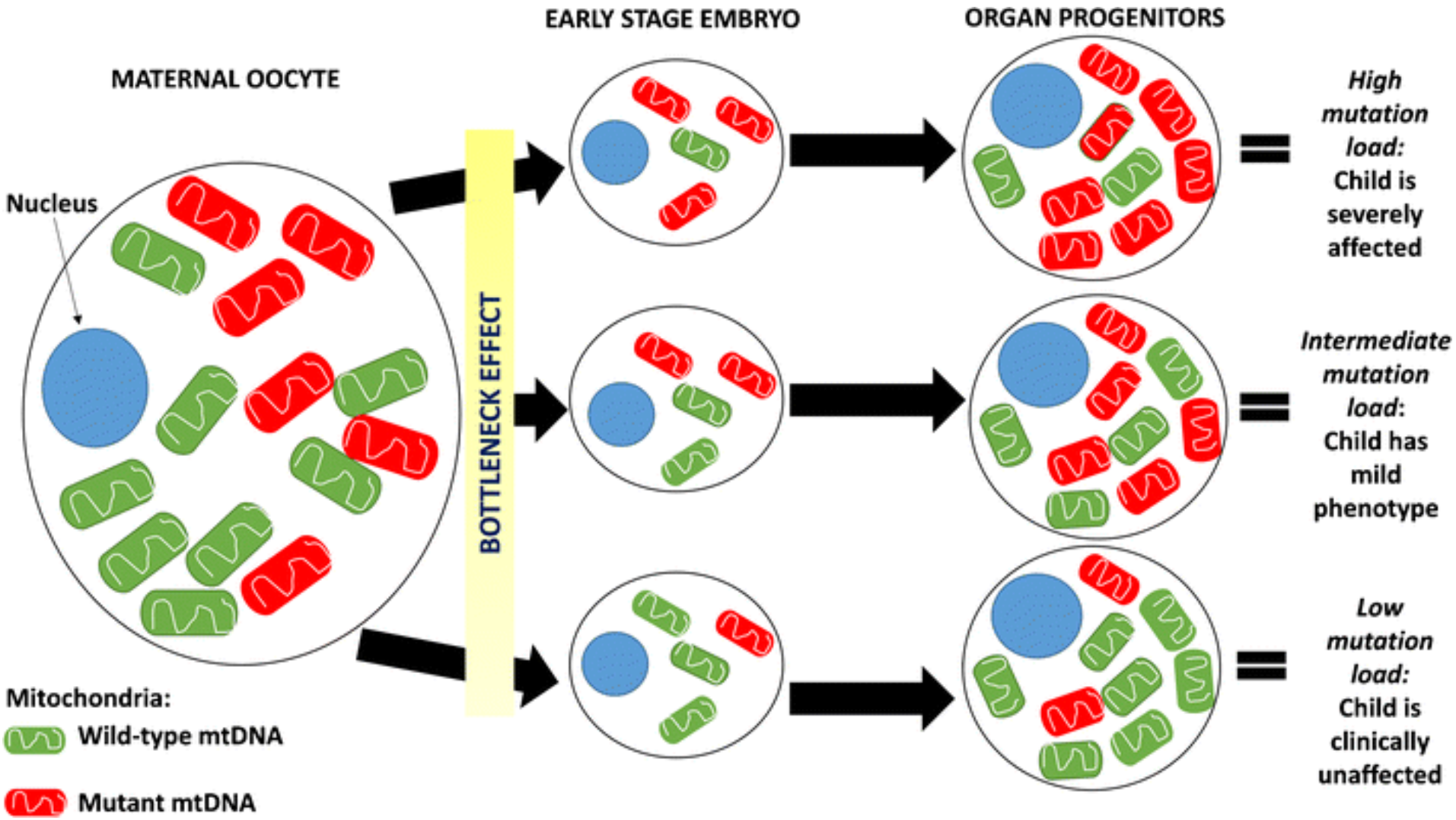
Depletion associated genes

All nuclearly encoded

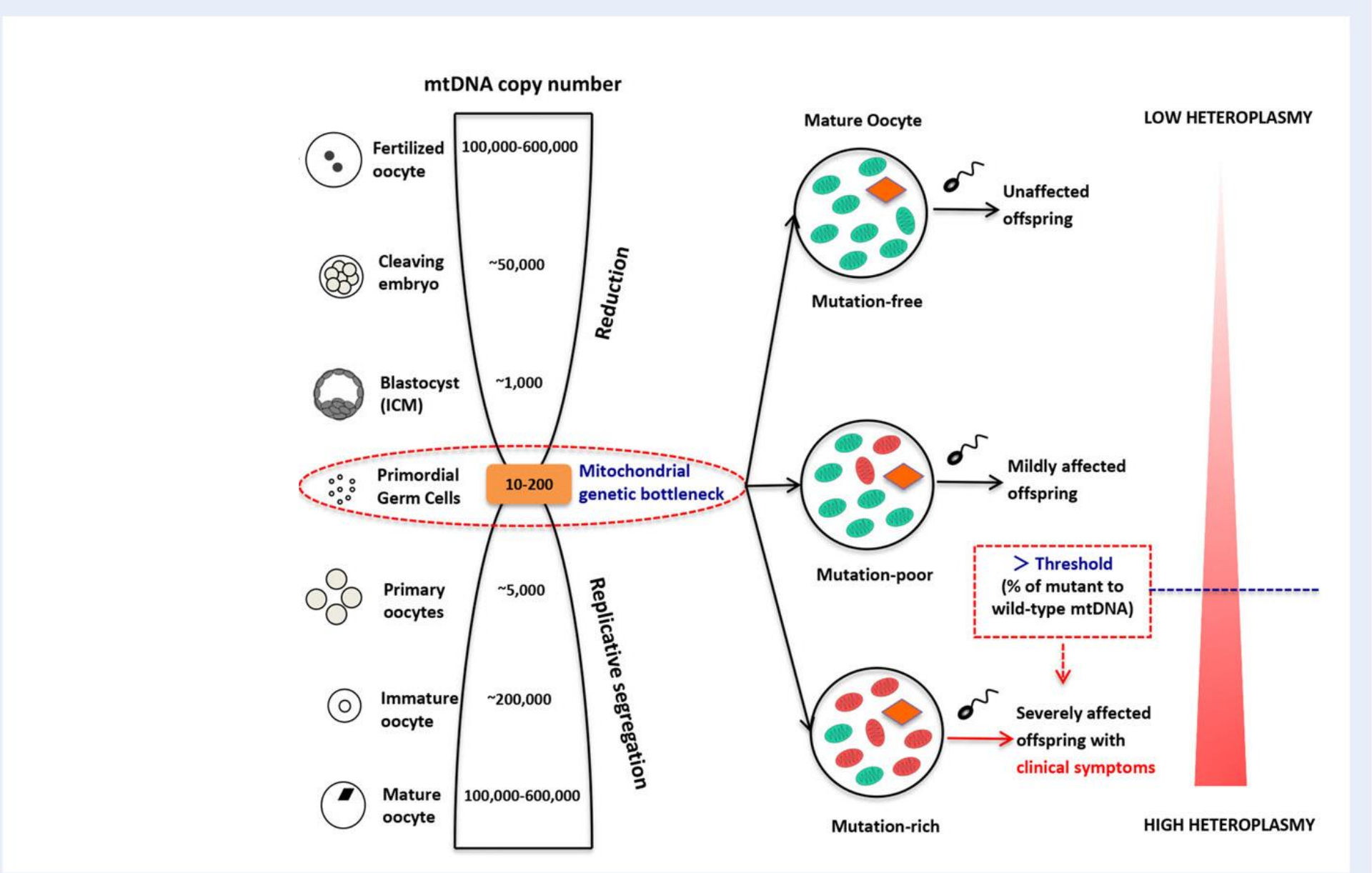


Particularities

- Bottleneck phenomenon



Bottleneck phenomenon



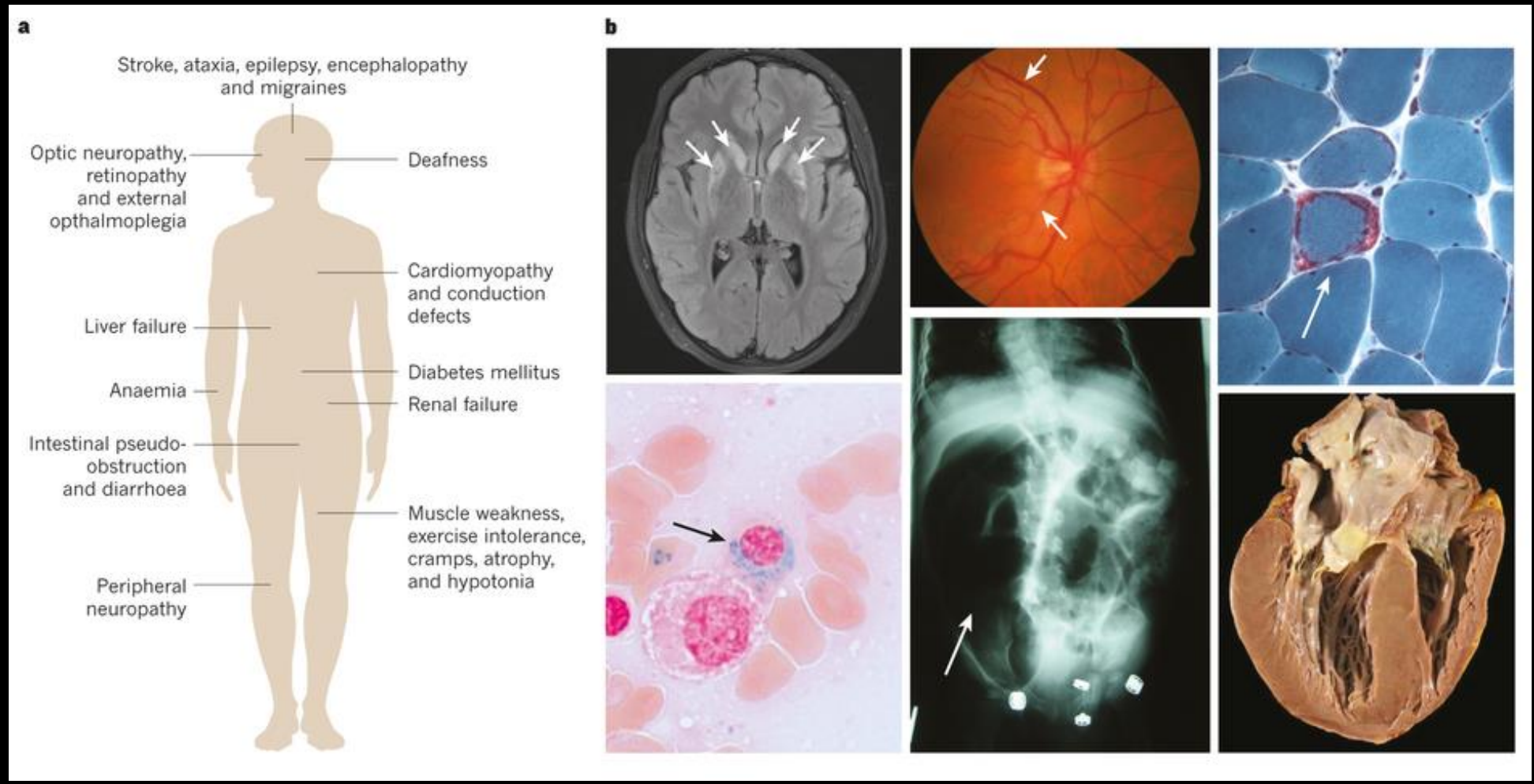
Mitochondrial diseases

- Prevalence: >1/5000 *

- Variable age of onset (neonatal – late adulthood)
- Often multisystemic
- Progressive

- No curative treatment

* Ng and Turnbull, 2016


a
Stroke, ataxia, epilepsy, encephalopathy and migraines
Optic neuropathy, retinopathy and external opthalmoplegia
Deafness
Cardiomyopathy and conduction defects
Liver failure
Diabetes mellitus
Anaemia
Renal failure
Intestinal pseudo-obstruction and diarrhoea
Muscle weakness, exercise intolerance, cramps, atrophy, and hypotonia
Peripheral neuropathy
b

Mitochondrial diseases

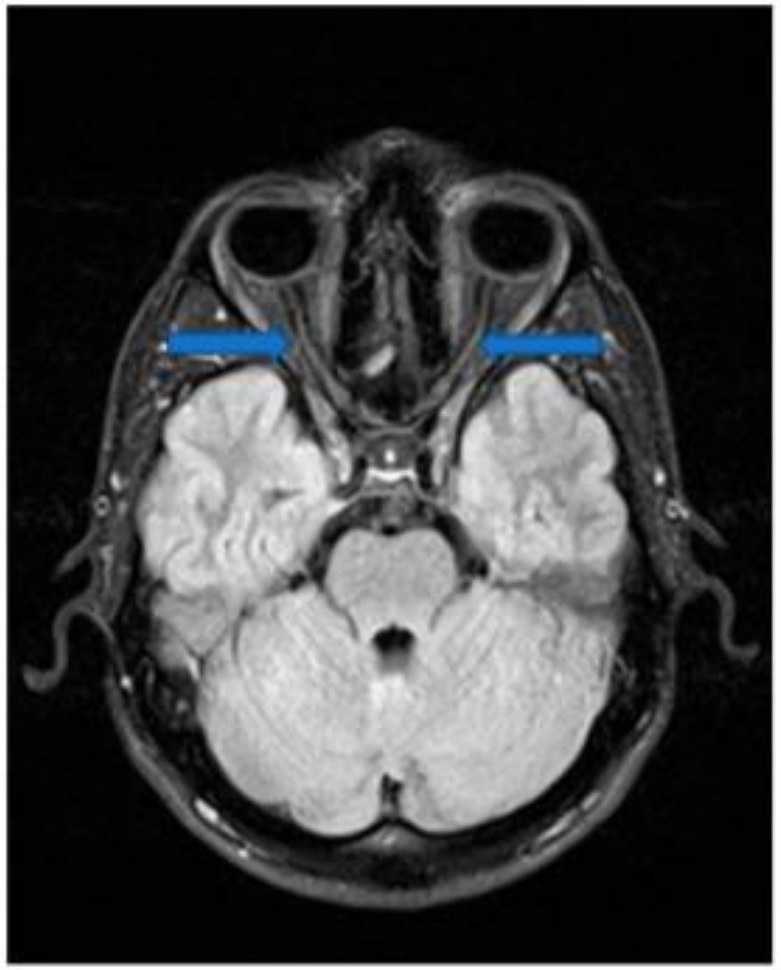
mtDNA encoded defeciencies

Mitochondrial diseases: LHON (Leber Hereditary Optic Neuropathy)

MT-ND1, MT-ND2, MT-ND3, MT-ND4, MT-ND4l, MT-ND5, MT-ND6, MT-CYB, MT-CO1, MT-CO3, MT-ATP6

<u>Clinical presentation</u>

Subacute vision loss
Male predominance
Young onset (15-35y)



Mitochondrial diseases: nonsyndromic deafness

M T - R N R 1

Clinical presentation

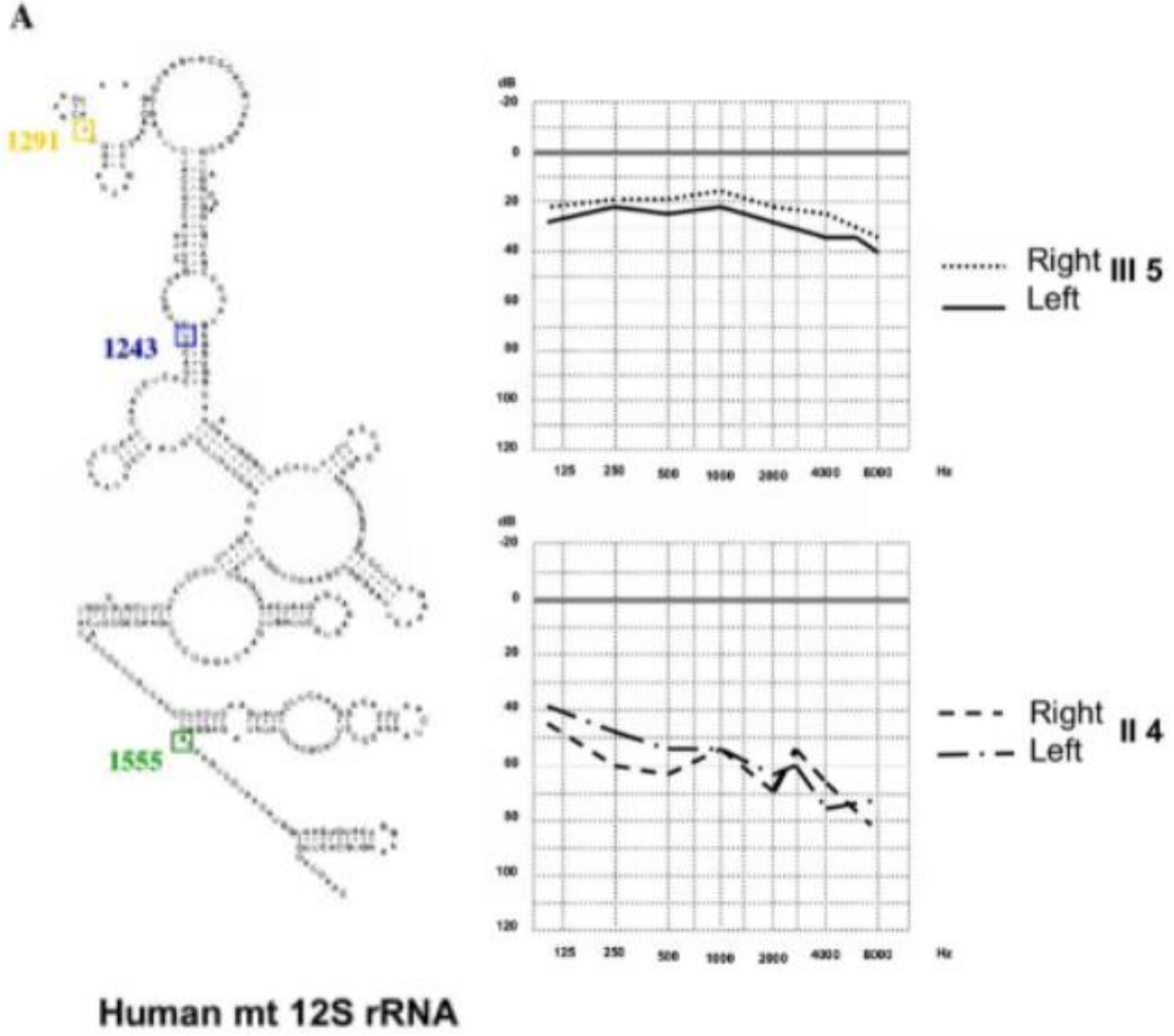
Deafness (isolated)

Sometimes caused by aminoglycoside trea

m.1555A>G

m.1243T>C *MT-RNR1*

m.1291T>C



Mitochondrial diseases: Leigh syndrome

MT-ND2, MT-ND3, MT-ND5, MT-ND6, MT-ATP6, MT-CO3, MT-TK

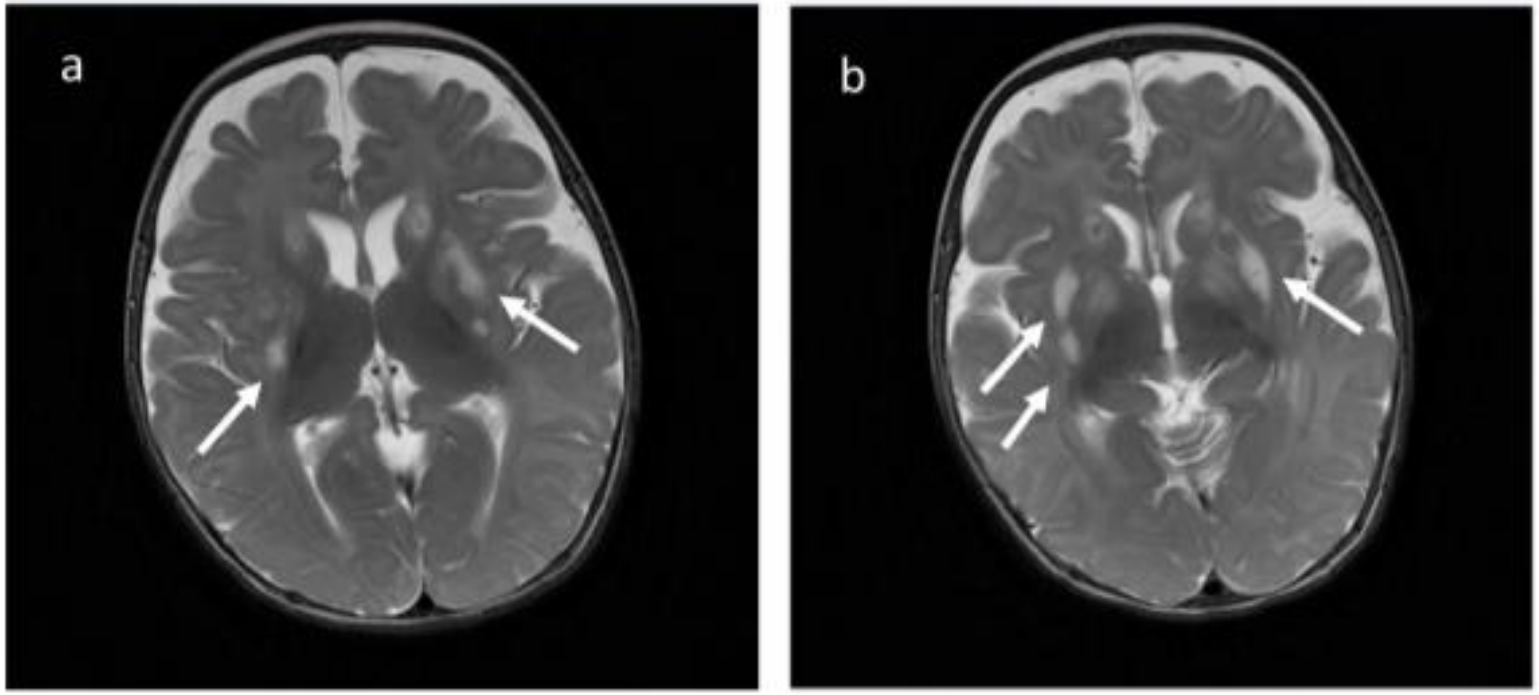
Many nuclear encoded genes

Clinical presentation

Typical symmetrical basal ganglia MRI lesions

1) Childhood presentation
- subacute necrotising encephalopathy
- developmental delay
- hypotonia
- epilepsy

2) Adult onset
- psychiatric features
- headache
- movement disorders



Baldo and Vilarinho, 2020

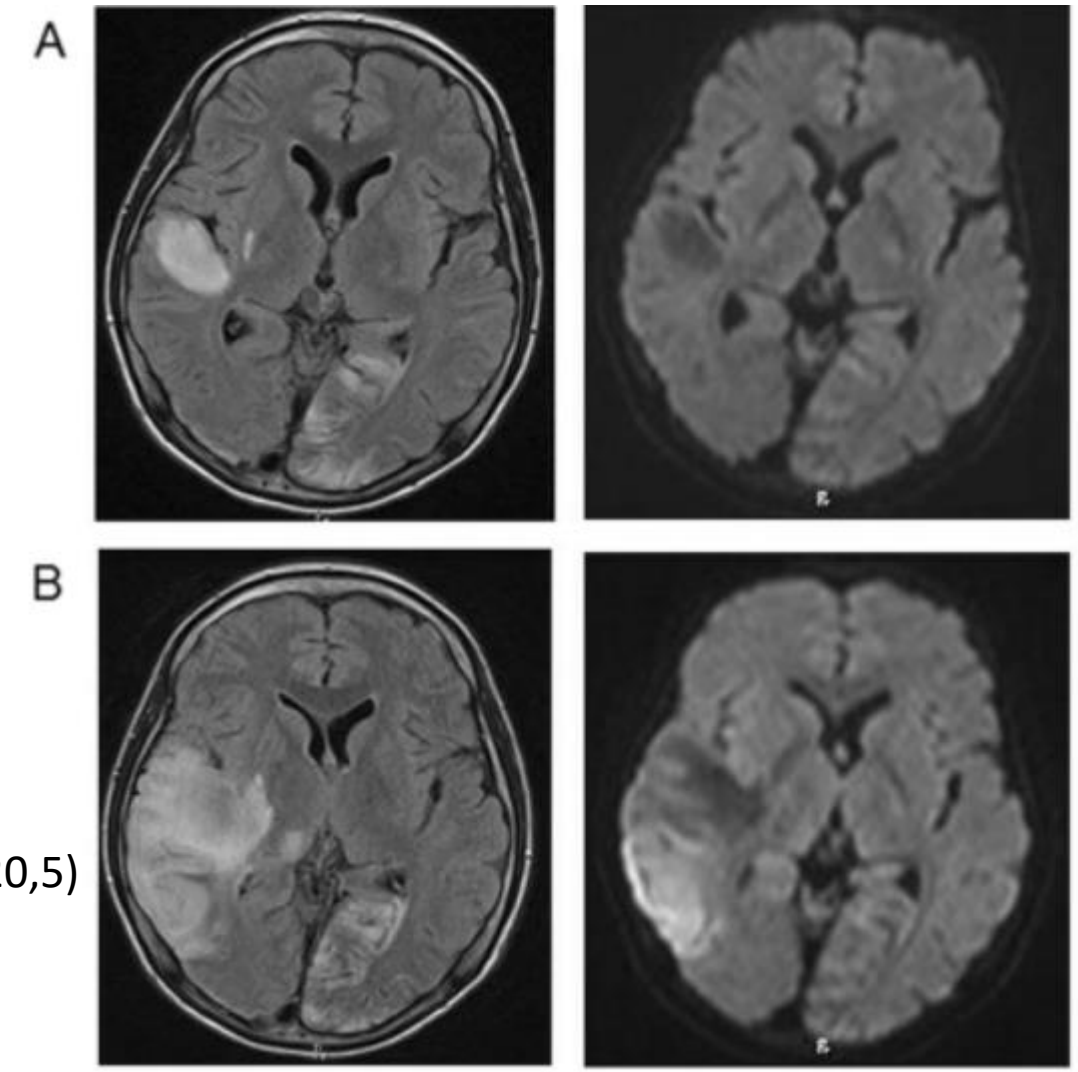
Mitochondrial diseases: MELAS

M	mitochondrial myopathy
E	encephalopathy
L	lactic
A	acidosis
S	stroke like episodes

MT-TL1, *MT-TQ*, *MT-TH*, *MT-TC*, *MT-TS1*, *MT-TS2*, *MT-TK*, *MT-ND1*, *MT-ND5*, *MT-ND6*

Clinical presentation

- teenage focal epilepsy
- progressive muscle weakness
- progressive cognitive decline
- cerebellar symptoms



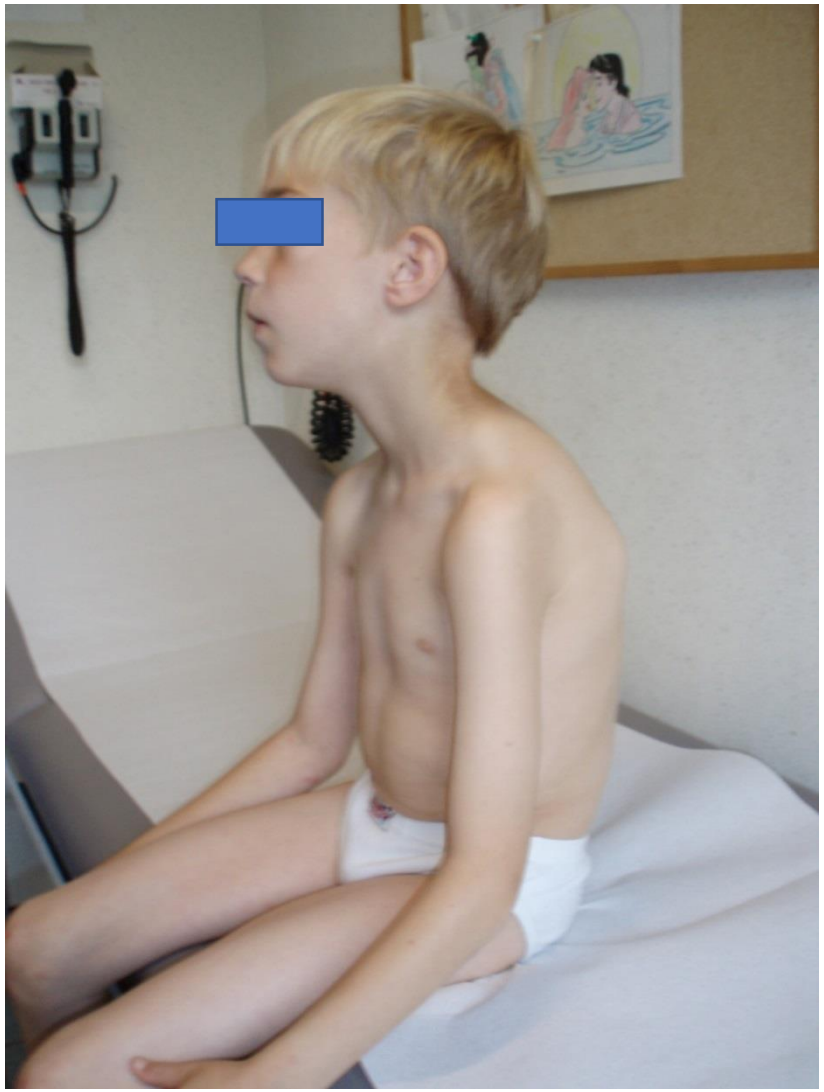
m.3243 A>G, *MT-TL1,*
22% blood
35% skin fibroblasts
37% saliva

Tissue specifity !

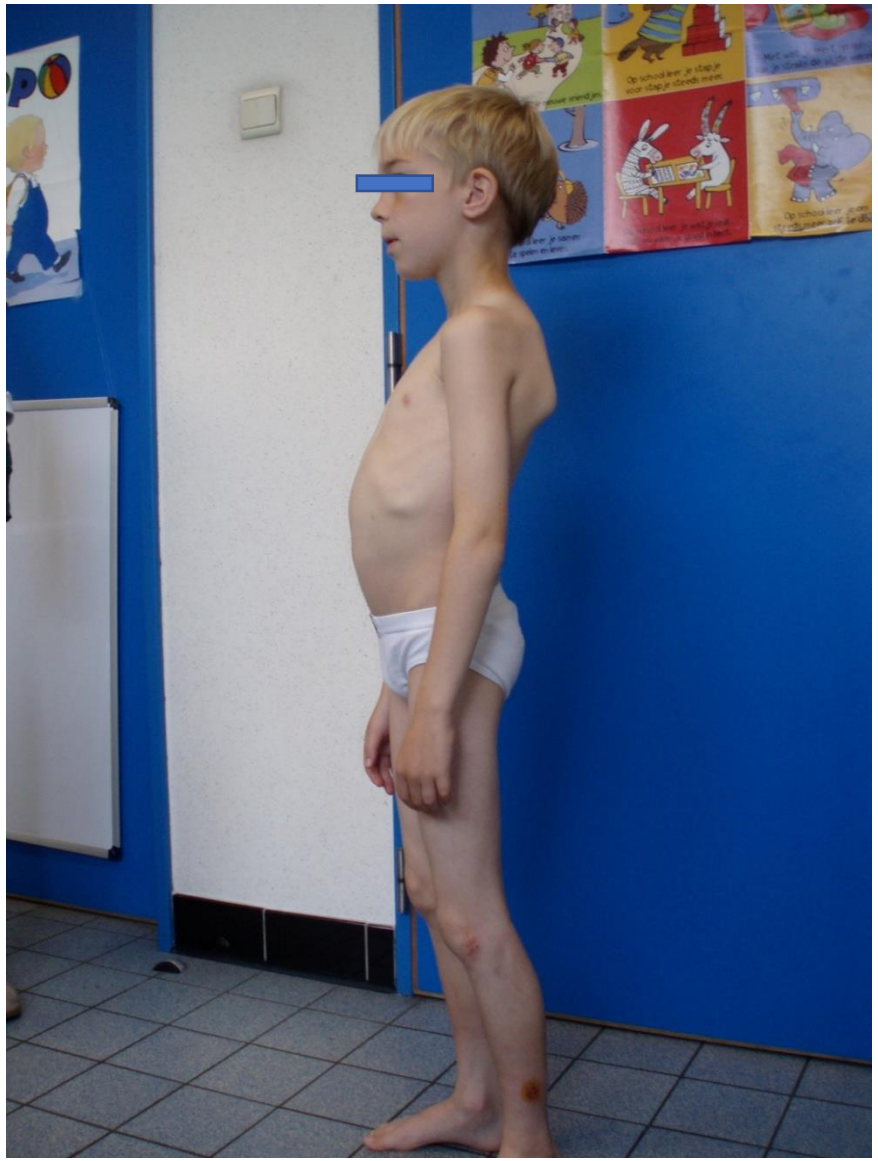
CSF lactate: 35,7mg/dl (ref: 13,7-20,5)

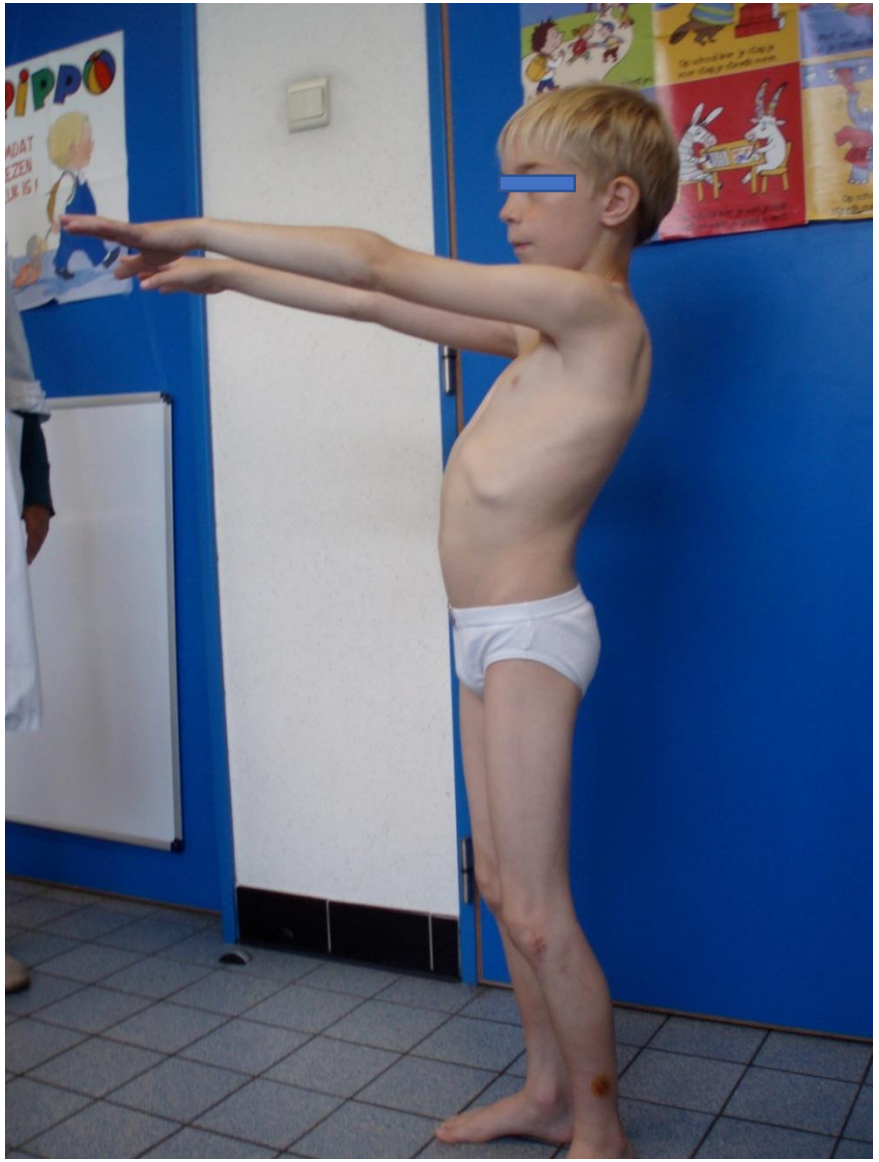
Ikeda et al., 2018

MELAS



MELAS





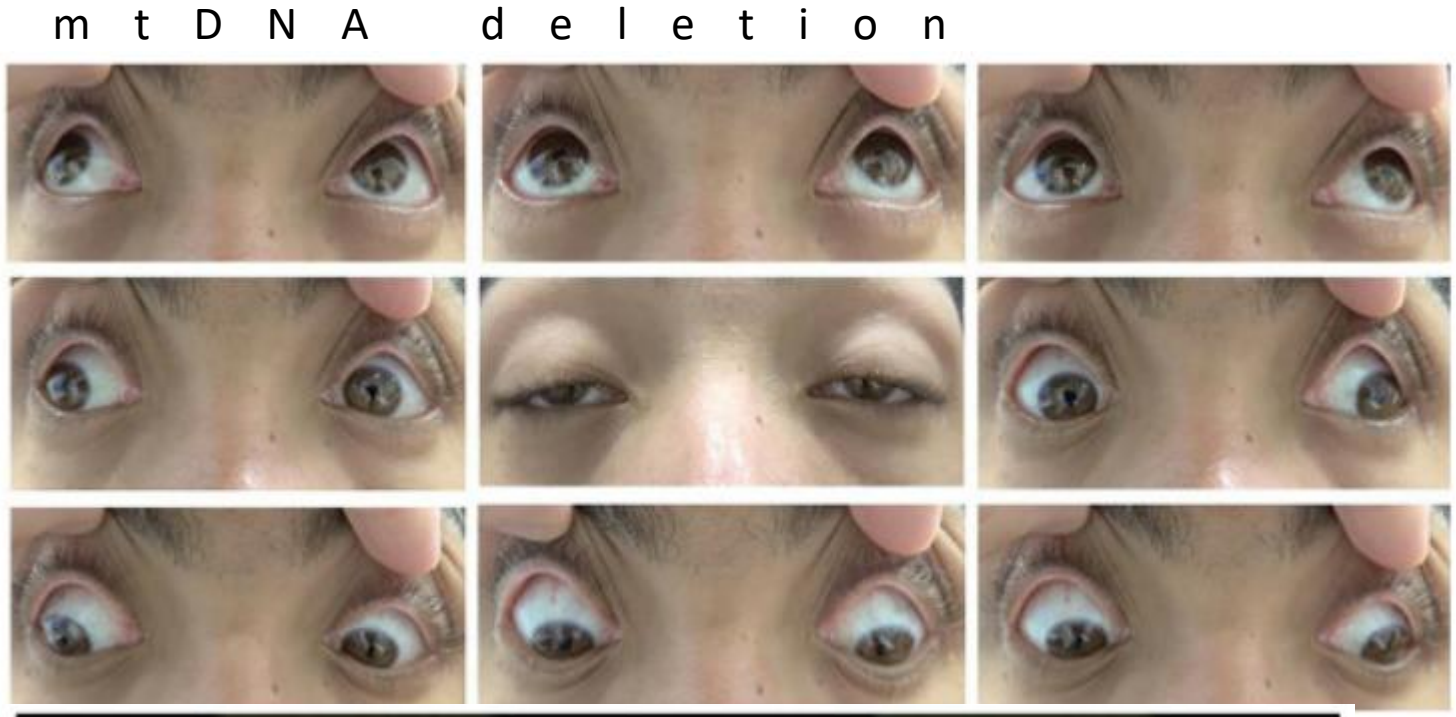
Mitochondrial diseases: deletions

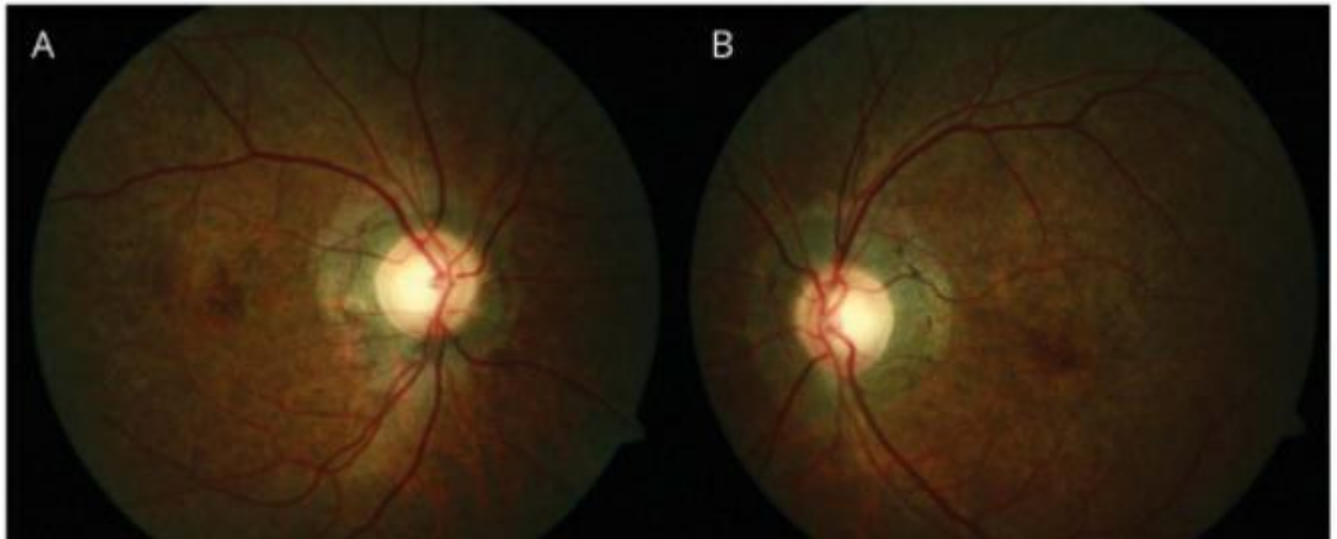
Kearns-Sayre syndrome

<u>Clinical presentation</u>

- teenage onset CPEO
- pigmentary retinopathy
- heart conduction problems
- ataxia
- deafness
- endocrine abnormalities

mtDNA deletion (1,0 -10 kb)





Nguyen et al., 2019

Mitochondrial diseases: deletions

m t D N A d e l e t i o n

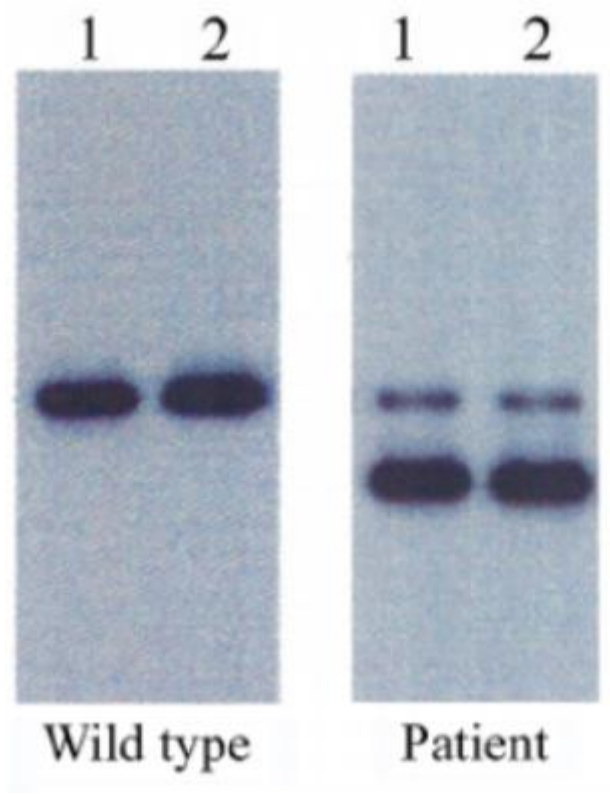
Pearson syndrome

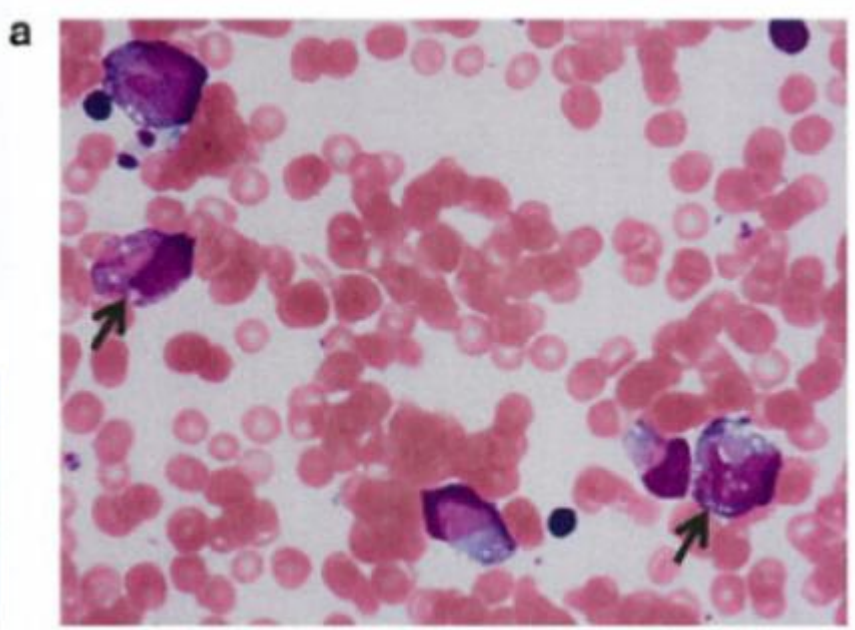
Clinical presentation

- childhood onset → early dismiss (<3y)
- bone marrow dysfunction
- exocrine pancreas dysfunction

- → sometimes evoluting towards Kearns sayre

mtDNA deletion

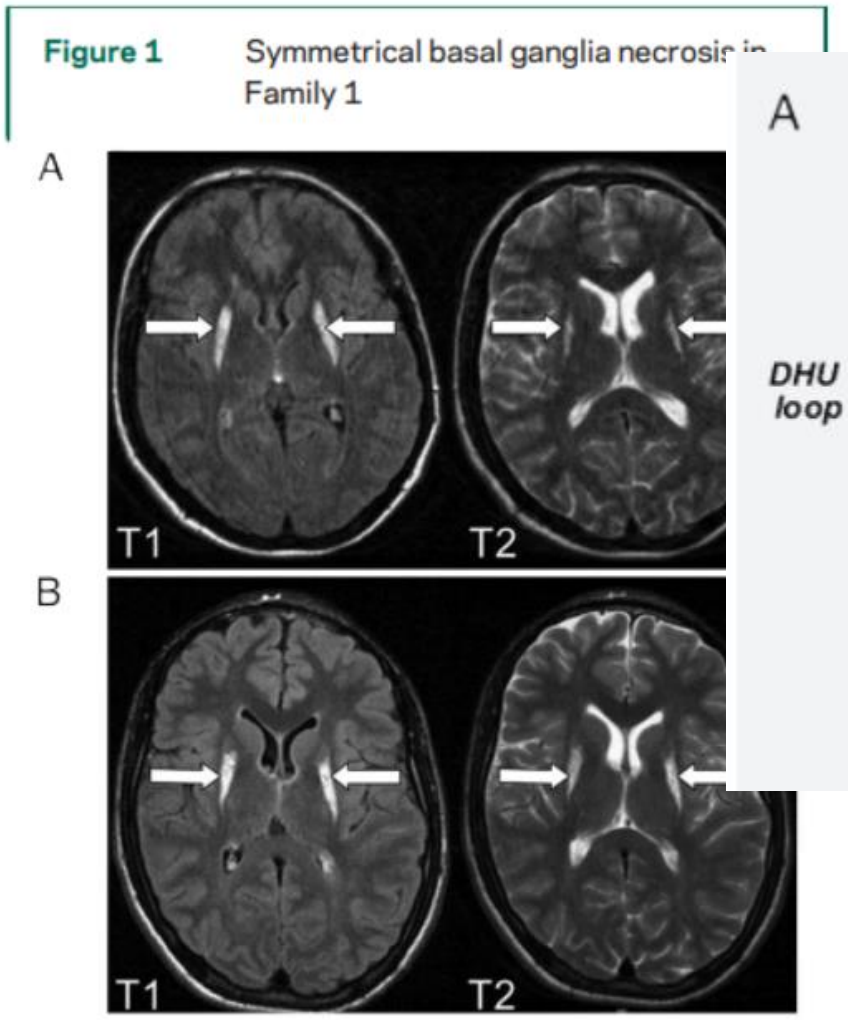


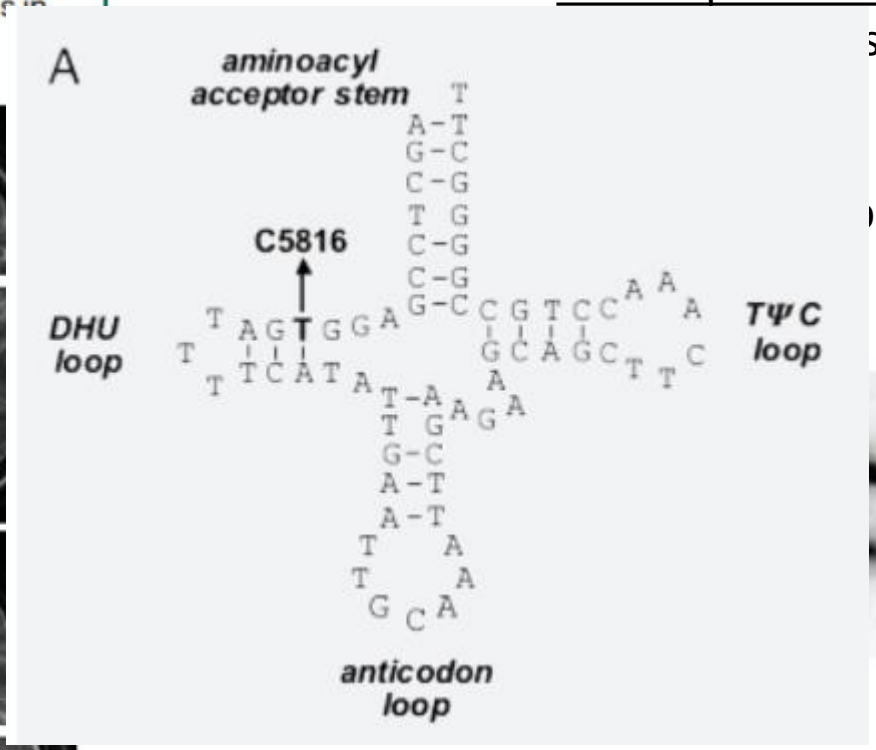


Nishimura et al., 2021

Mitochondrial diseases: tRNA

M T - T C

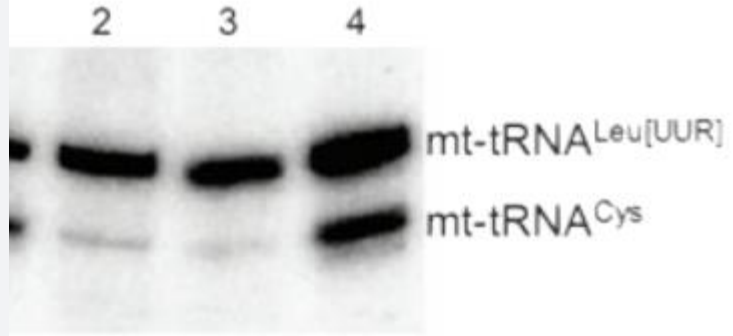




Clinical presentation

s with

sy



m.5816A>G, *MT-TC,*
100% muscle and blood

Homoplasmy

McFarland et al., 2007

Mitochondrial diseases: tRNA

M T - T D

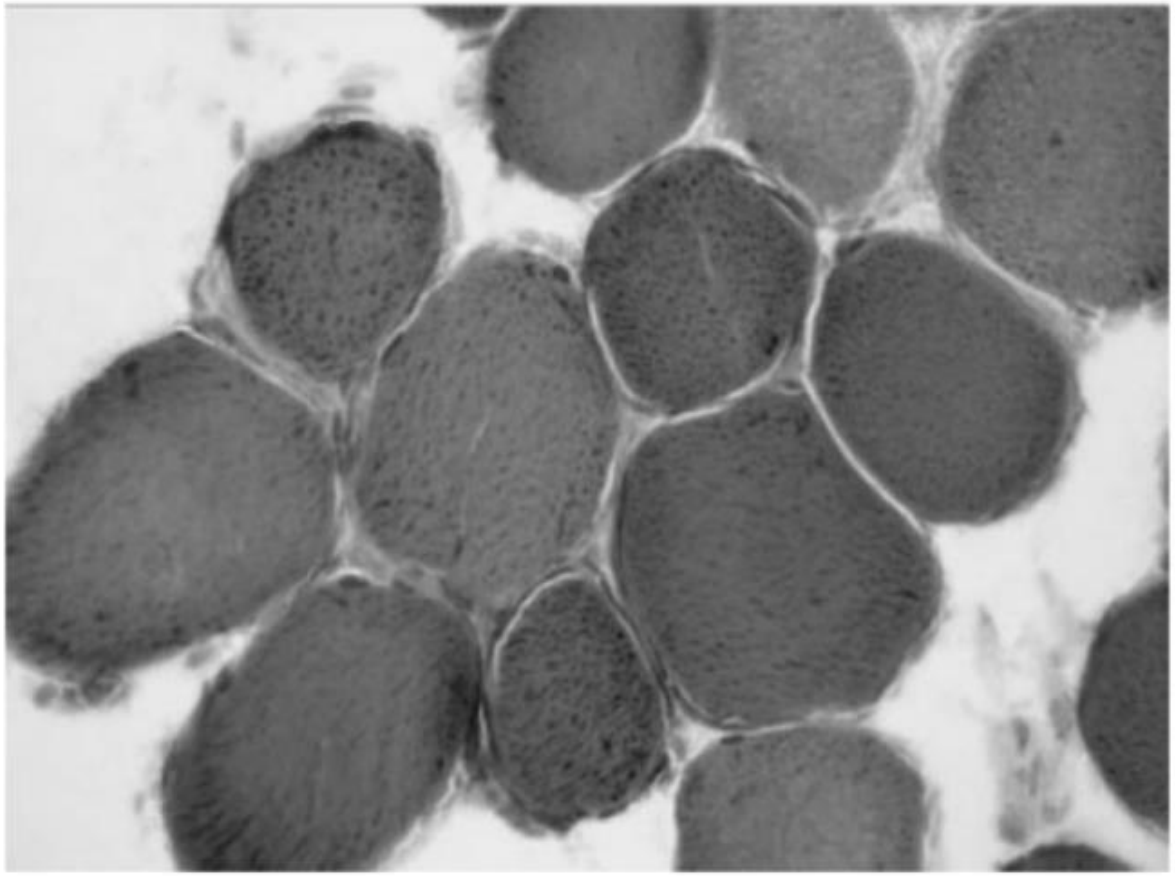
Clinical presentation

childhood onset
exercise intolerence
progressive

m.7526 A>G, *MT-TD*
100% skeletal muscle
3% blood and skin fibroblasts!

Tissue specifity !

Maternal testing: undetectable mutation



Seneca et al., 2005

Mitochondrial diseases: tRNA

M T - T F

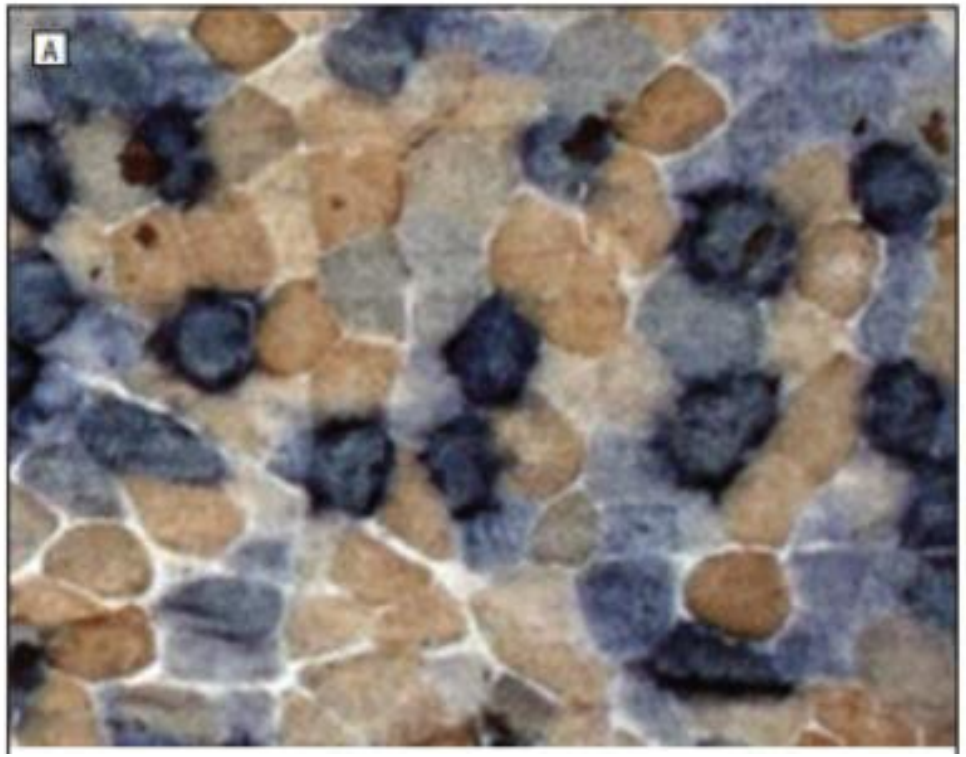
Clinical presentation

Adult onset exercise intolerence, progressive

Hearing loss

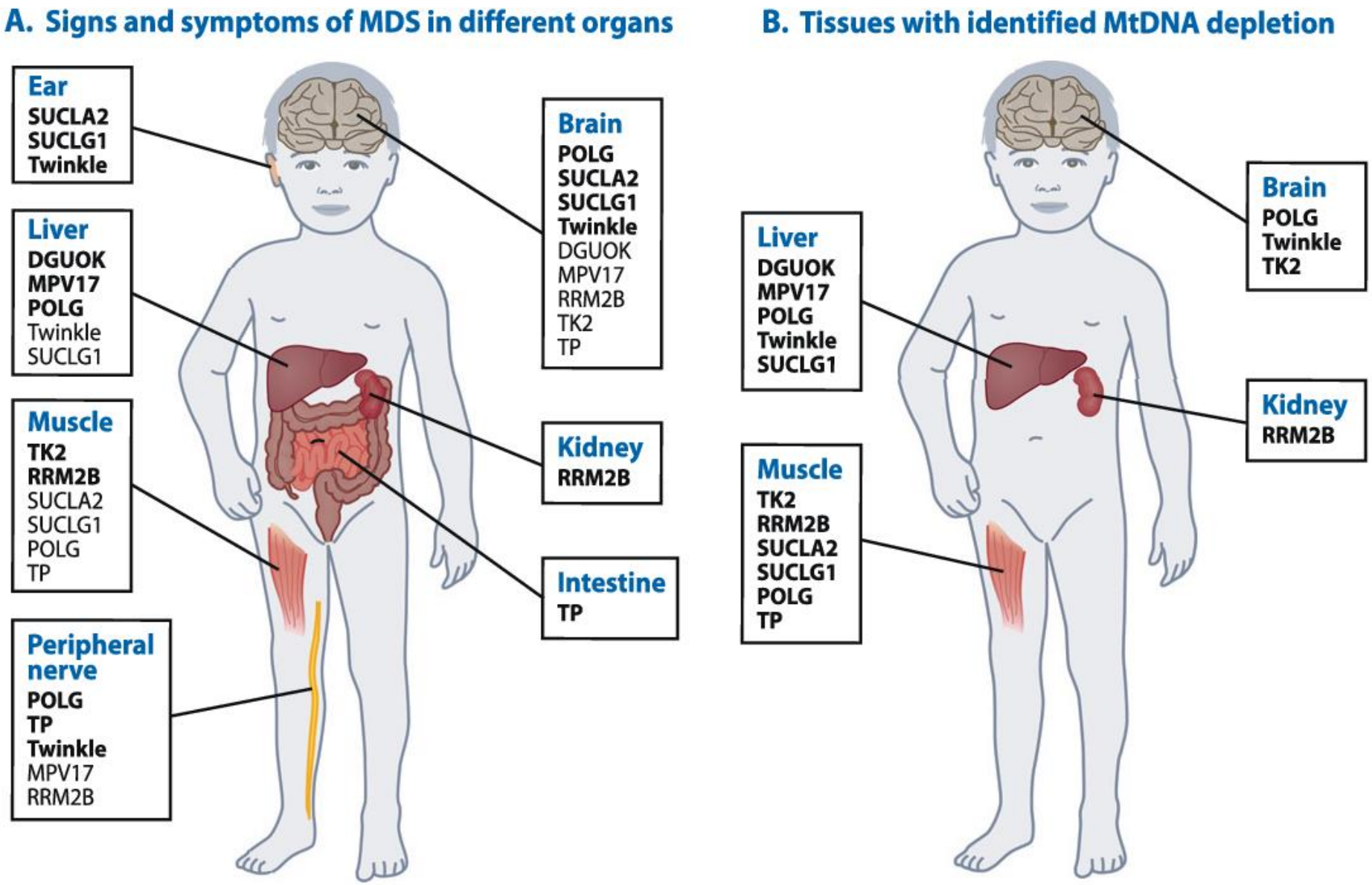
Variable age of onset!

m.622 G>A, *MT-TF*
88% skeletal muscle
66% blood
36% urine



Deschauer et al., 2006

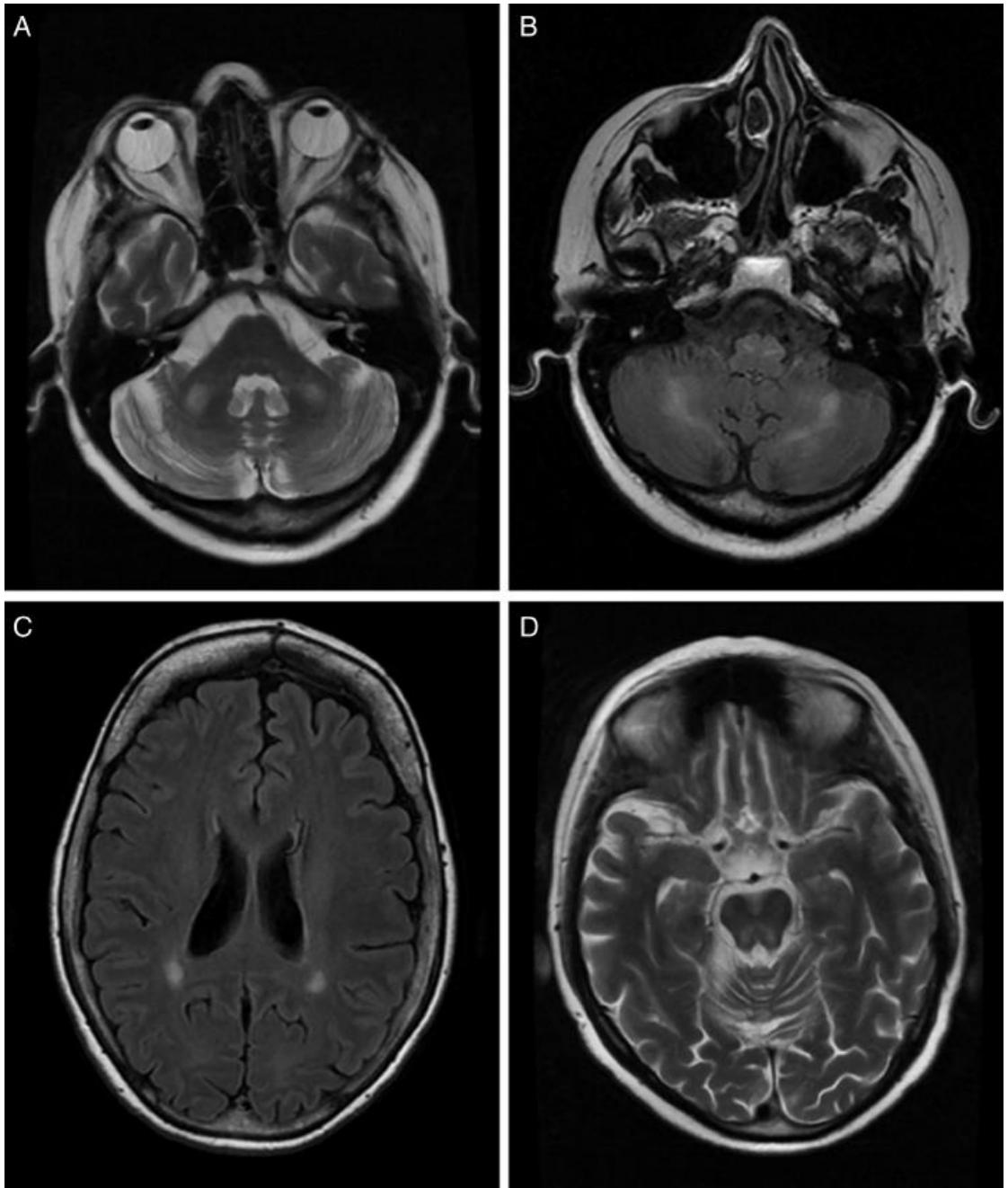
mtDNA depletion syndromes



Mitochondrial diseases

nDNA encoded defeciencies

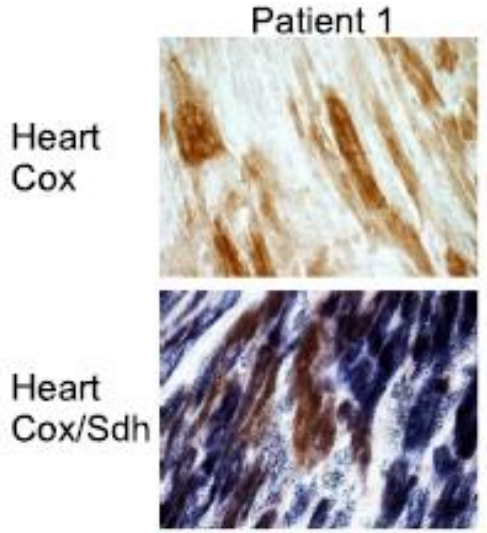
POLG deficiency

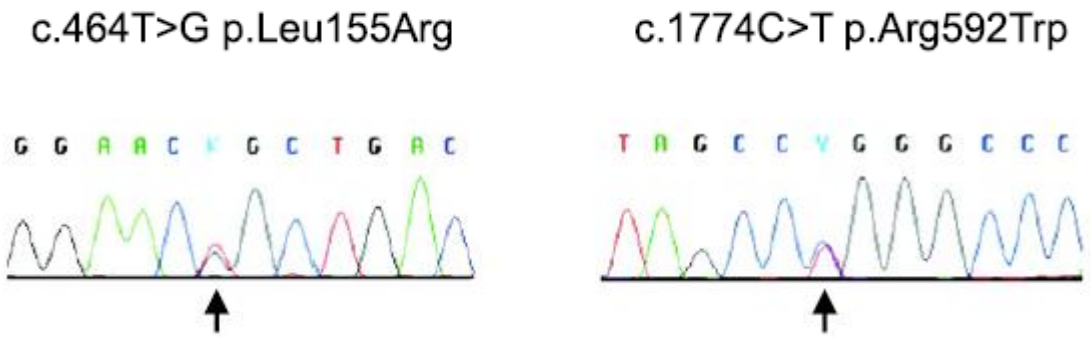


AARS2

- hypertrofic cardiomyopathy
- Delayed motore development
- lactate

- ➔ WES: AARS2



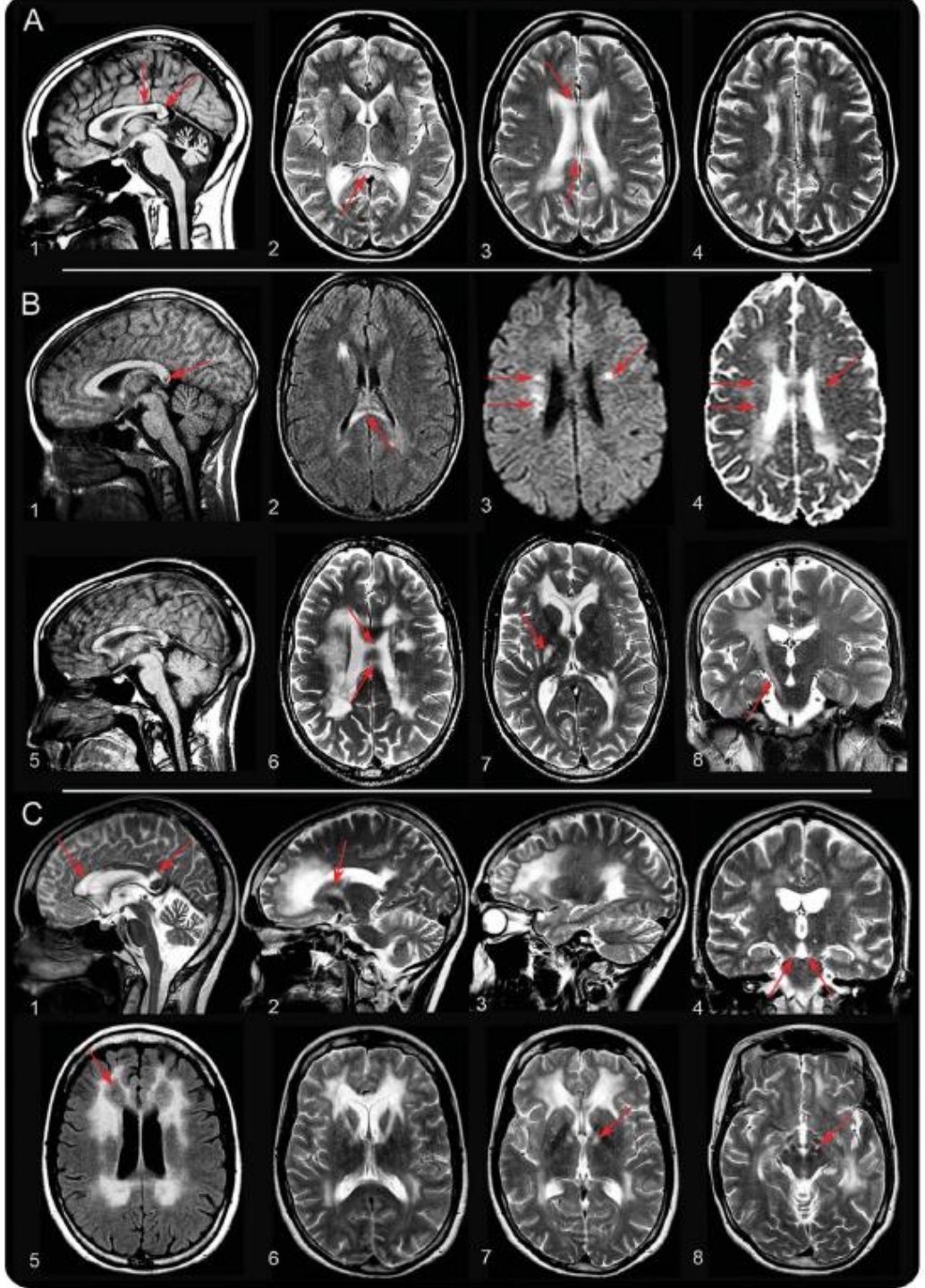


Götz et al., 2011

AARS2

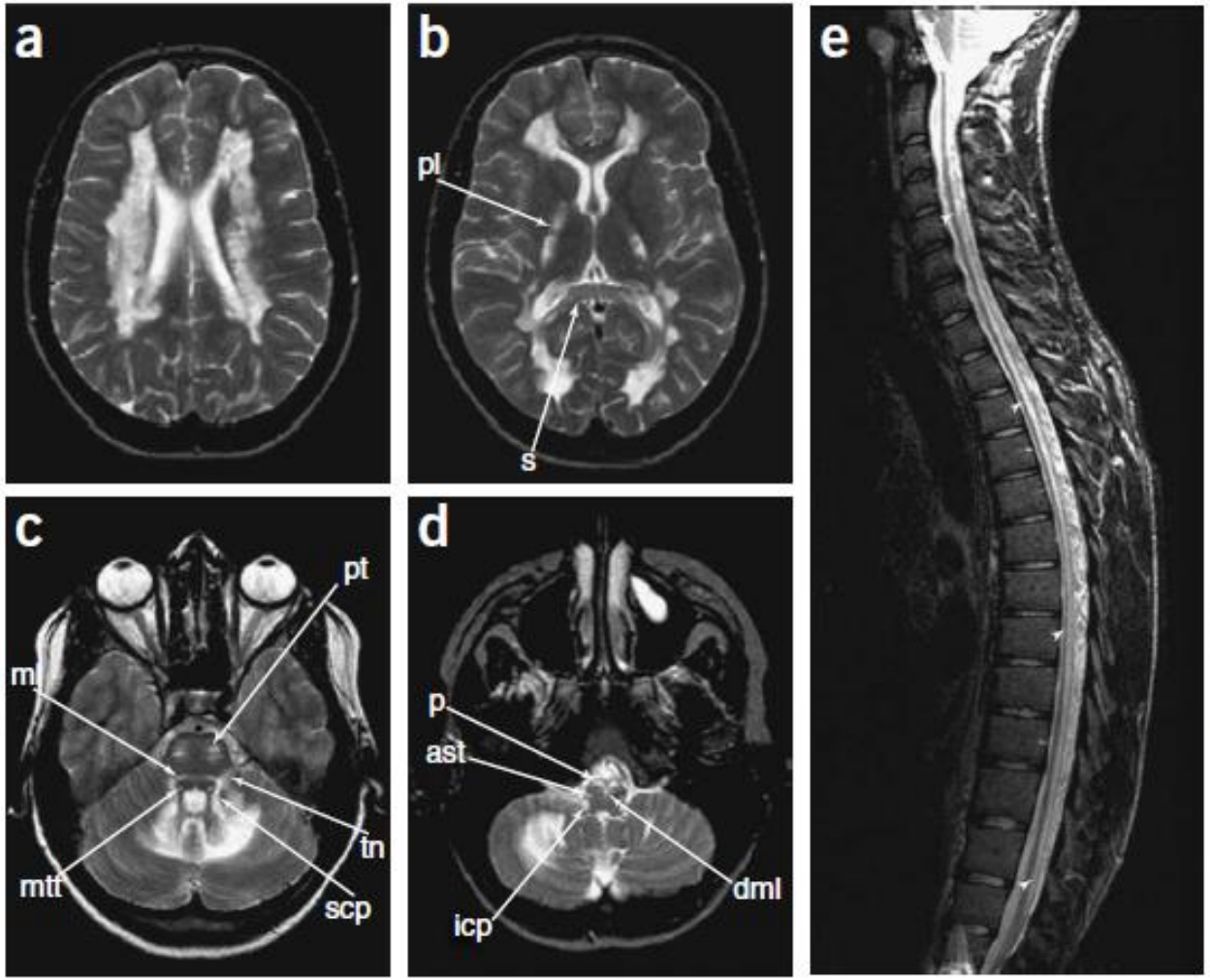
- Progressive leuko-encephalopathie
- Ovarian failure

- NO cardiomyopathie



Dallabona et al., 2014

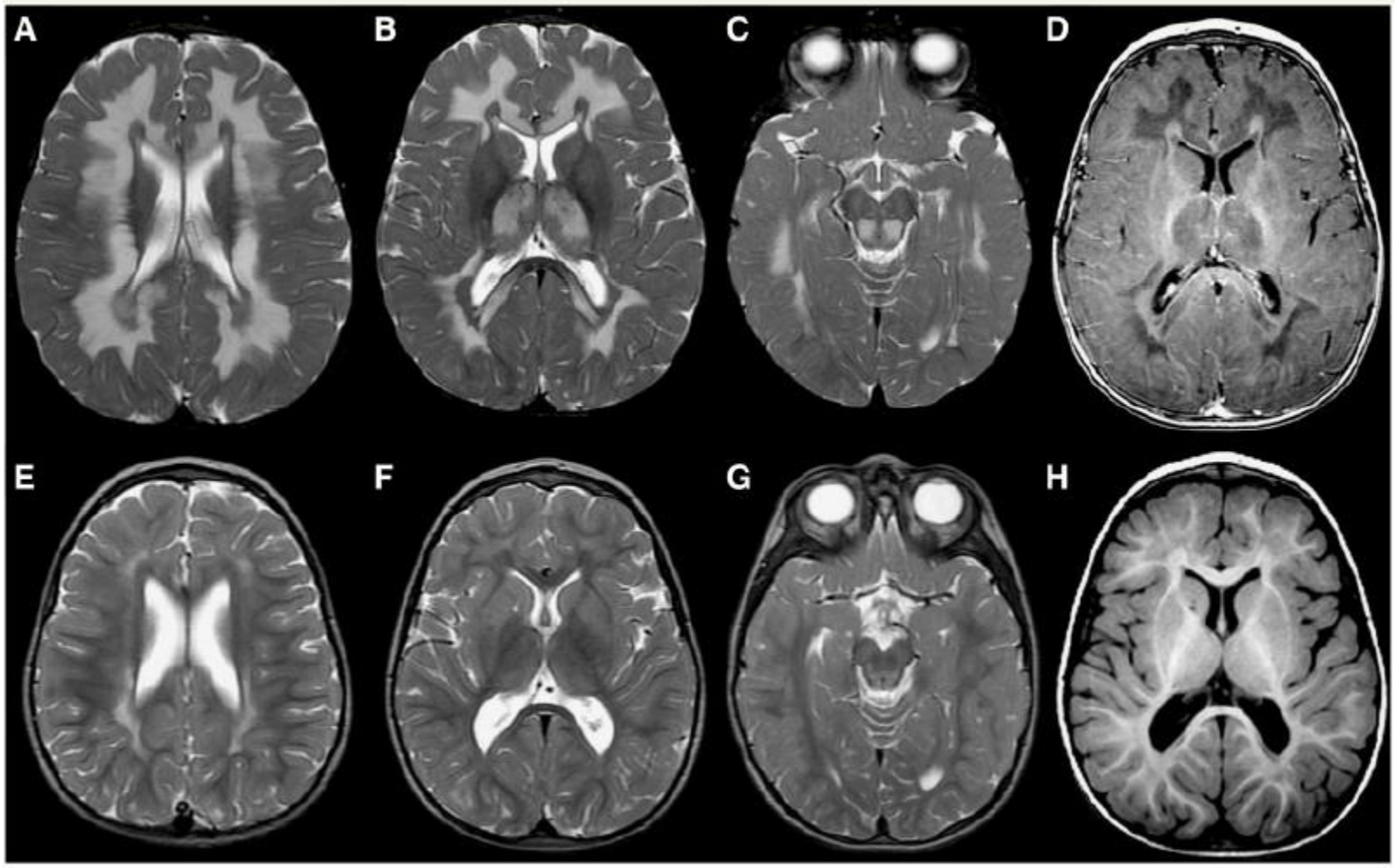
DARS2



LBSL

Scheper et al., 2007

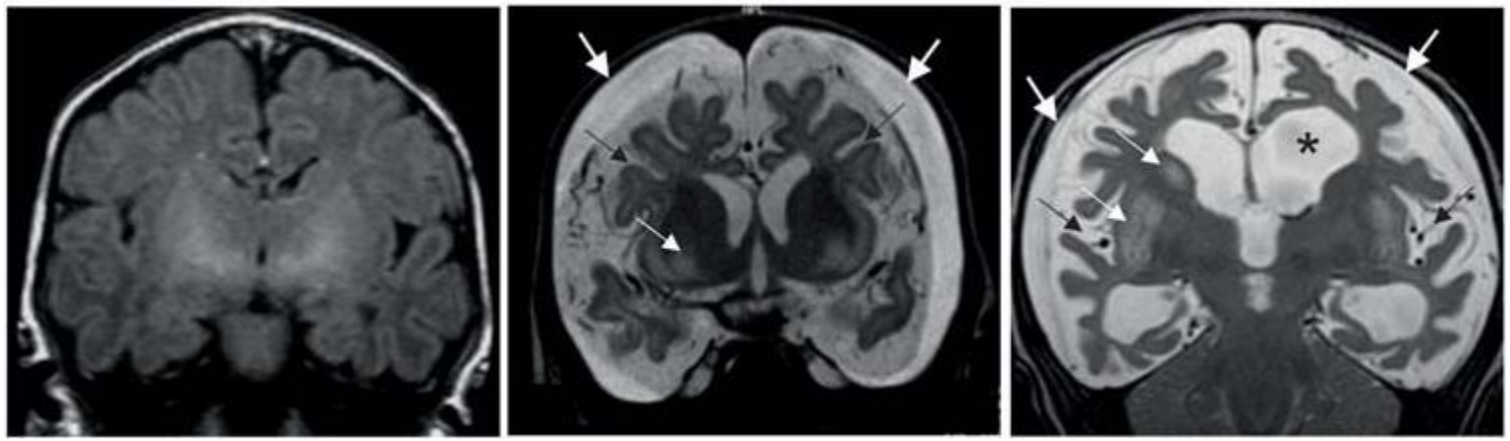
EARS2



Steenweg et al., 2012

LTBL

FARS2



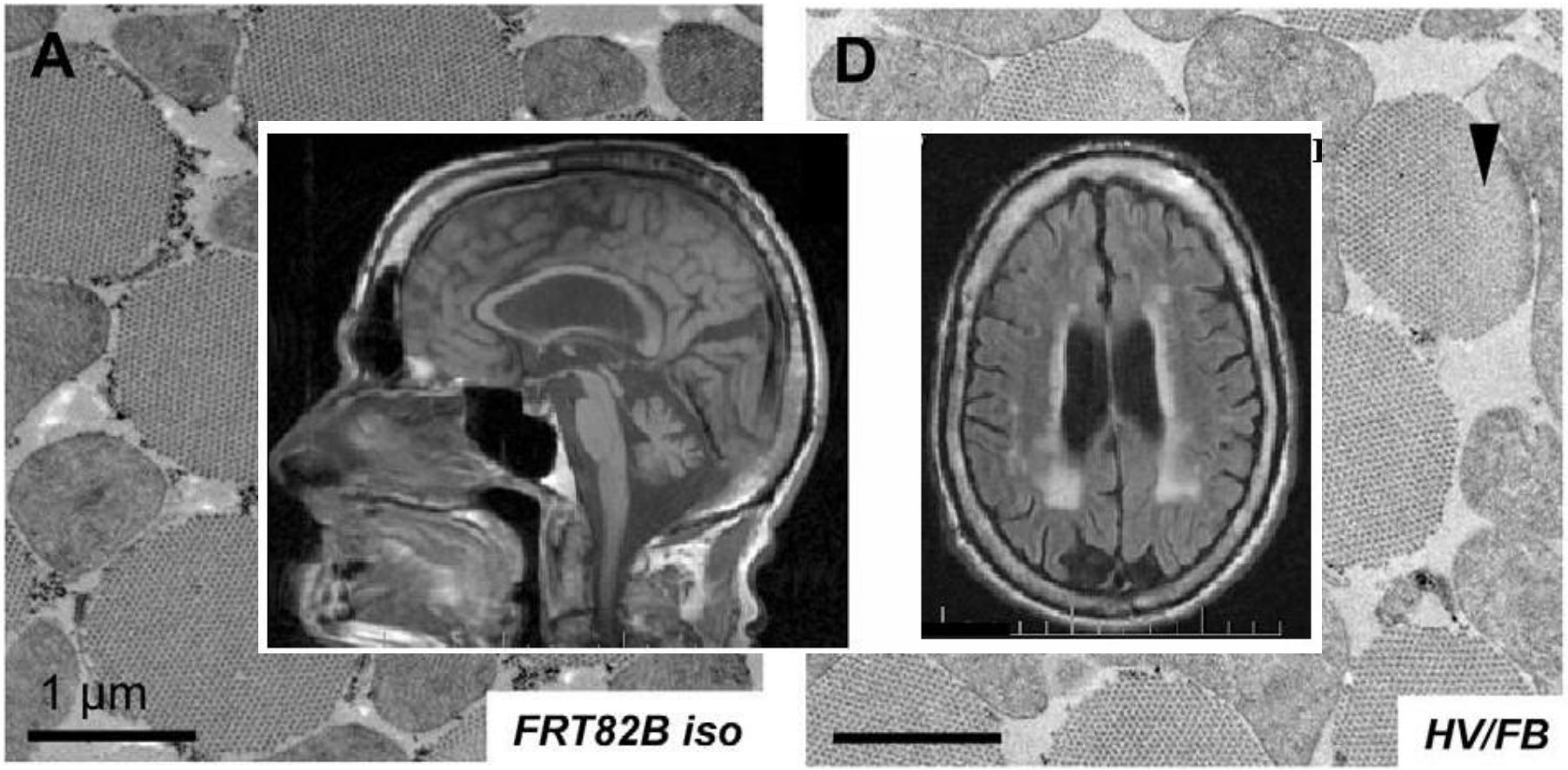
Alpers-like syndroom

Elo et al., 2012

MARS2

ARSAL:

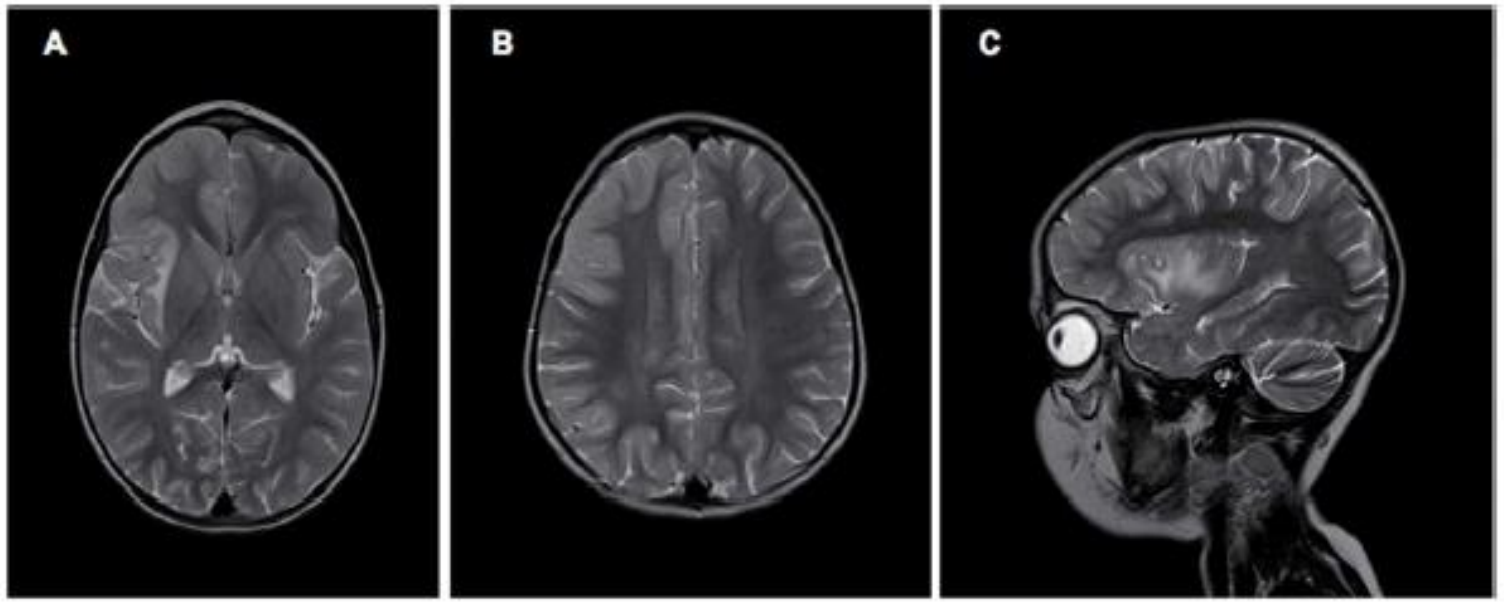
Autosomal recessive spastic ataxia with leuko-encephaloopathy



Bayat et al., 2012

VARS2

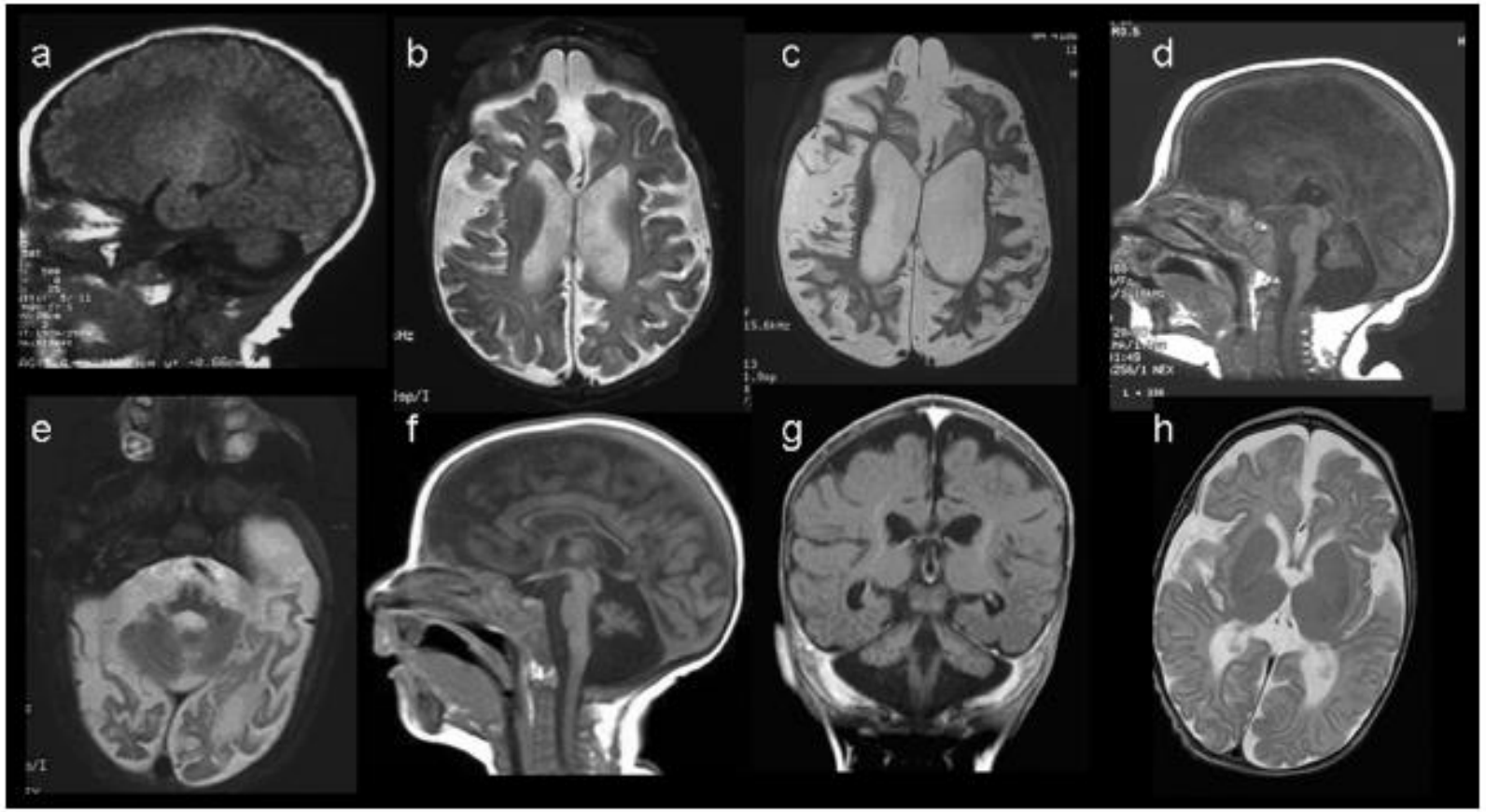
Microcephaly and epilepsy



Diodato et al., 2014

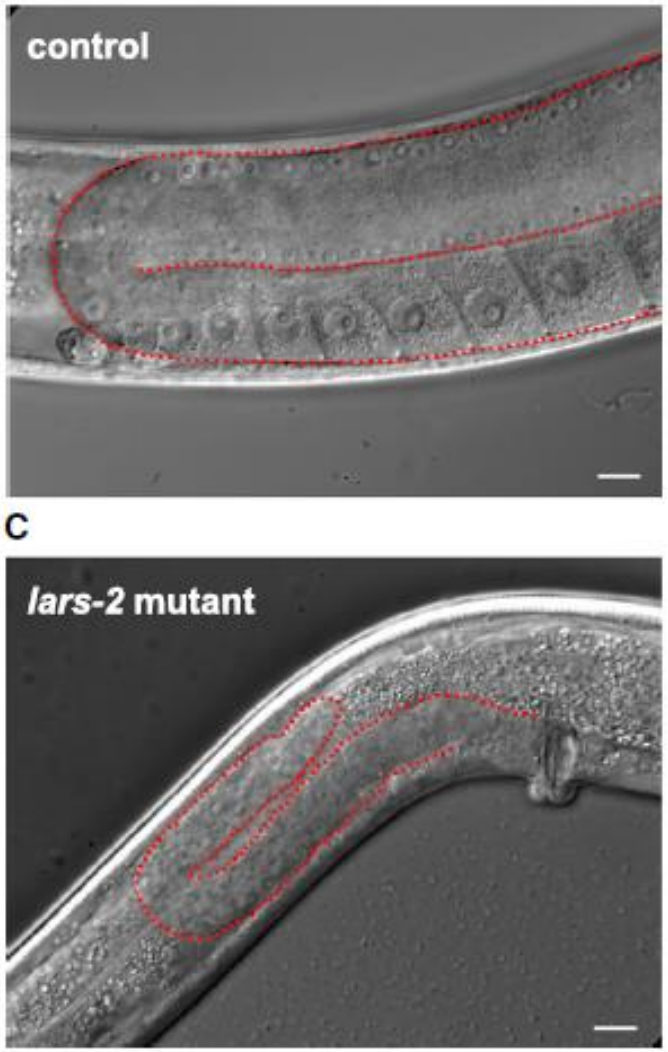
RARS2

Pontocerebellar hypoplasie type 6



Edvarson et al., 2007

HARS2 and LARS2



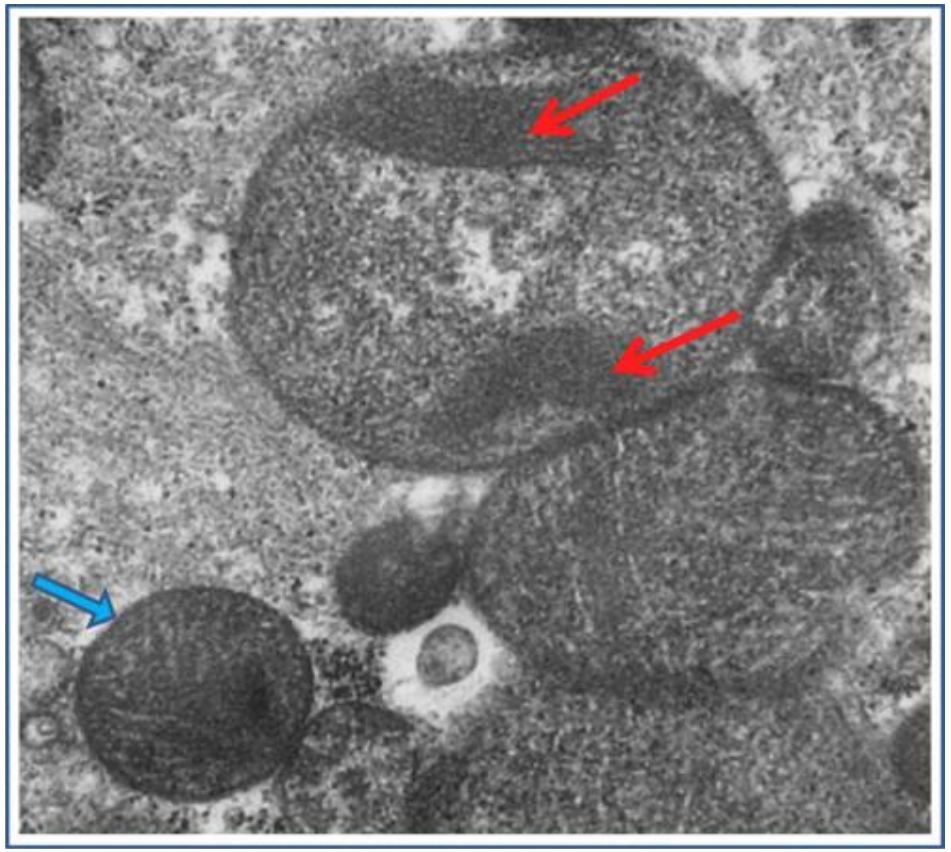
Pierce et al., 2013

-Perrault-syndrome:
overian failure
and sensironeural
deafness

SARS2

HUPRA syndroom

Hyper**u**ricemia, **p**ulmonary hypertension, and progressive **r**enal failure

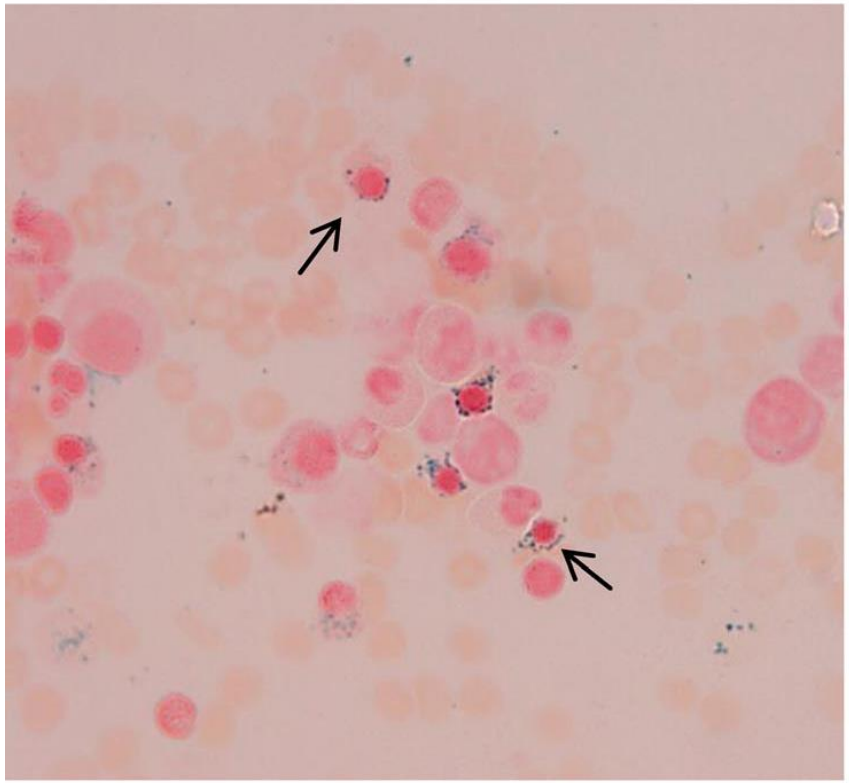


Belostotsky et al., 2011

YARS2

MLASA

Myopathy, lactic acidosis, sideroblastic anemia

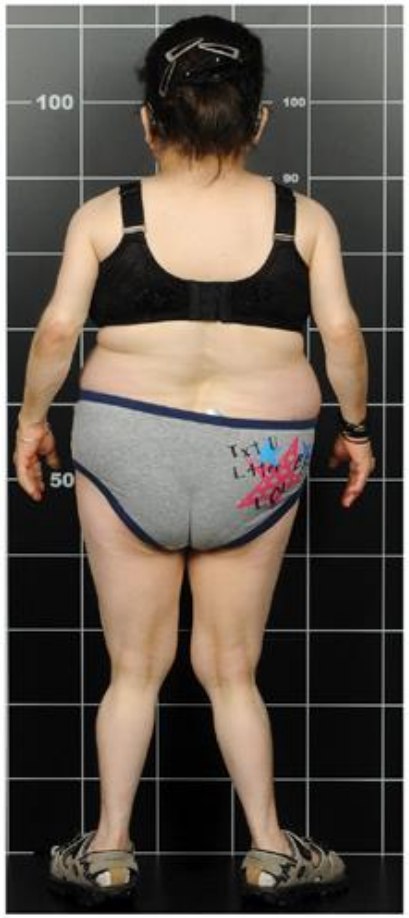


Riley et al., 2010

IARS2

CAGSSS

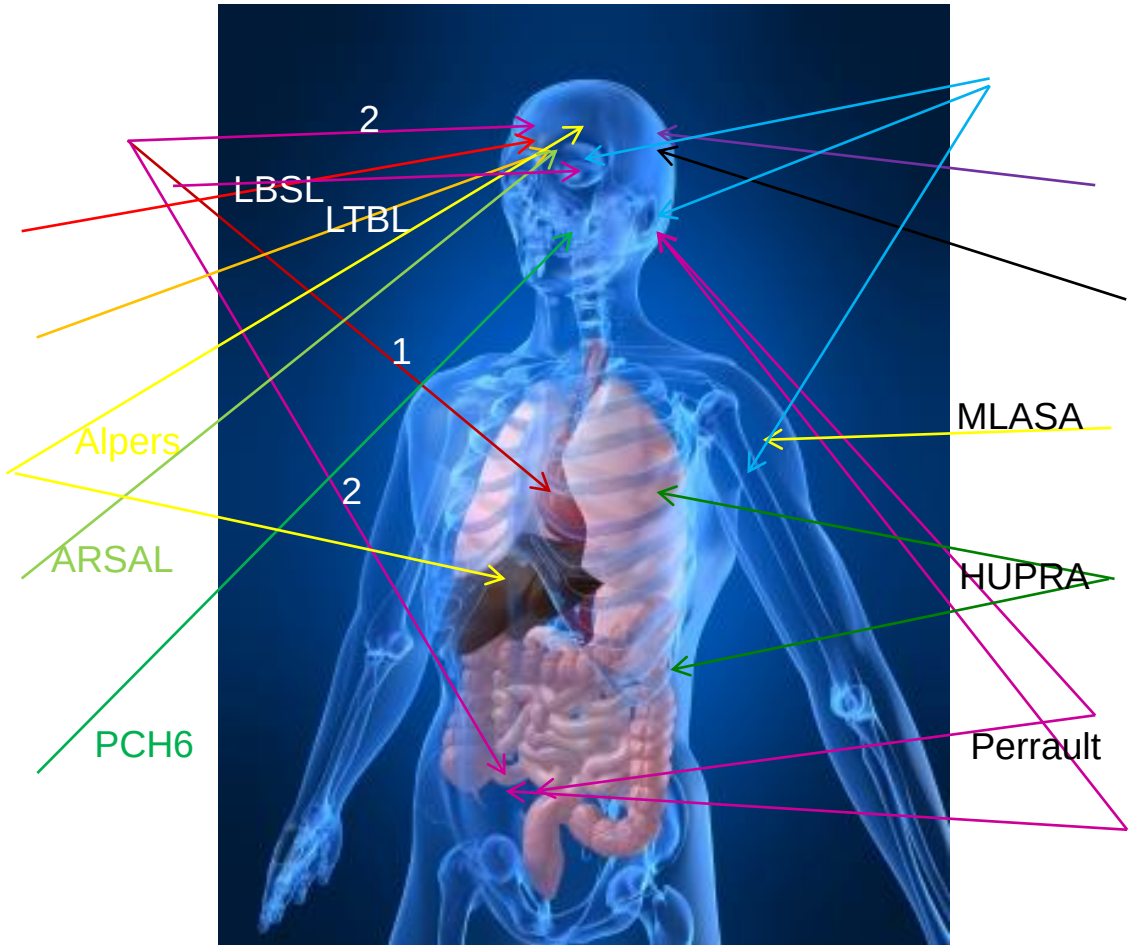
cataracts (CA), growth hormone deficiency (G), sensory neuropathy (S), sensorineural hearing loss (S), and skeletal dysplasia (S)



Schwartzentruber et al., 2014

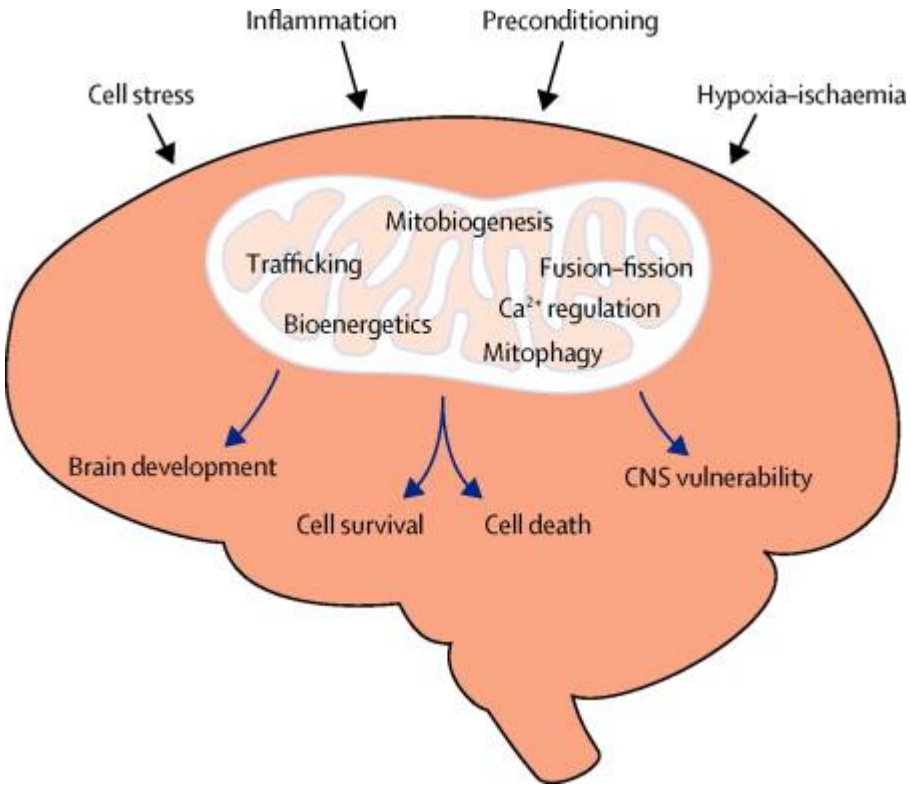
Mitochondrial tRNA synthetases (aaRS2)

AARS2	FARS2	KARS	PARS2	TARS2
CARS2	GARS	LARS2	GATC	VARS2
DARS2	HARS2	MARS2	RARS2	WARS2
EARS2	IARS2	NARS2	SARS2	YARS2



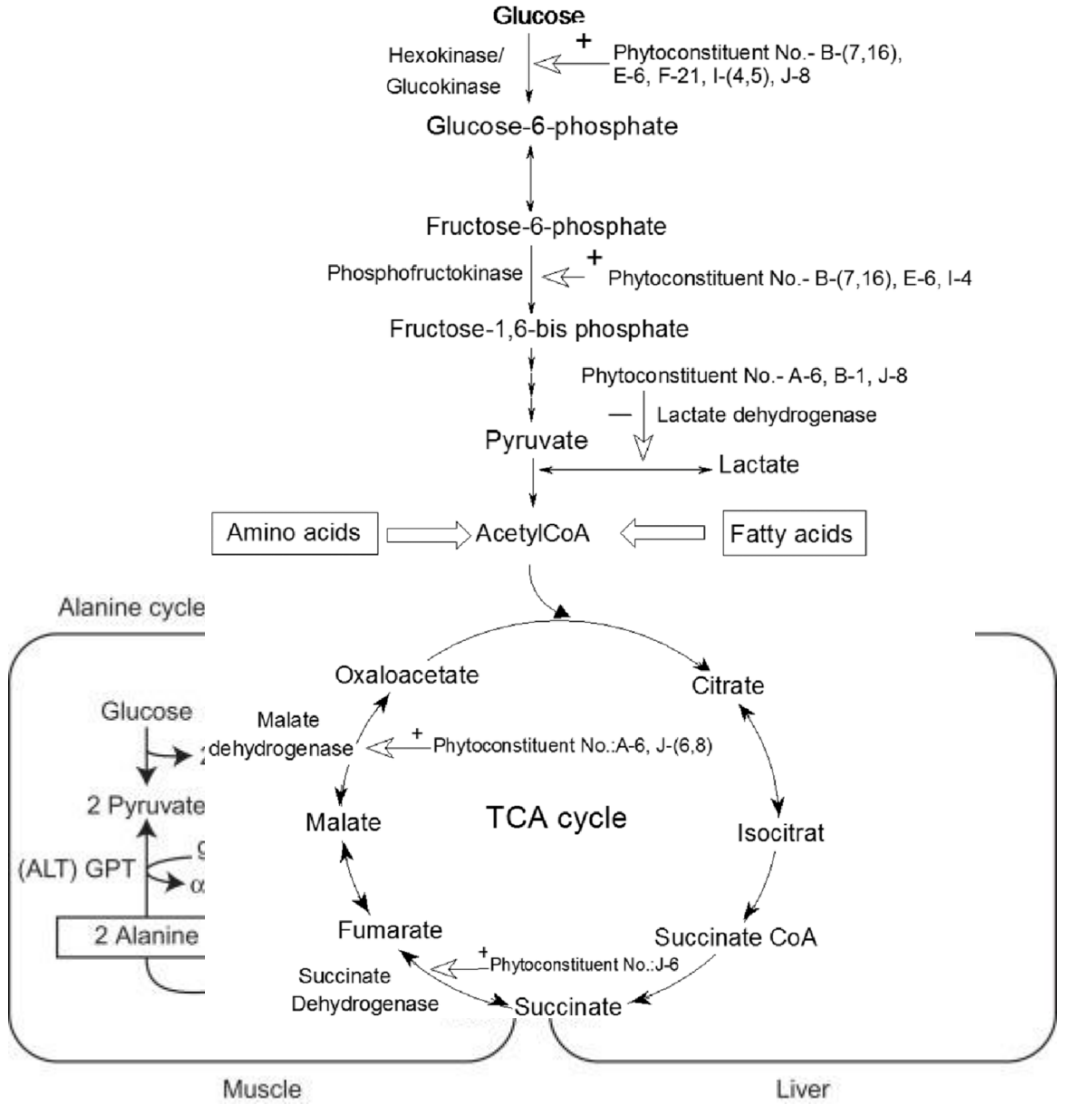
Diagnostics

- Always part of your DD!



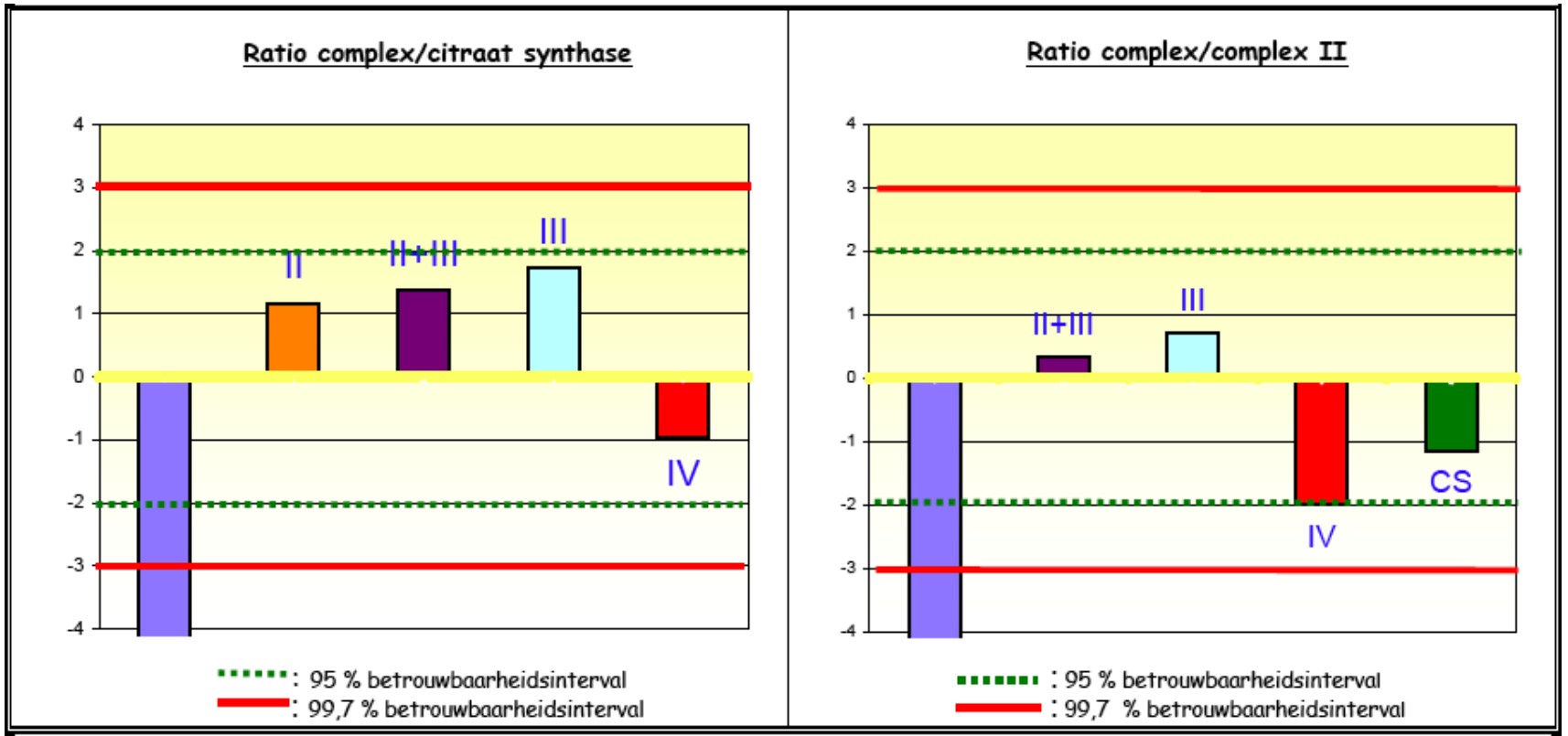
Diagnostics

- Biochemical analyses
  - Lactate: CSF, serum, urine
  - Pyruvate
  - OA: Krebs-intermediates
  - Alanine
  - FGF21



Diagnostics

- Biochemical analyses
  - Tissue (muscle-liver-skin-heart) analysis for spectrophotopetric enzyme analysis

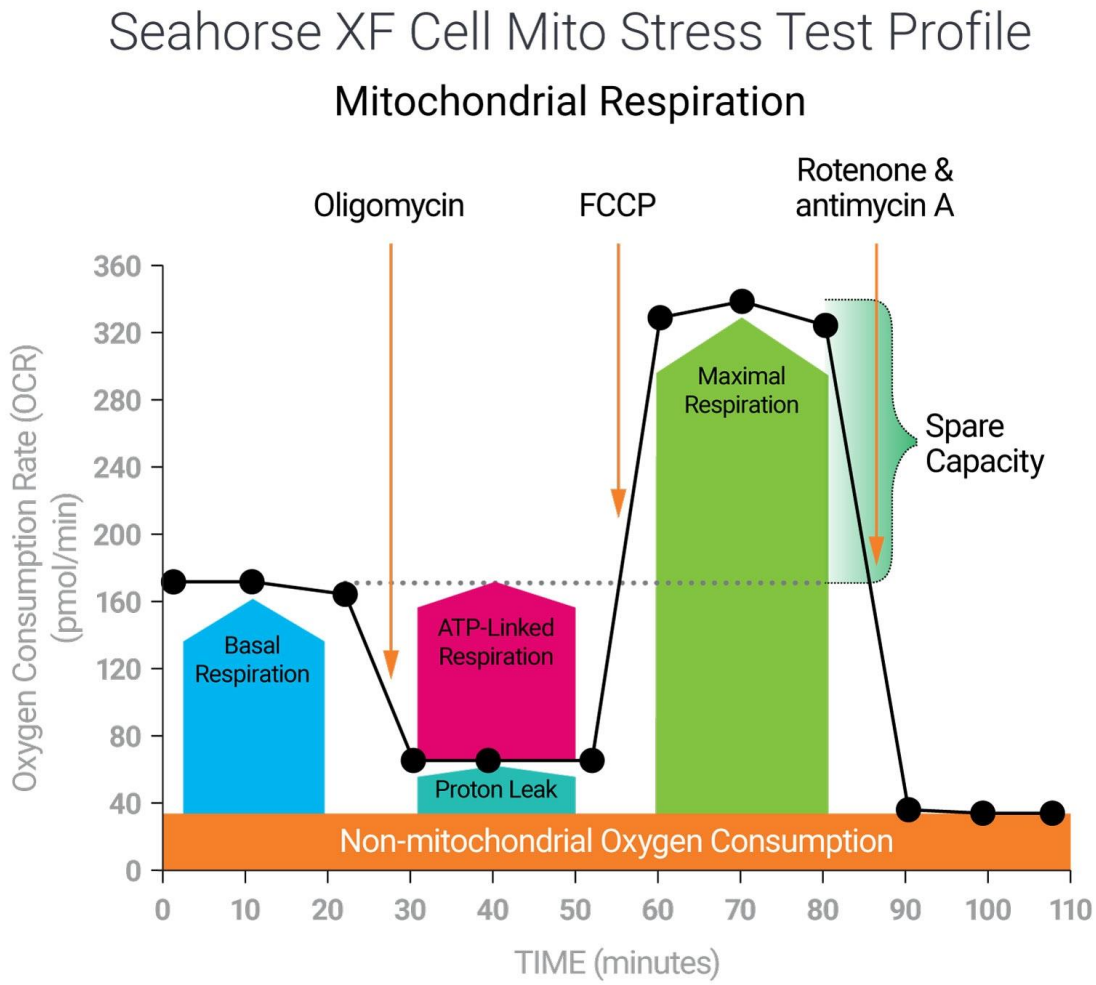


Diagnostics

Possible results:	Interpretaion
• Isolated complex deficiency →	mtDNA complex subunit Nuclear complex subunit Nuclear assembly factor
• Combined complex defiency (I+III+IV) →	mtDNA depletion mtDNA deletion Nuclear genes: trasncription and translation
• Combined complex defciency (I+II) →	Fe-S cluster genes

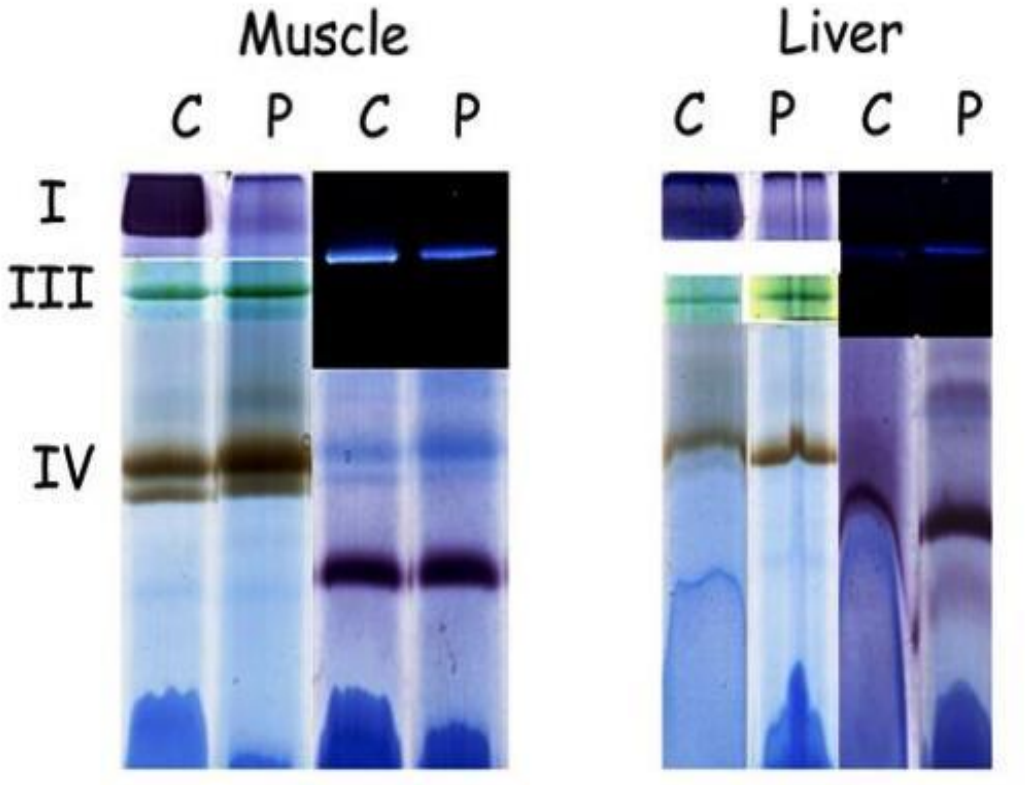
Diagnostics

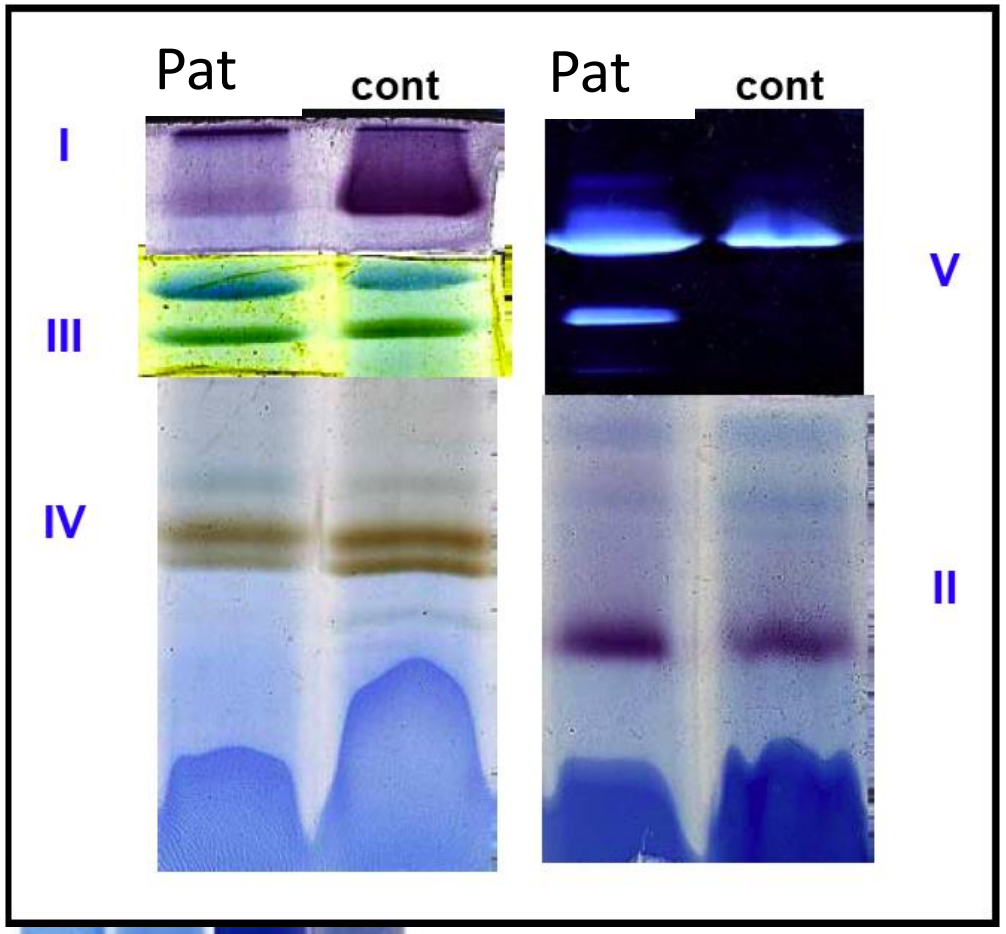
- Biochemical analyses (skin)
  - Oxygen consumption analysis
  - More in vivo mimics



Diagnostics

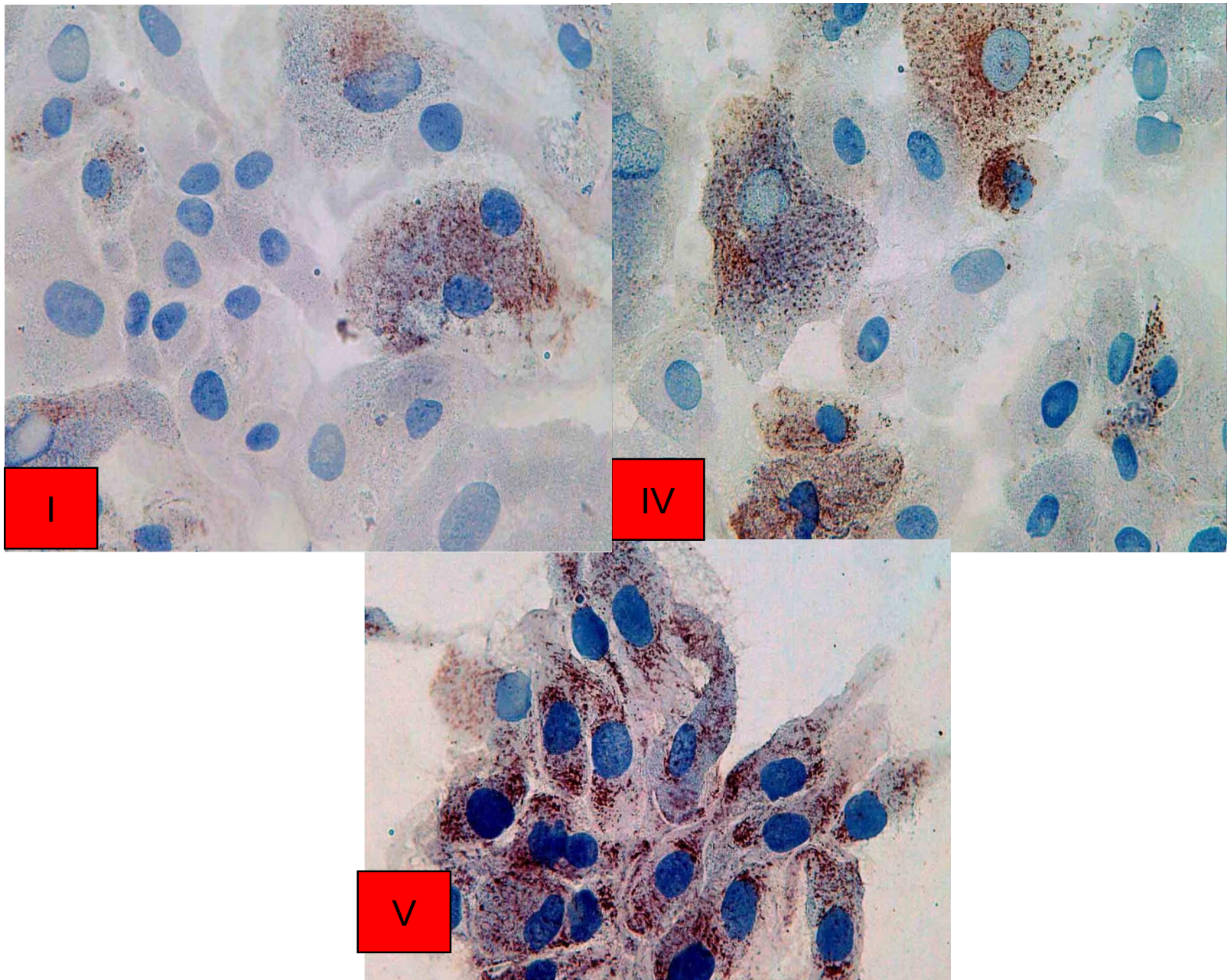
- Biochemical analyses: BN-PAGE



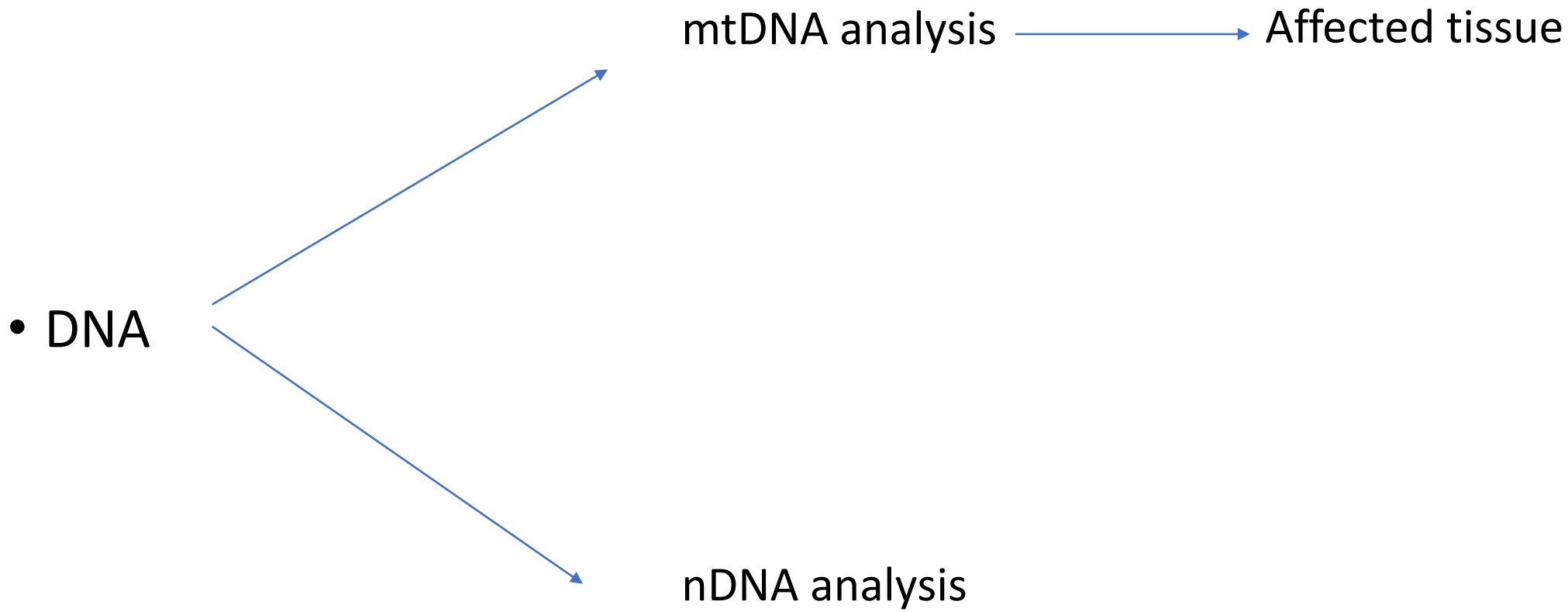


Diagnostics

- Biochemical analyses:
  - immunocytostaining



Diagnostics



Conclusion

- Not unfrequently occuring diseases
- All organs can be involved, at any age
- Often severely and progressive
- No cure >> prevent, if possible