La richiesta di competenza neurologica nel prossimo futuro

Sesta edizione

Le malattie mitocondriali:

la diagnosi genetica

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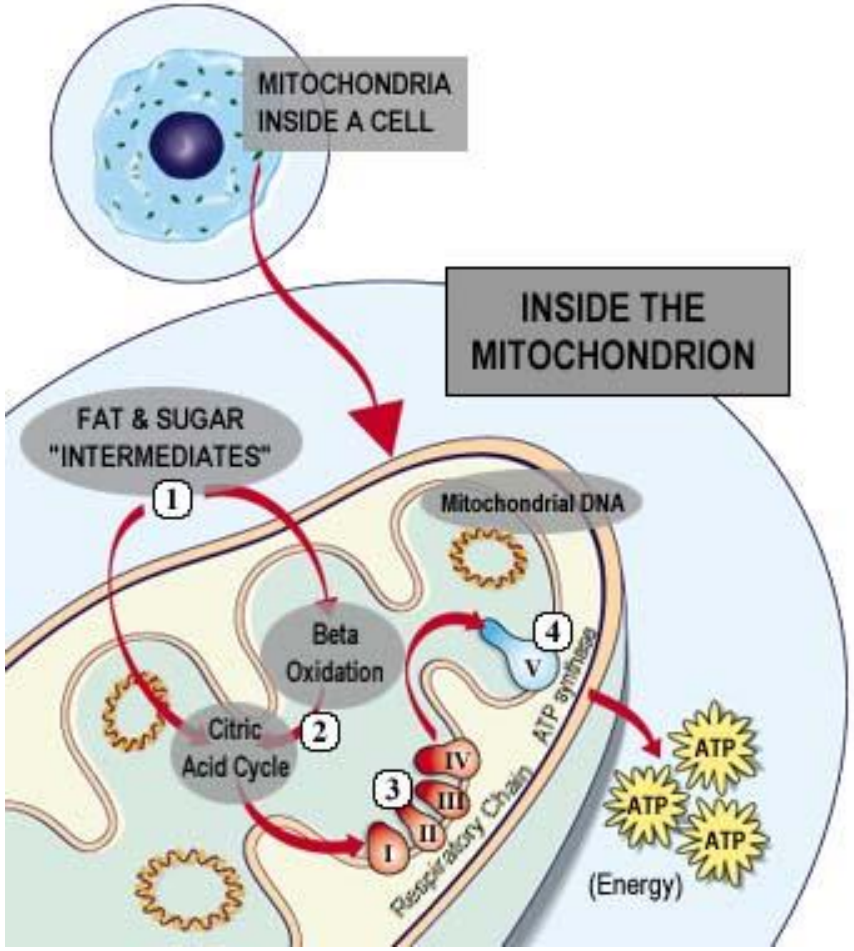
Sistema Socio Sanitario

Regione Lombardia

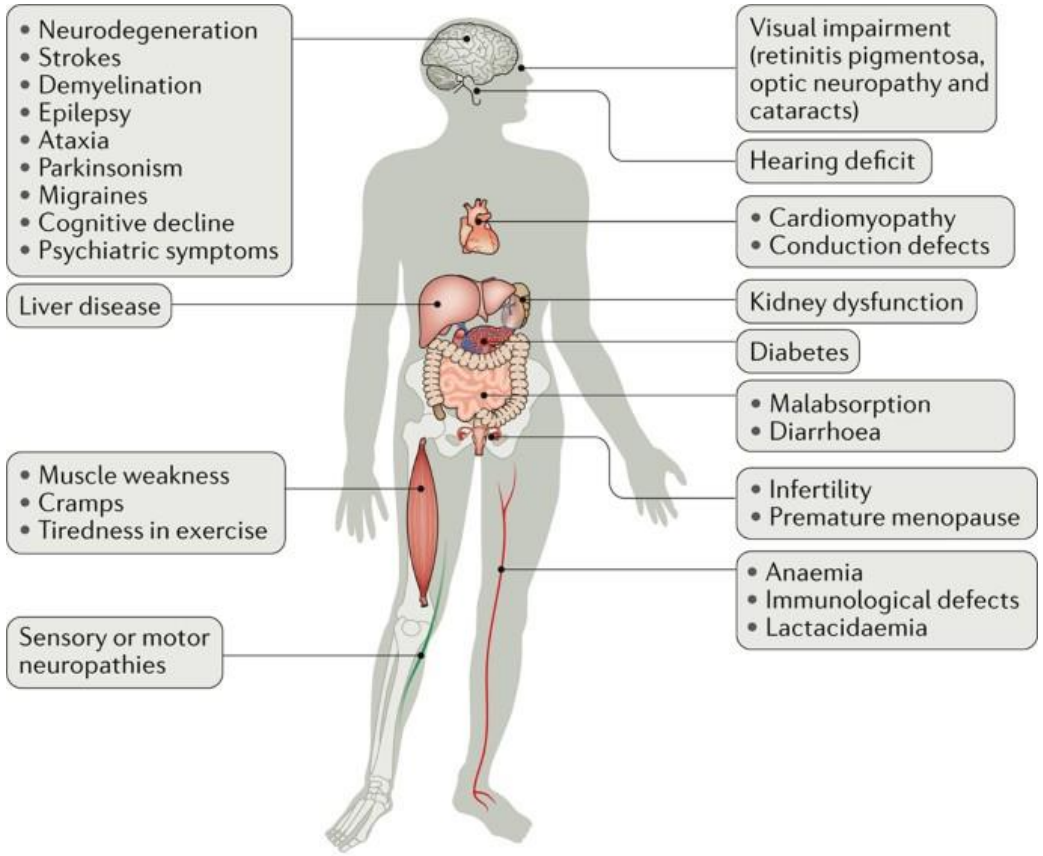
MITOCHONDRIAL DISORDERS

Mitochondrial Disorders affect the mitochondrial respiratory chain (MRC) and Oxidative Phosphorylation (OXPHOS)



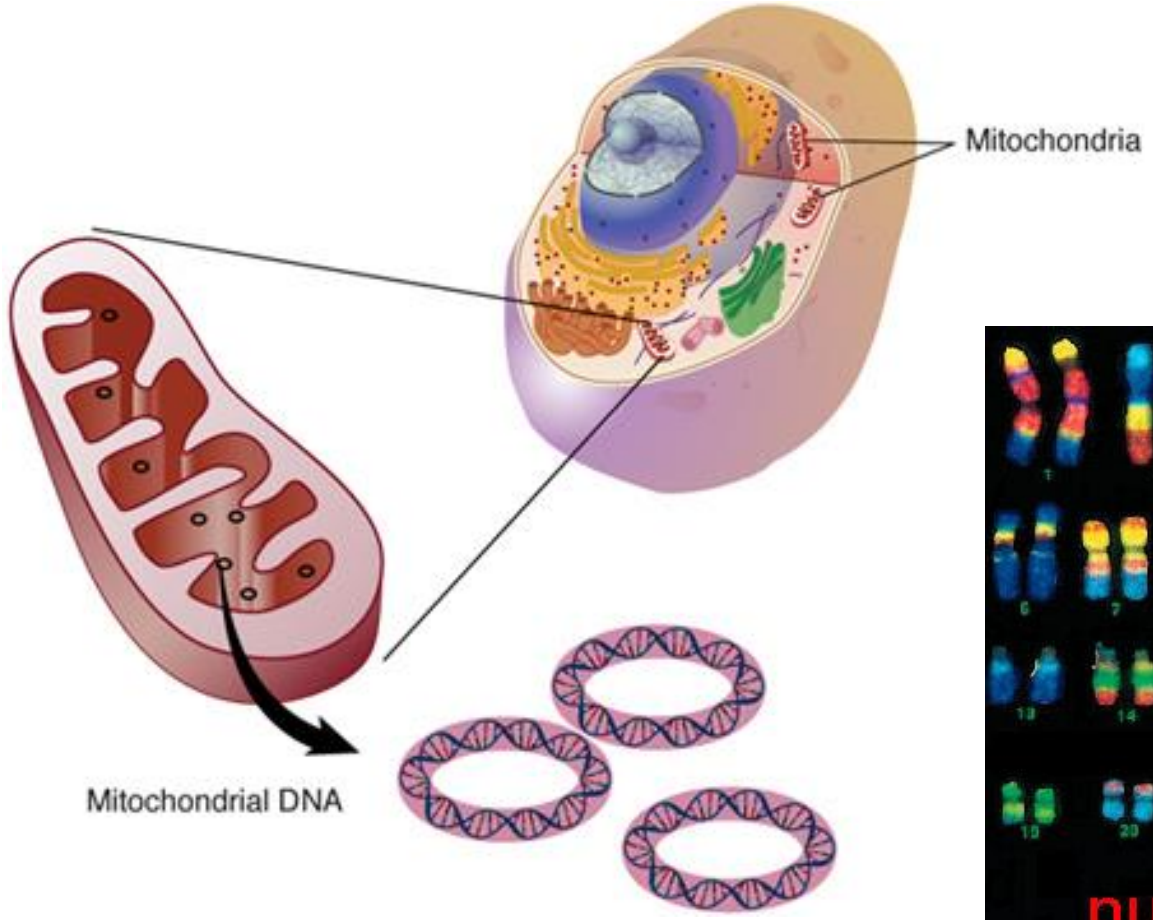
Mitochondrial medicine

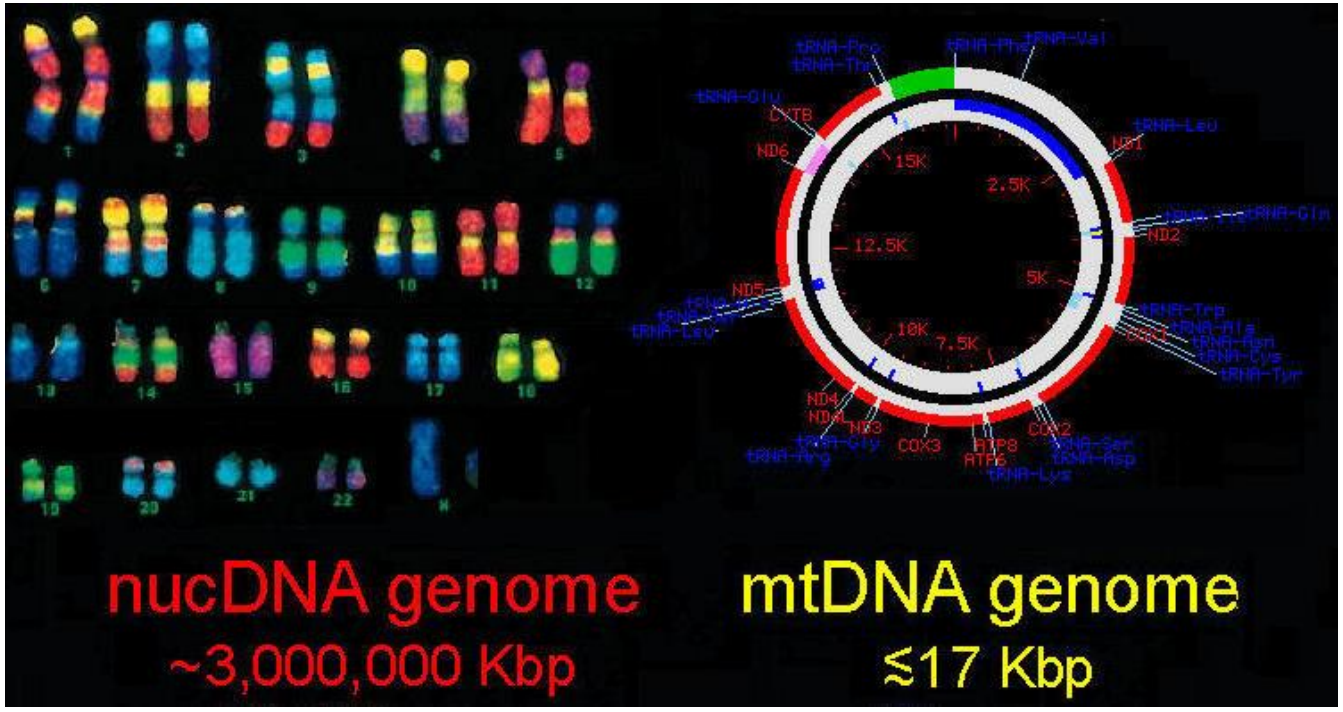
"any tissue, any symptom, any age"

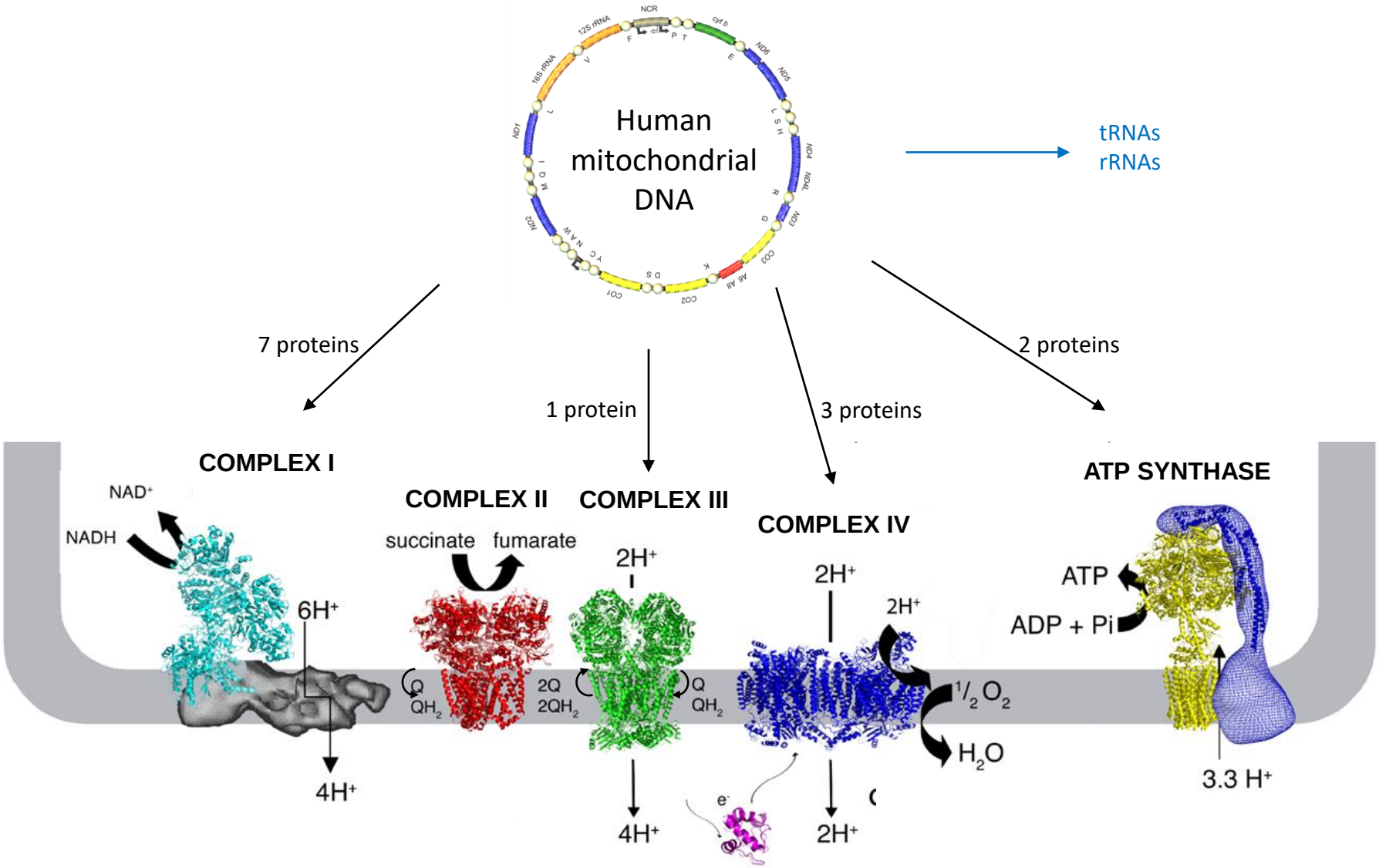


Nature Reviews | Molecular Cell Biology

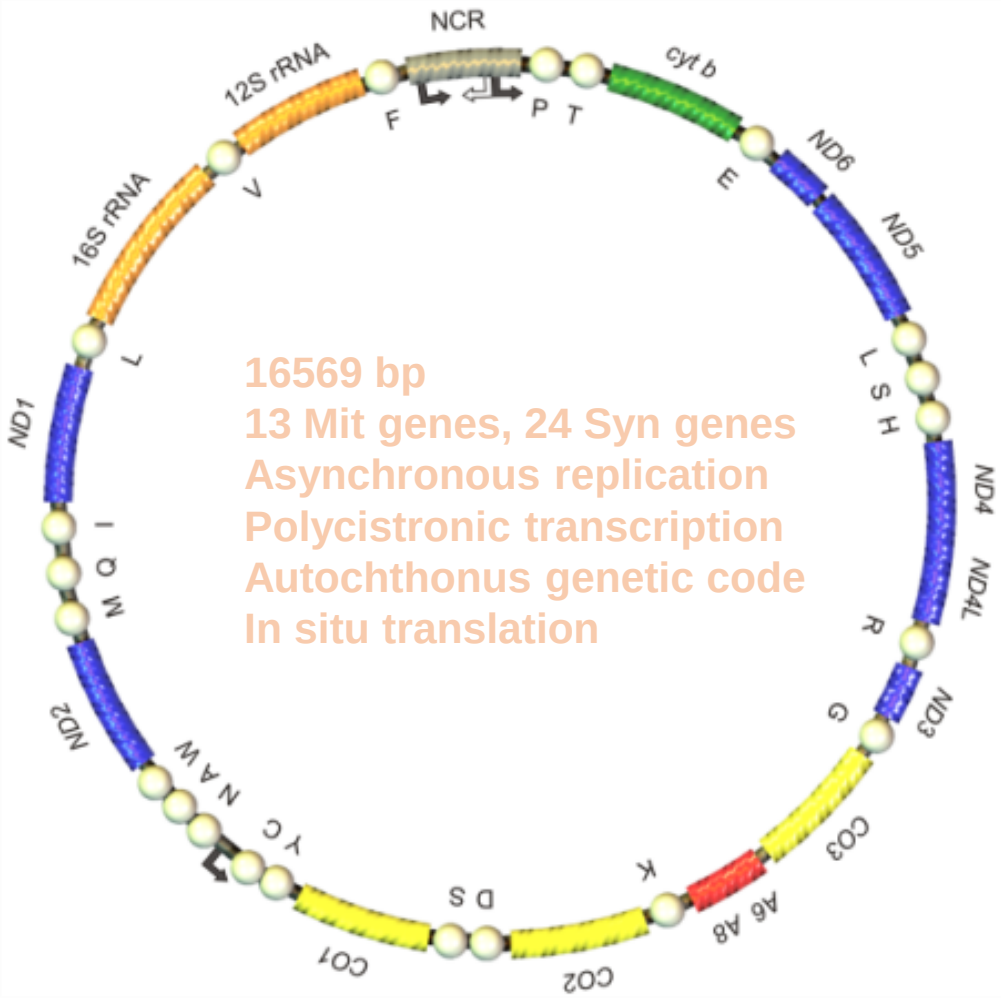
MITOCHONDRIAL DNA - MTDNA






Human mitochondrial DNA
tRNAs
rRNAs
7 proteins
1 protein
3 proteins
2 proteins
COMPLEX I
COMPLEX II
COMPLEX III
COMPLEX IV
ATP SYNTHASE
NAD+
NADH
6H+
4H+
succinate
fumarate
2H+
Q
QH2
2Q
2QH2
e-
ATP
ADP + Pi
1/2 O2
H2O
3.3 H+

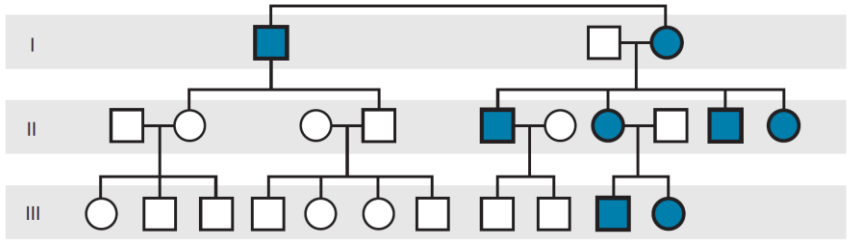
MTDNA FEATURES



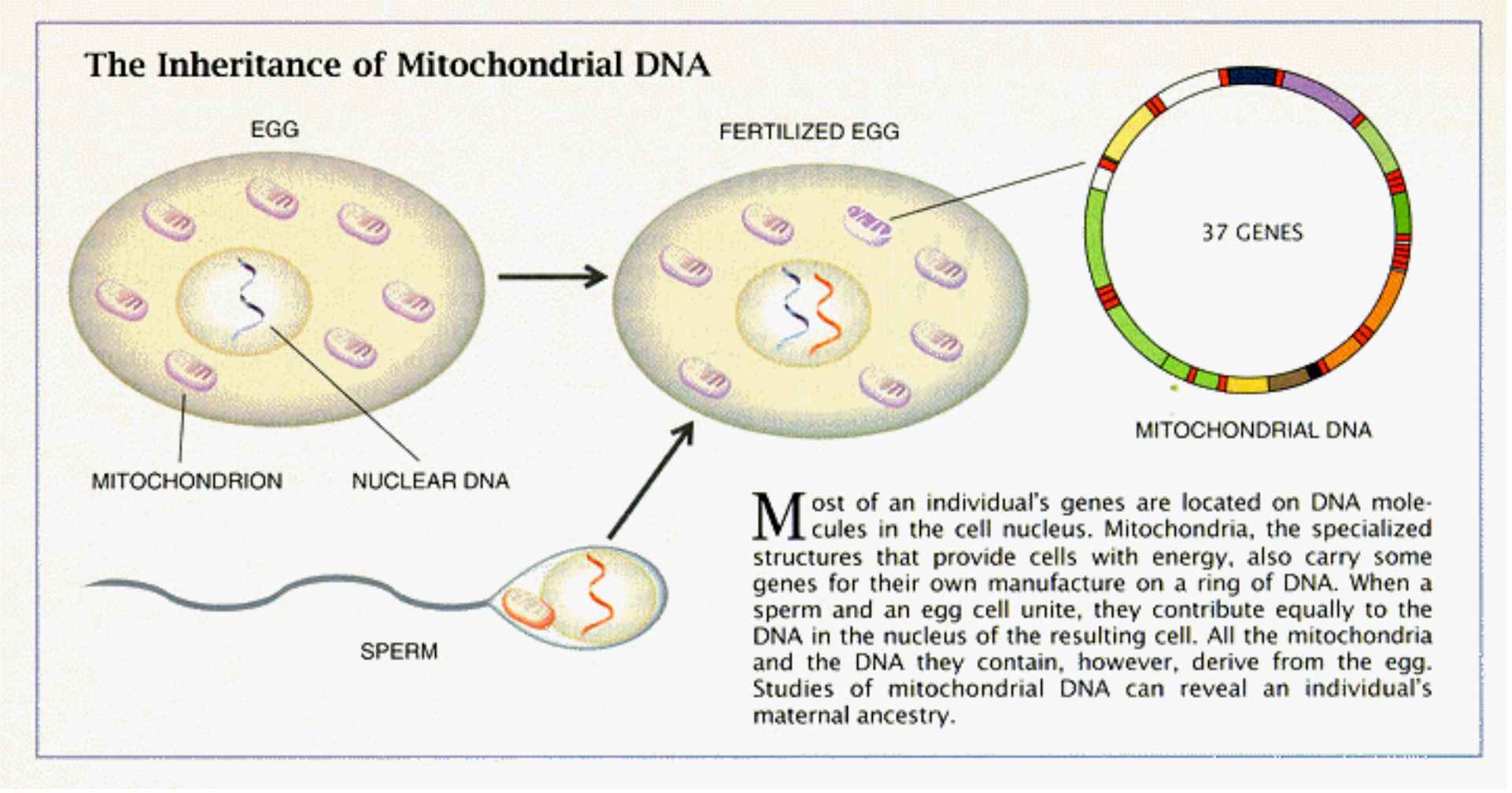
Mitochondrial DNA rules

- Maternal inheritance
- Heteroplasmy
- Mitotic Segregation
- Threshold Effect

MTDNA RULES



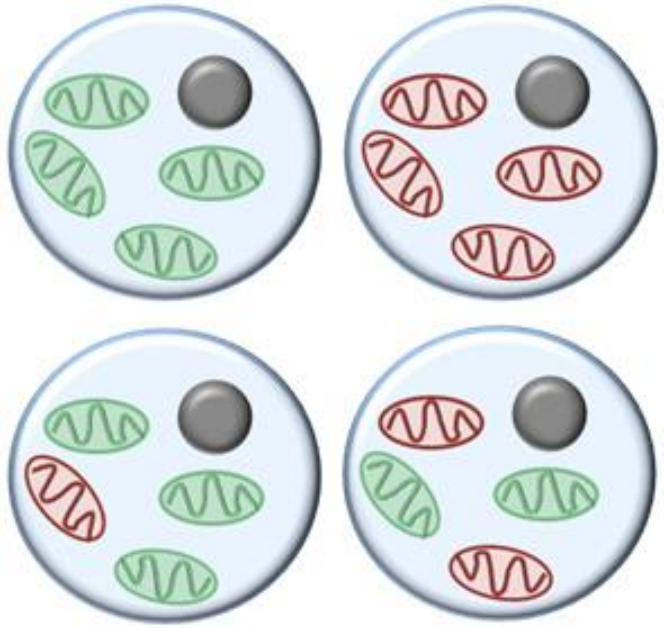
- **Maternal inheritance**



MTDNA RULES

- **Polyplasmy**

Each human cell has thousands of mitochondria and within each single mitochondrion are present multiple copies (2 to 10) of mtDNA. Usually all these copies are identical, a status known as homoplasmy.



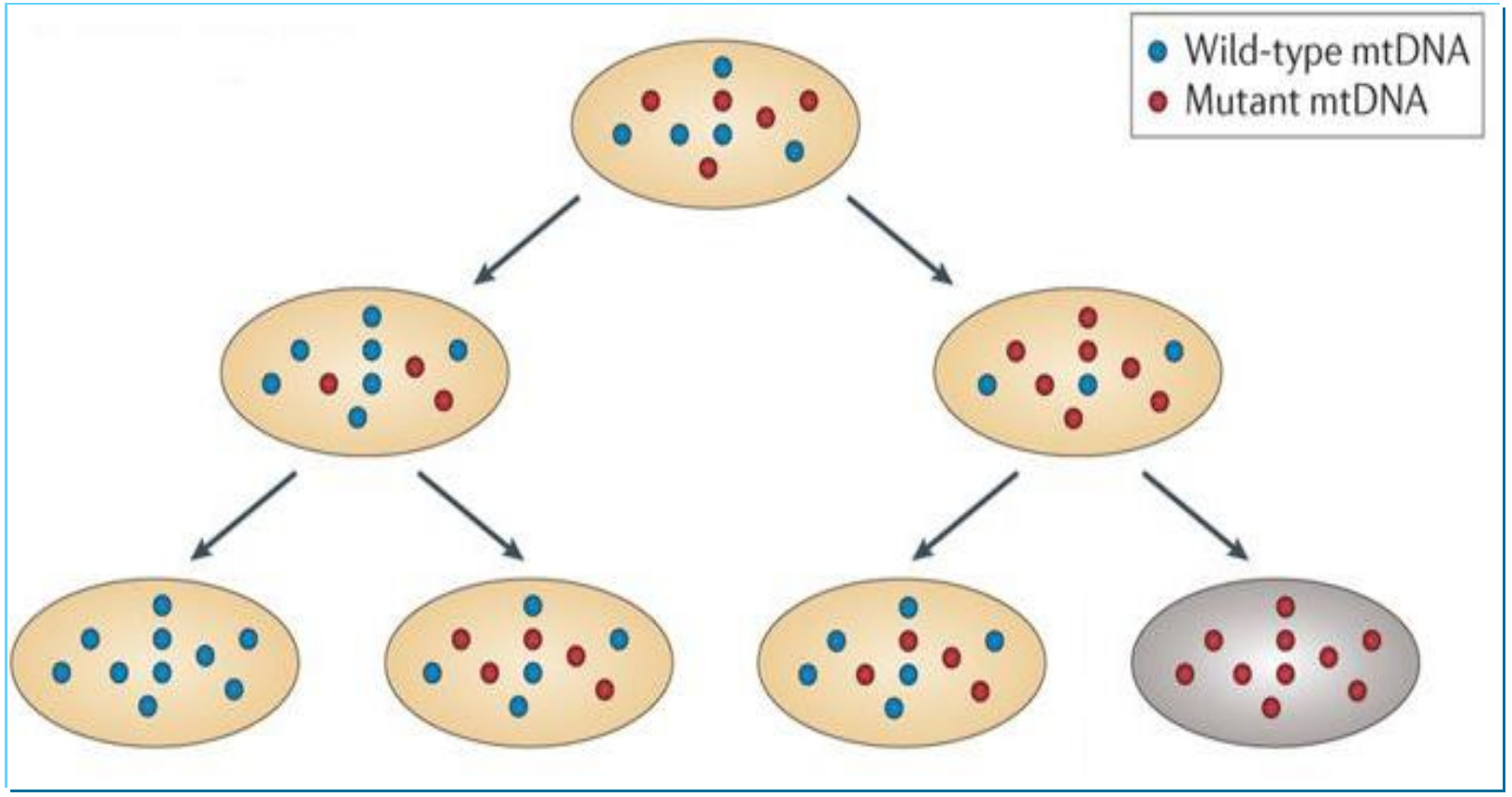
Homoplasmy

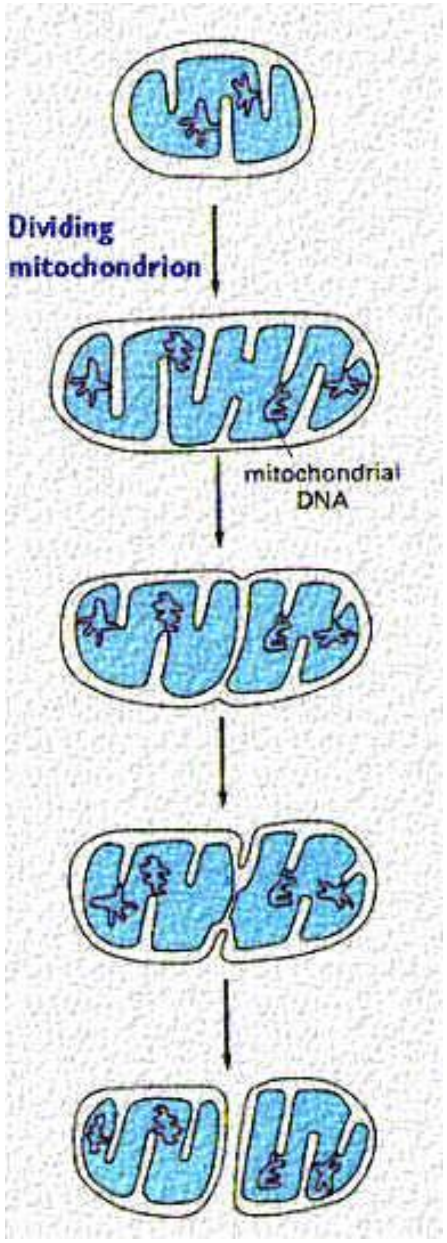
Heteroplasmy

However, mutant mtDNA could coexist with wt mtDNA in the same cell. This coexistence is called heteroplasmy.

MTDNA RULES

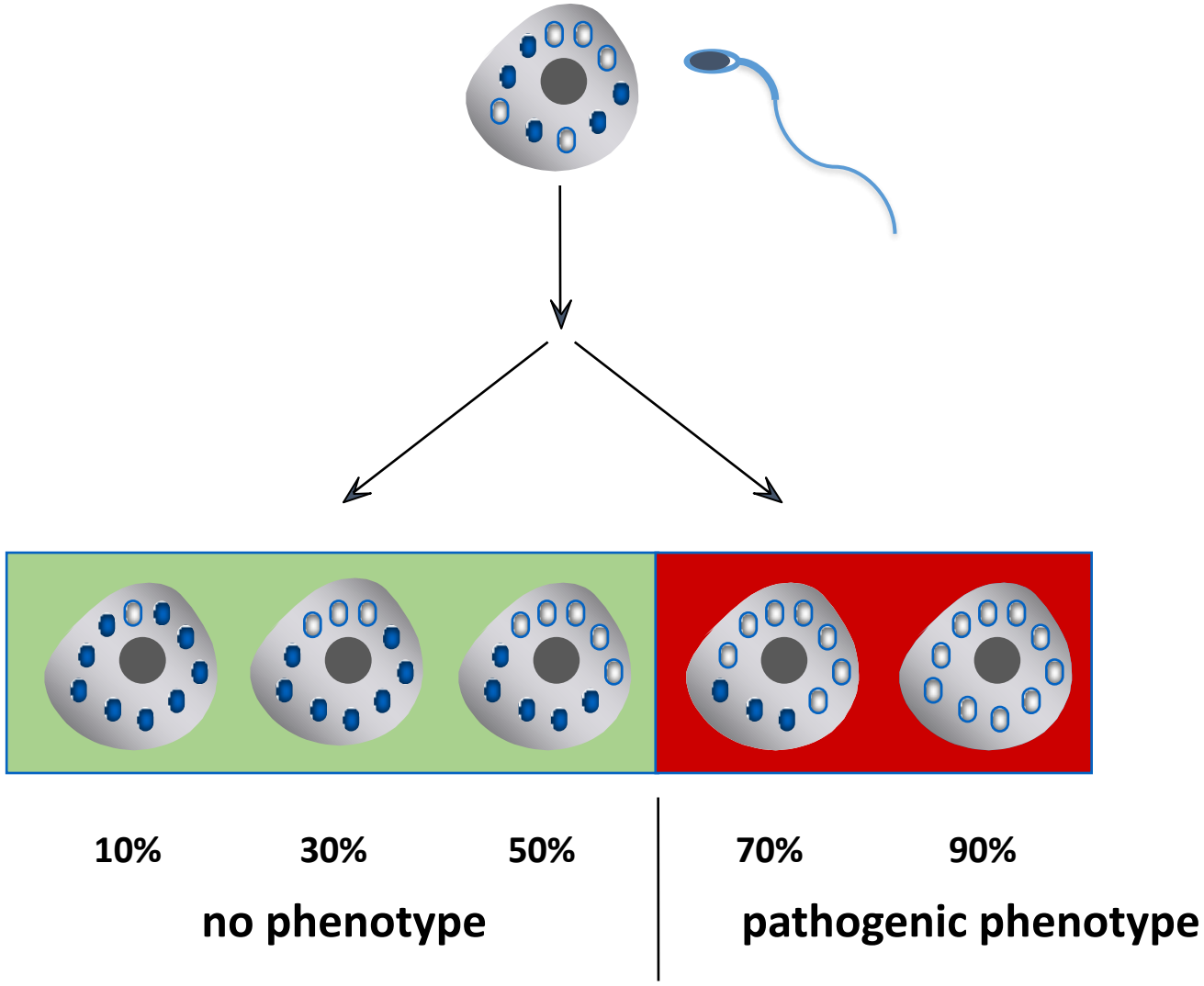
- **Mitotic/random Segregation**



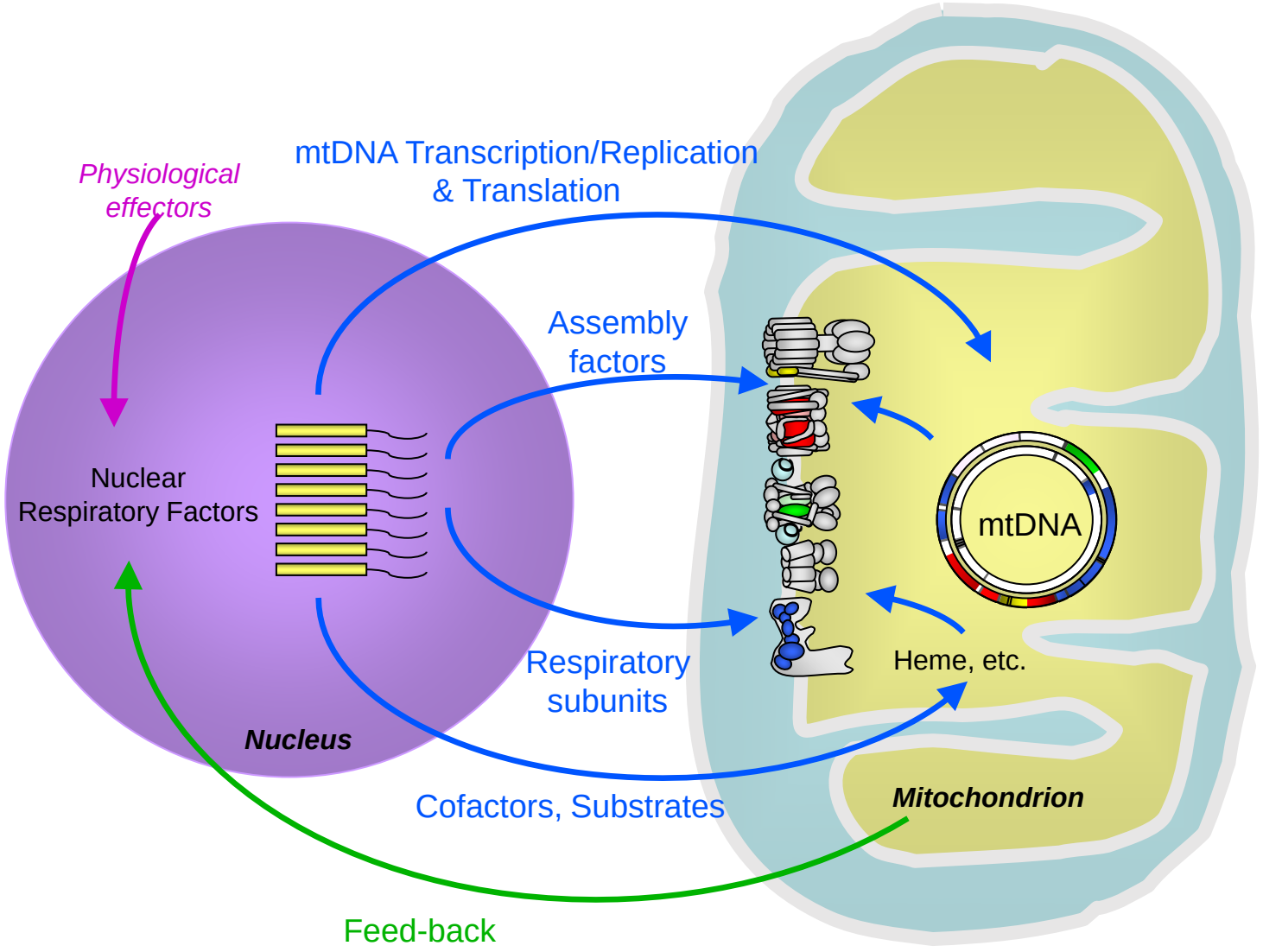


MTDNA RULES

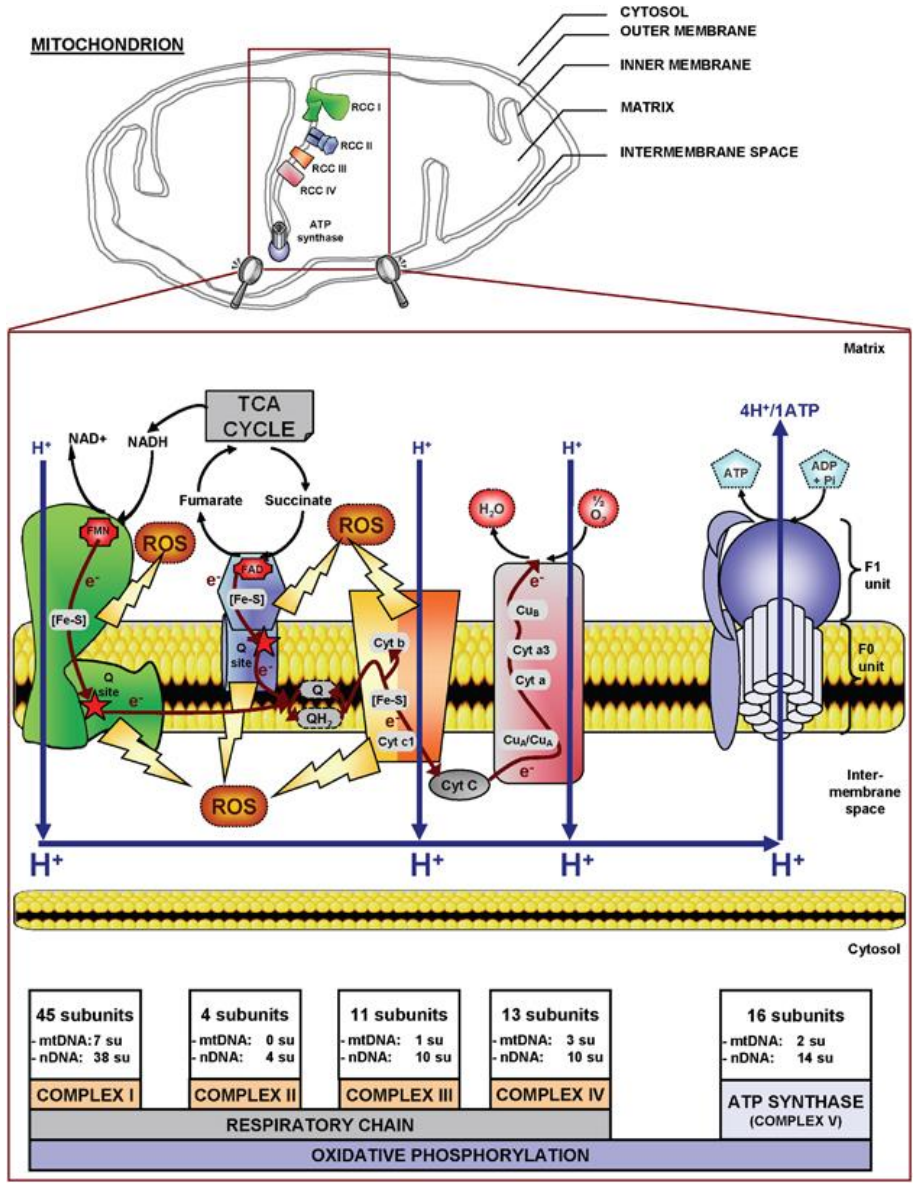
- **Threshold Effect**



Mitochondrial Disorders: not only mtDNA!

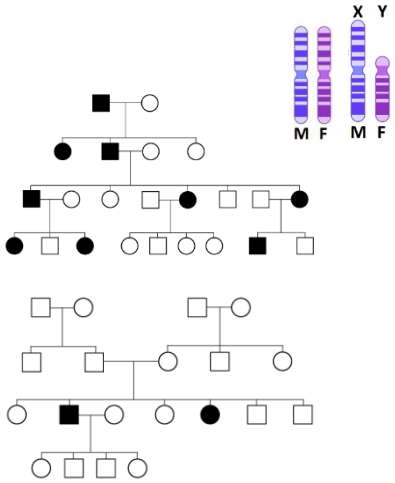


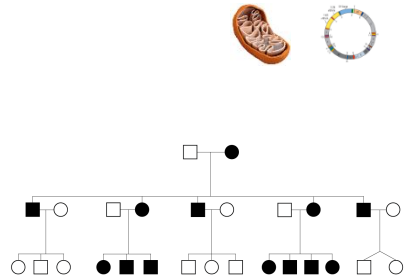
MITOCHONDRIAL DISORDERS: GENETIC HETEROGENICITY

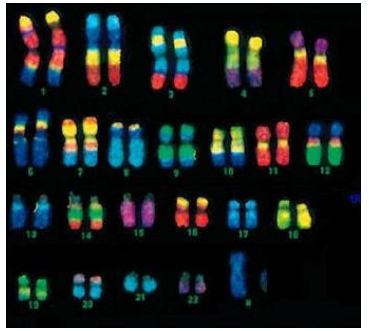


Mitochondrial medicine

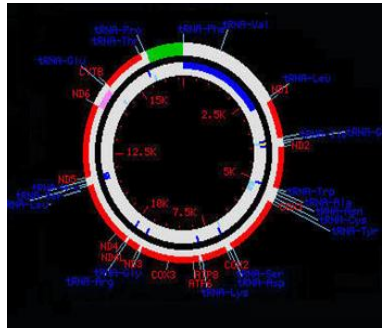
"any mode of inheritance"







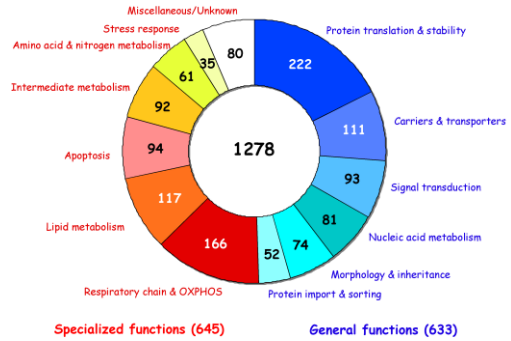
nDNA



mtDNA

MITOCHONDRIAL DISORDERS: GENETIC ANALYSIS

Mitochondrial DNA defects ← Genetic classification → Nuclear DNA mutations



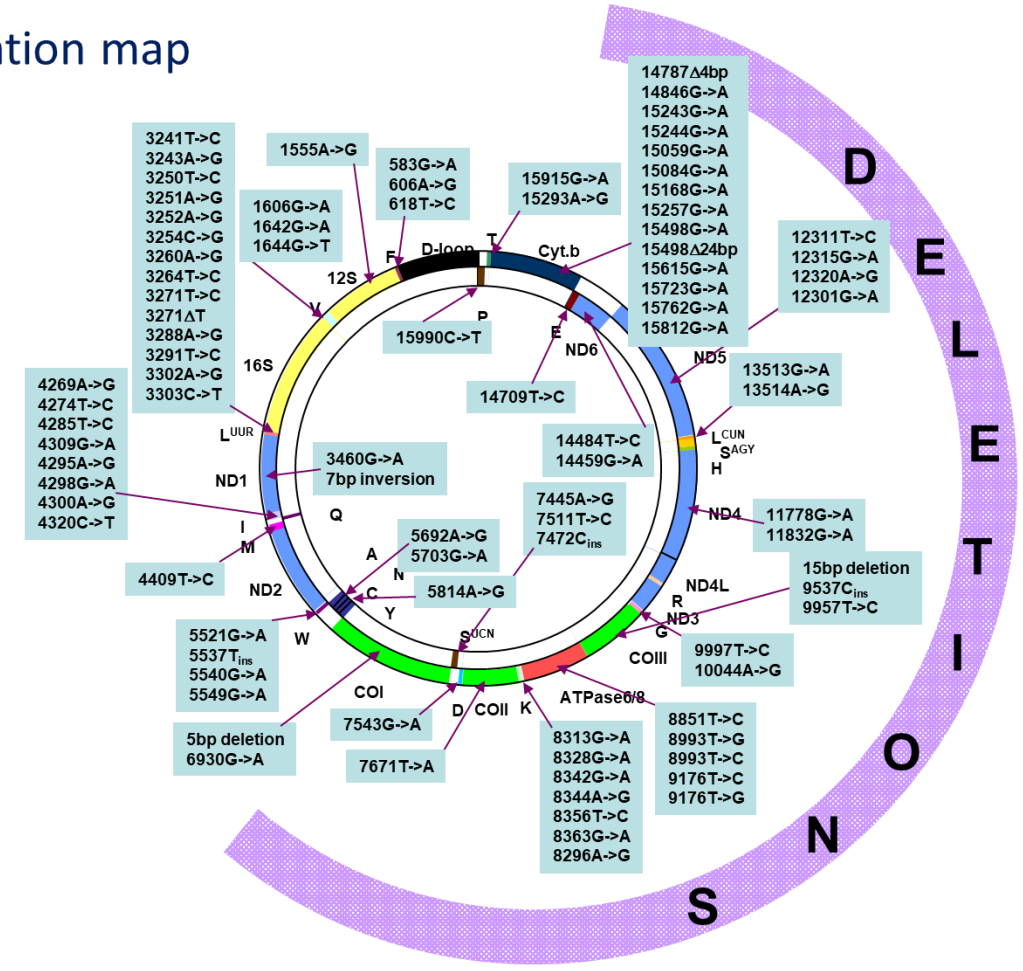
Mitochondrial DNA rules

- Maternal inheritance
- Heteroplasmy
- Mitotic Segregation
- Threshold Effect

Mendelian rules

MITOCHONDRIAL DNA DEFECTS

Mutation map

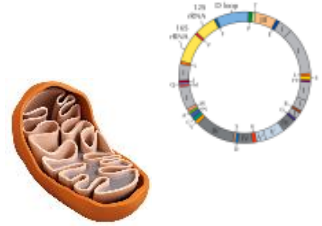


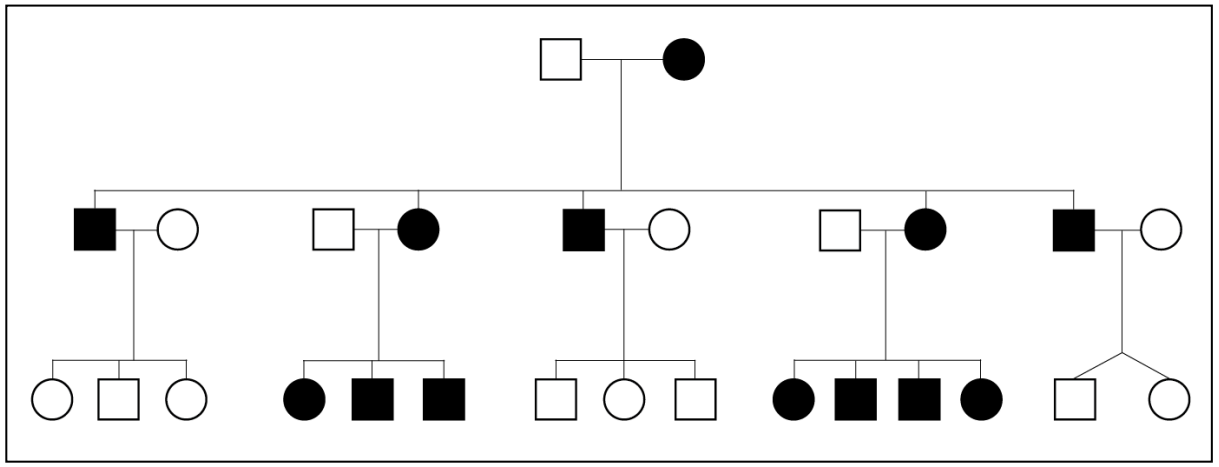
- Protein-encoding OXPHOS subunit genes
- Protein synthesis genes (rRNAs, tRNAs)
- Large deletions

Heteroplasmic mutations

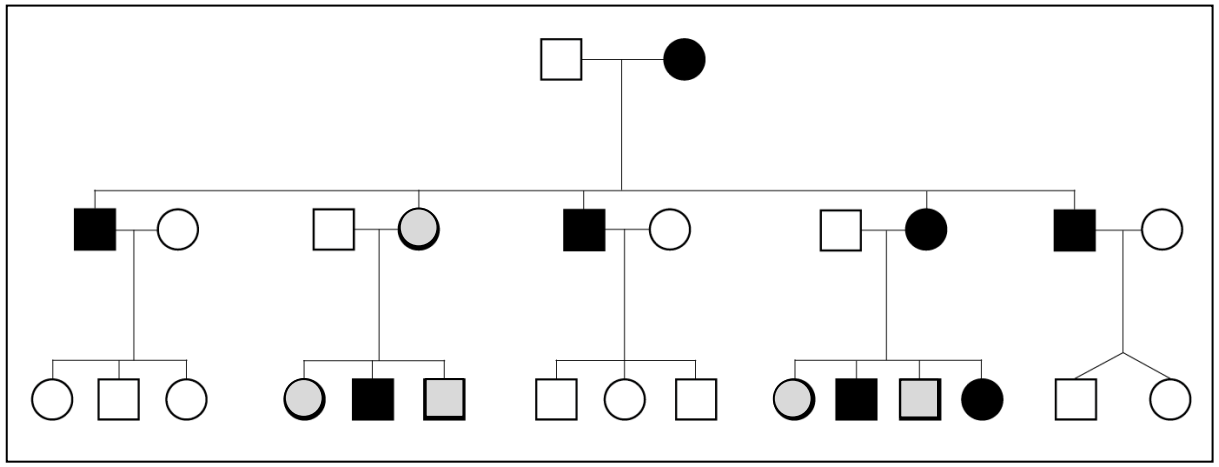
Homoplasmic mutations

MITOCHONDRIAL DNA DEFECTS



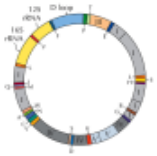


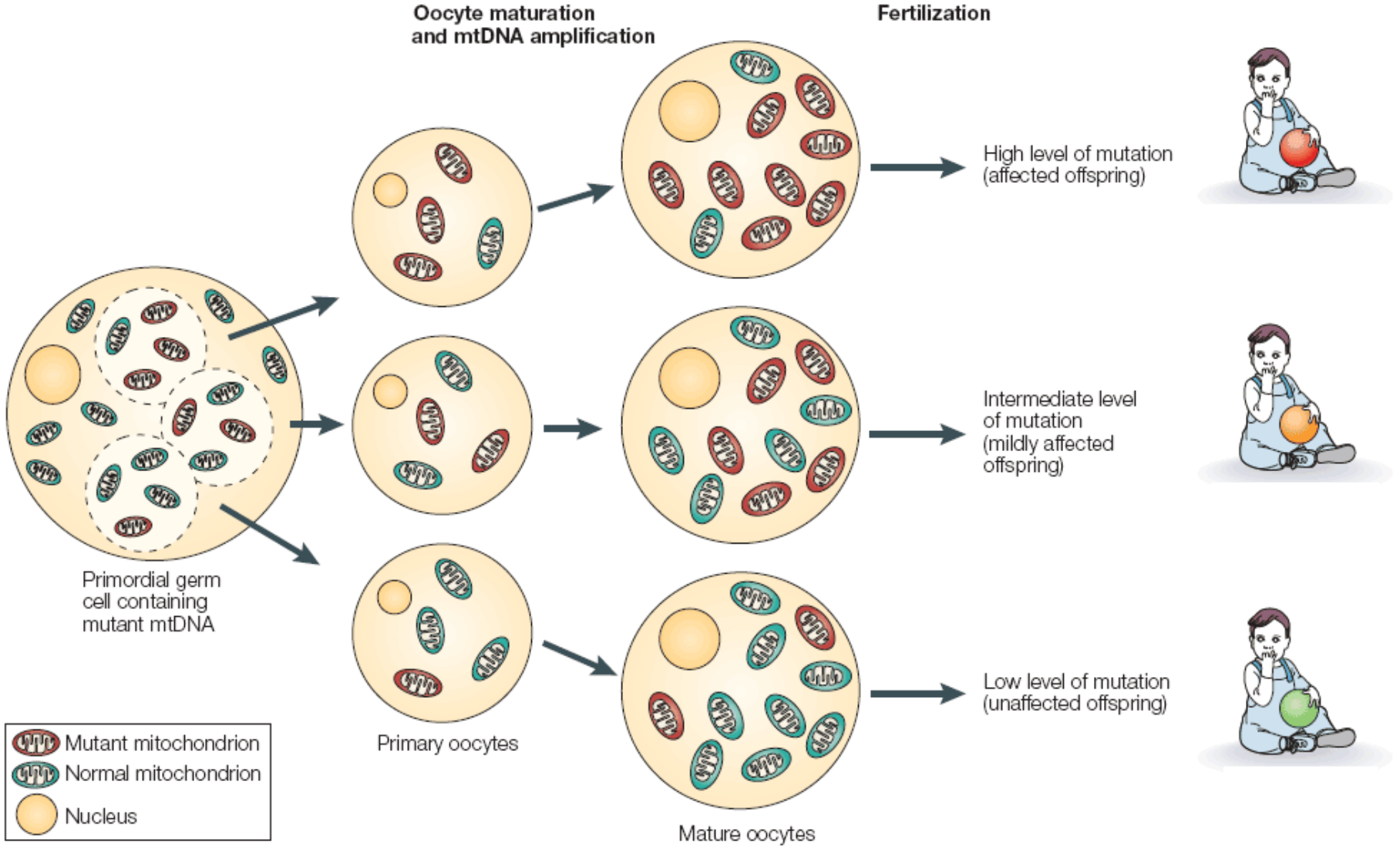
- *Homoplasmic mutation*
- *Complete penetrance*



- *Heteroplasmic mutation*
- *Random segregation/Threshold effect*

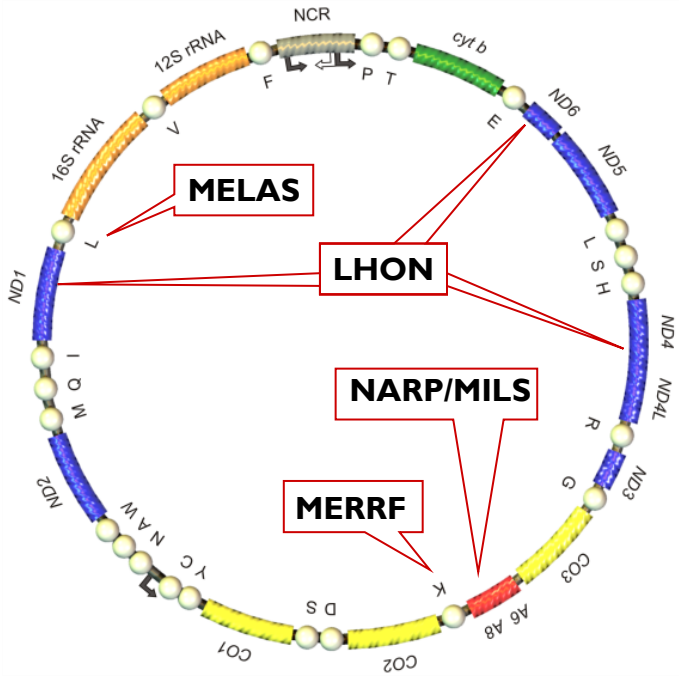
MITOCHONDRIAL DNA DEFECTS





MITOCHONDRIAL DNA DEFECTS

- **Protein-encoding OXPHOS subunit genes**
- **Protein synthesis genes (rRNAs, tRNAs)**
- Large deletions



1988: first mtDNA mutations

Today > 200 mutations reported; the most common are:

- m.3460G>A *MT-ND1*
- m.11778G>A *MT-ND4*
- m.14484T>C *MT-ND6*
- m.3243A>G *MT-TL1*, $tRNA^L$
- m.8344A>G *MT-TK*, $tRNA^K$
- m.8993T>G *MT-ATP6*

Heteroplasmic mutations

LHON: Leber Hereditary Optic Neuropathy

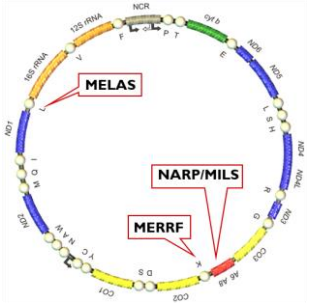
MELAS: mitochondrial Myopathy, Encephalopathy, Lactic Acidosis, and Stroke-like episodes.

MERRF: Myoclonic Epilepsy associated with Ragged-Red Fibers. Additional symptoms: ataxia, hearing loss, lipomas

NARP: Neuropathy, Ataxia, Retinitis Pigmentosa.

MILS: Maternally Inherited Leigh Sindrome; early-onset progressive neurodegenerative disorder with a characteristic neuropathology (necrotizing encephalopathy, infantile subacute)

MITOCHONDRIAL DNA DEFECTS



Heteroplasmic point mutations

Maternally inherited
Qualitative correlation btw mutation and phenotype e.g. MERRF, MELAS, NARP, MMC, MM, etc.
Quantitative correlation btw % of mutation load and clinical severity

m.8993T>G (p.L156R) in ***MTATP6***

Mutation load:	
• Up to 60%	unaffected
• 60-90%	NARP
• >90%	MILS

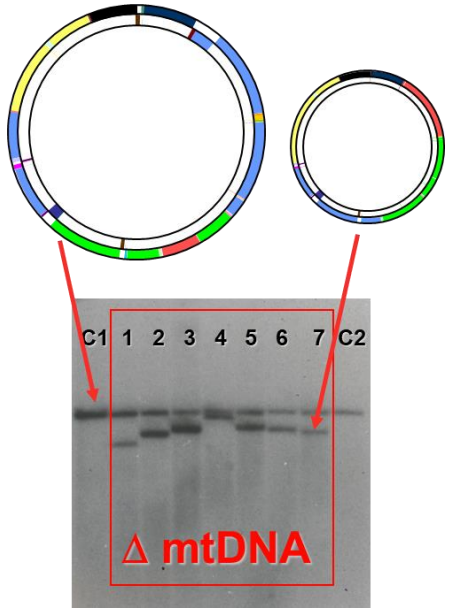
Warning for mtDNA analysis

- Heteroplasmy may be highly variable in various tissues in the same subject
- **The analysis has to be performed on DNA extracted from affected tissues, tipically from muscle**
- Need to determine heteroplasmy level
- Useful to assess heteroplasmy level in different tissues
- Useful to assess heteroplasmy level in family members (maternal relatives)

Prenatal or preimplantation analysis

MITOCHONDRIAL DNA DEFECTS

- Protein-encoding OXPHOS subunit genes
- Protein synthesis genes (rRNAs, tRNAs)
- **Large deletions**

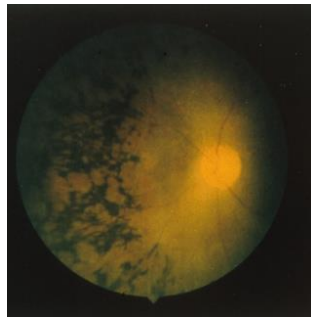


Single mtDNA deletions

<u>Sporadic</u>
Heteroplasmic
Correlation with tissue distribution of ΔmtDNA
No clear quantitative correlation btw % and size of ΔmtDNA and clinical severity

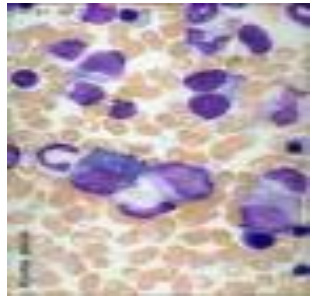
sporadic PEO: Δ only in muscle

PEO, proximal mit. myopathy



Kearns Sayre s.: Δ mostly in muscle

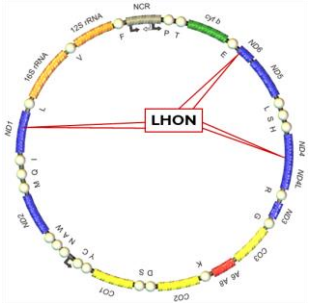
juvenile onset, PEO, mit. myopathy
ataxia, progressive neurological failure
retinitis pigmentosa, heart blocks



Pearson's: Δ everywhere

neonatal pancytopenia
pancreatic failure
may evolve into KSS

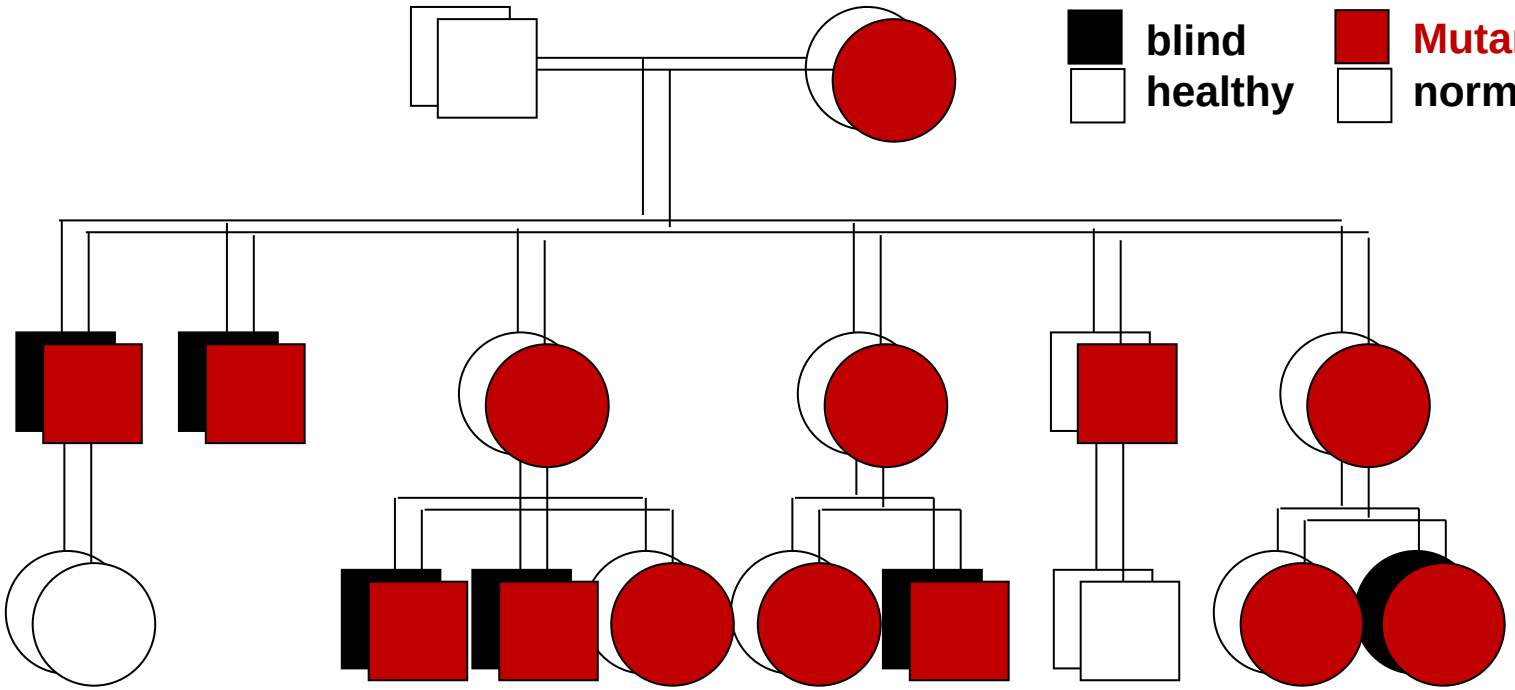
MITOCHONDRIAL DNA DEFECTS



Homoplasmic point mutations

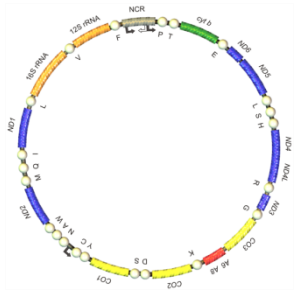
Maternally inherited
Qualitative correlation btw mutation and phenotype
Incomplete (sex dependent) penetrance

LHON: Leber Hereditary Optic Neuropathy



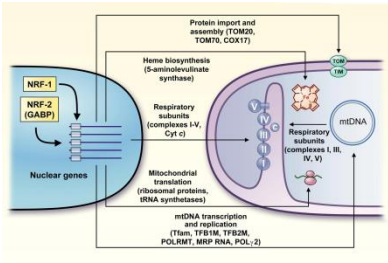
Mutation is necessary but not sufficient to produce the disease phenotype

MITOCHONDRIAL DISORDERS: GENETIC ANALYSIS



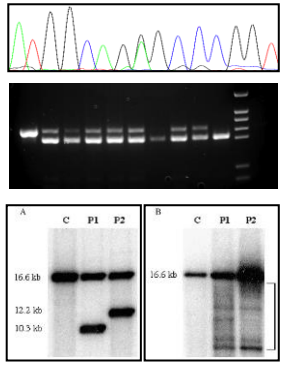
Mitochondrial disorders

- **mtDNA mutations**
  - **Sporadic**
    Large-scale deletions or duplications
    → Protein-encoding and RNA genes
  - **Maternal**
    Point mutations microdeletions
    → Protein-encoding or tRNA genes
- **Defects of intergenomic signalling**
  - **Mendelian**
    Associated with mtDNA mutations
    → mtDNA depletion
    → Multiple mtDNA deletions
- **Nuclear DNA mutations**
  - **Mendelian**
    Biochemical defects no mtDNA lesions
    → Multiple defects
    → Generalized defects
    → Tissue-specific defects



MITOCHONDRIAL DISORDERS: GENETIC ANALYSIS

Mitochondrial DNA defects ← Genetic classification → Nuclear DNA mutations

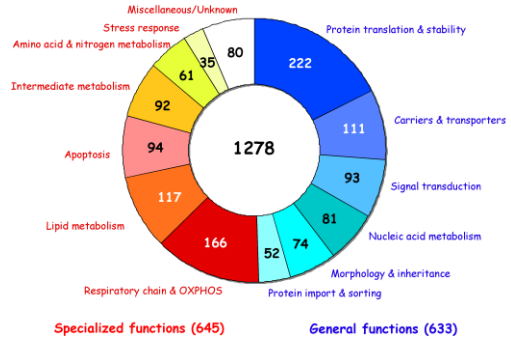


Direct sequencing of the whole mtDNA

RFLP for heteroplasmy quantification

Southern blot - Long range PCR - qPCR

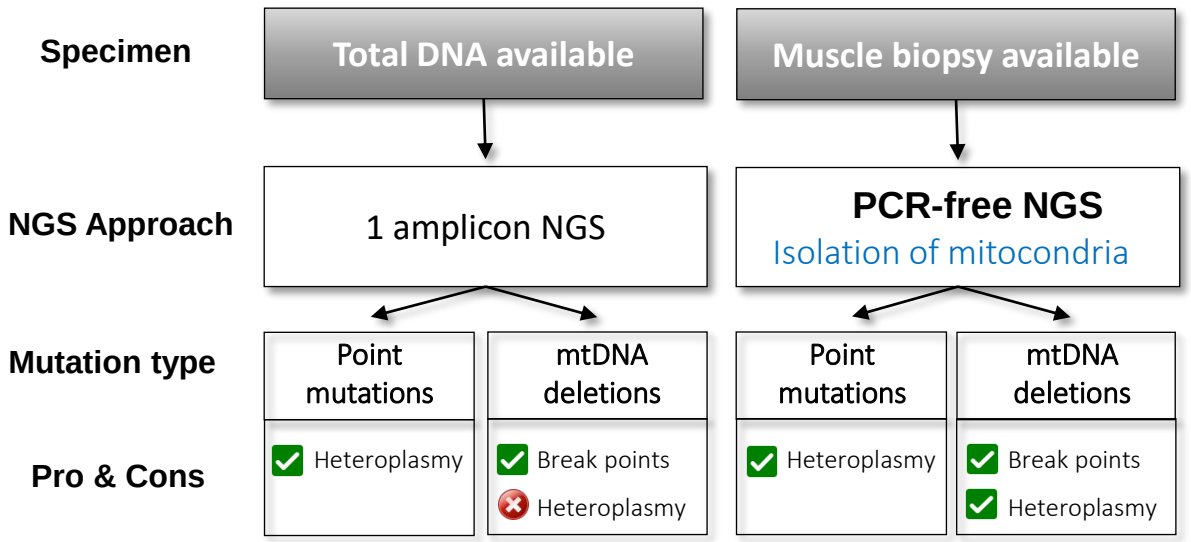
NGS strategies for:
- Sequencing
- Quantification of heteroplasmy
- Detection of large deletions



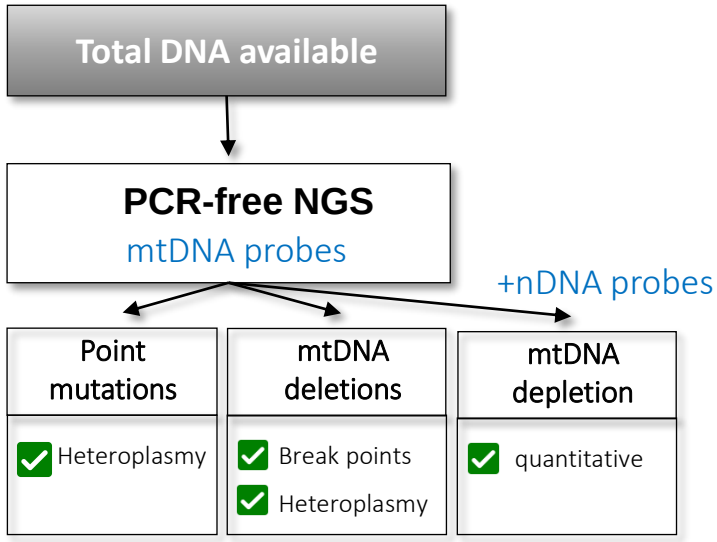
NGS strategies:
- **Targeted gene panels** (~300 genes associated with MD)
- **Clinical Exome** (~3000-5000 genes associated with human diseases)
- **Whole exome** (Exons of all nuclear genes, ~20000–25000)
- **Whole genome** (entire nuclear DNA)

NEW NGS APPROACHES FOR MTDNA ANALYSIS

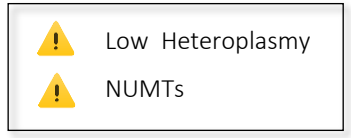
- Short read NGS



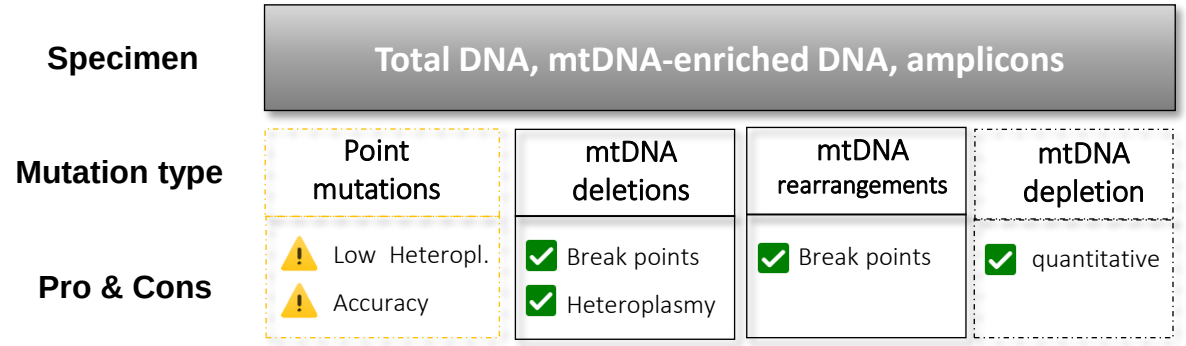
Legati et al. 2021



➢ **WGS (WES)**

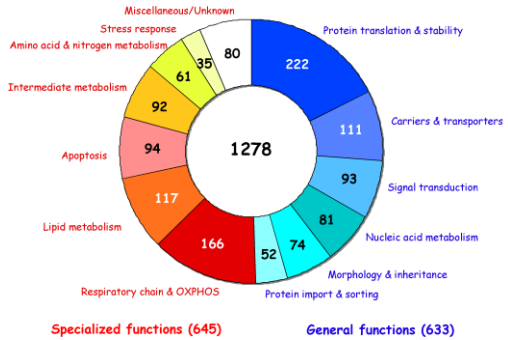


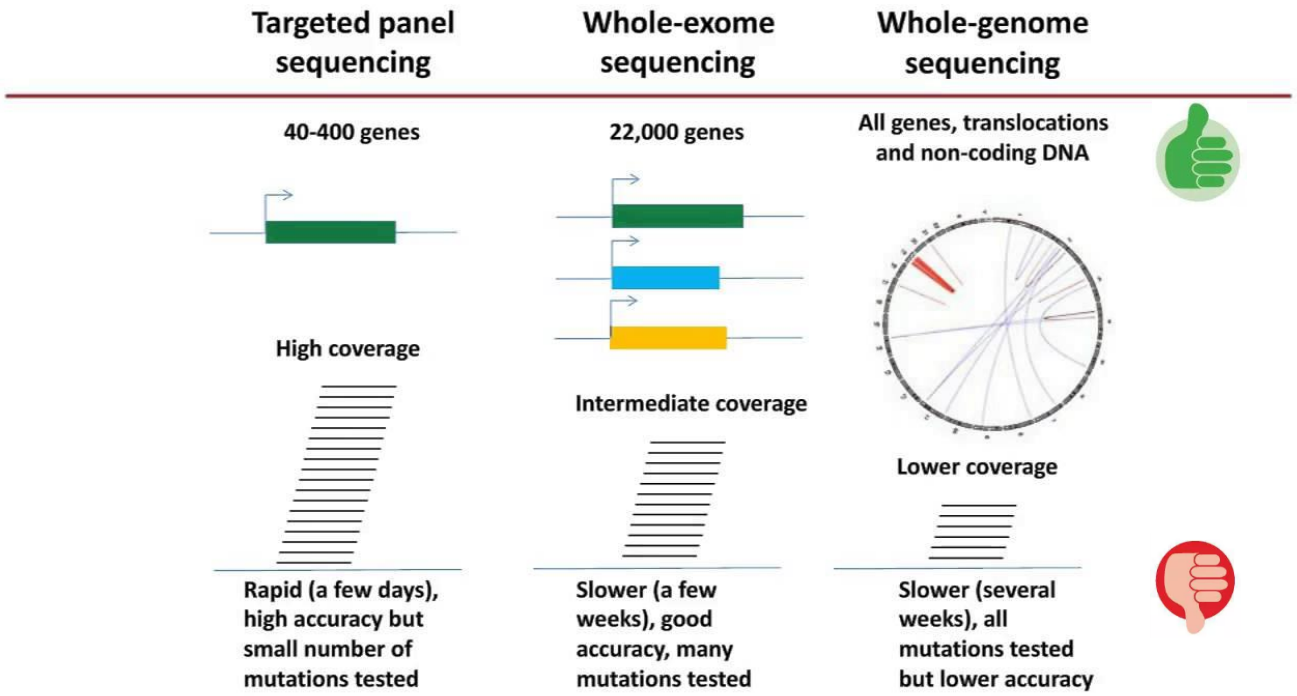
- Long read NGS



MITOCHONDRIAL DISORDERS: NUCLEAR DNA ANALYSIS

Genetic classification → Nuclear DNA mutations





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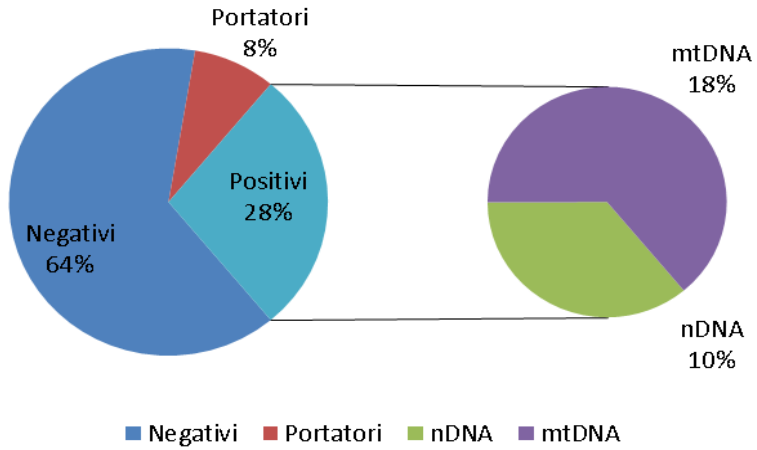
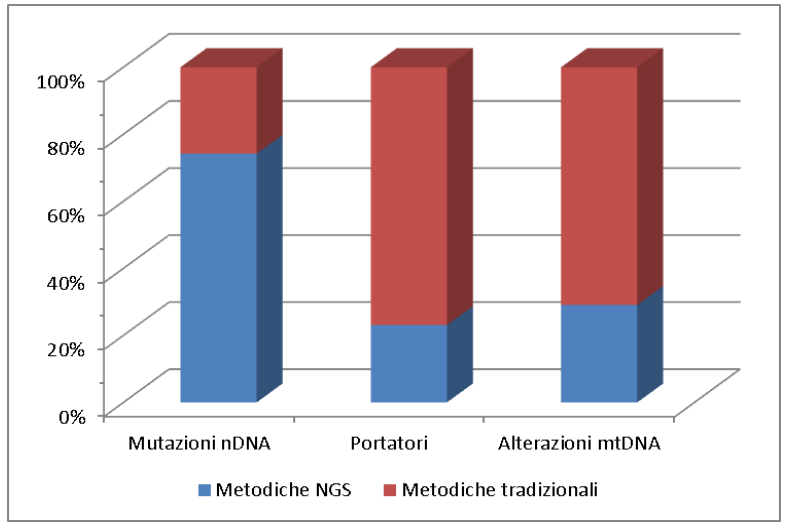
TARGETED GENE PANELS

- Increased diagnostic yield
- New phenotypes
- Concurrent variants/
  Digenic cases
- (New disease-genes)



IRCCS Besta – children cohort



Human Mutation
Variation, Informatics, and Disease

Homozygous mutations in *C1QBP* as cause of progressive external ophthalmoplegia (PEO) and mitochondrial myopathy with multiple mtDNA deletions

Silvia Marchet, Andrea Legati, Alessia Nasca, Ivano Di Meo, Manuela Spagnolo, Nadia Zanetti, Eleonora Lamantea, Alessia Catania, Costanza Lamperti, Daniele Ghezzi

MUSCLE &NERVE

New missense variants of *NDUFA11* associated with late-onset myopathy

Lorenzo Peverelli MD, Andrea Legati PhD, Eleonora Lamantea MSc, Alessia Nasca MSc, Alberto Lerario MD, Valentina Galimberti PhD, Daniele Ghezzi PhD, Costanza Lamperti MD,

Bi-allelic pathogenic variants in *NDUFC2* cause early-onset Leigh syndrome and stalled biogenesis of complex I

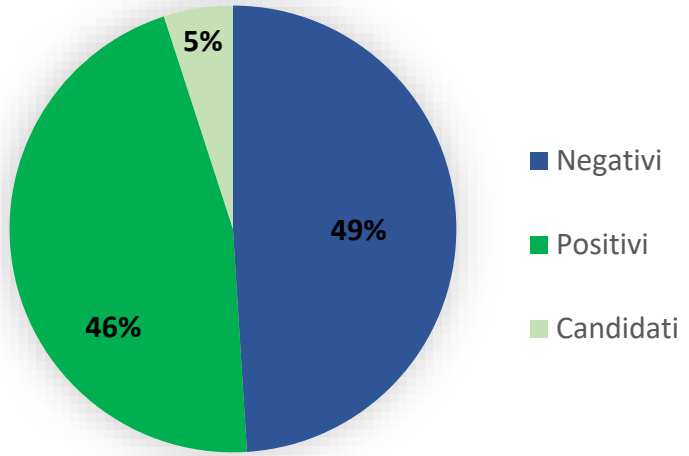
Ahmad Alahmad, Alessia Nasca, Juliana Heidler, Kyle Thompson, Monika Oláhová, Andrea Legati, Eleonora Lamantea, Jana Meisterknecht, Manuela Spagnolo, Langping He, Seham Alameer, Fahad Hakami, Abeer Almehdar, Anna Ardissone, Charlotte L Alston, Robert McFarland, Ilka Wittig, Daniele Ghezzi, Robert W Taylor

EMBO Mol Med (2020) 12: e12619

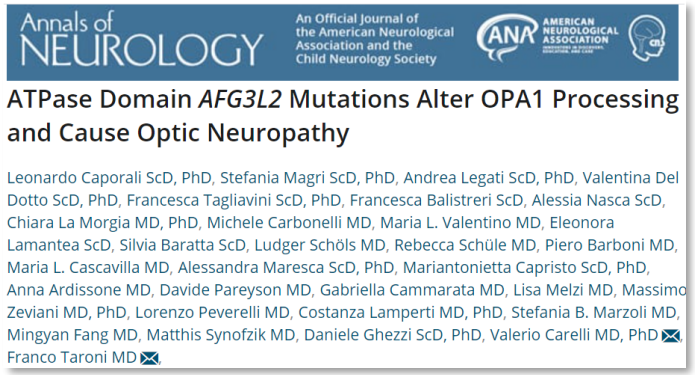
WHOLE EXOME SEQUENCING

- Further increased diagnostic yield

Wortmann et al. 2015:	*39%*
Taylor et al. 2016:	*52%*
Stenton et al. 2021	***55%***



- New disease-genes
- New phenotypes / new pathomechanisms


Annals of NEUROLOGY
An Official Journal of the American Neurological Association and the Child Neurology Society

ATPase Domain *AFG3L2* Mutations Alter OPA1 Processing and Cause Optic Neuropathy

Leonardo Caporali ScD, PhD, Stefania Magri ScD, PhD, Andrea Legati ScD, PhD, Valentina Del Dotto ScD, PhD, Francesca Tagliavini ScD, PhD, Francesca Balistreri ScD, Alessia Nasca ScD, Chiara La Morgia MD, PhD, Michele Carbonelli MD, Maria L. Valentino MD, Eleonora Lamantea ScD, Silvia Baratta ScD, Ludger Schöls MD, Rebecca Schüle MD, Piero Barboni MD, Maria L. Cascavilla MD, Alessandra Maresca ScD, PhD, Mariantonietta Capristo ScD, PhD, Anna Ardissone MD, Davide Pareyson MD, Gabriella Cammarata MD, Lisa Melzi MD, Massimo Zeviani MD, PhD, Lorenzo Peverelli MD, Costanza Lamperti MD, PhD, Stefania B. Marzoli MD, Mingyan Fang MD, Matthis Synofzik MD, Daniele Ghezzi ScD, PhD, Valerio Carelli MD, PhD, Franco Taroni MD

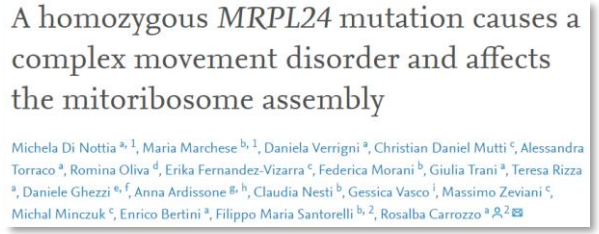

Human Mutation
Variation, Informatics, and Disease

Novel *NDUFA12* variants are associated with isolated complex I defect and variable clinical manifestation

Alessandra Torraco, Alessia Nasca, Daniela Verrigni, Alessandra Pennisi, Maha S. Zaki, Giorgia Olivieri, Zahra Assouline, Diego Martinelli, Reza Maroofian, Teresa Rizza, Michela Di Nottia, Federica Invernizzi, Eleonora Lamantea, Daniela Longo, Henry Houlden, Holger Prokisch, Agnès Rötig, Carlo Dionisi-Vici, Enrico Bertini, Daniele Ghezzi, Rosalba Carrozzo, Daria Diodato


Impaired complex I repair causes recessive Leber's hereditary optic neuropathy

Sarah L. Stenton, Natalia L. Sheremet, Claudia B. Catarino, Natalia A. Andreeva, Zahra Assouline, Piero Barboni, Ortal Barel, Riccardo Berutti, Igor Bychkov, Leonardo Caporali, Mariantonietta Capristo, Michele Carbonelli, Maria L. Cascavilla, Peter Charbel Issa, Peter Freisinger, Sylvie Gerber, Daniele Ghezzi, Elisabeth Graf, Juliana Heidler, Maja Hempel, Elise Heon, Yulya S. Itkis, Elisheva Javasky, Josseline Kaplan, Robert Kopajtich, Cornelia Kornblum, Reka Kovacs-Nagy, Tatiana D. Krylova, Wolfram S. Kunz, Chiara La Morgia, Costanza Lamperti, Christina Ludwig, Pedro F. Malacarne, Alessandra Maresca, Johannes A. Mayr, Jana Meisterknecht, Tatiana A. Nevinitsyna, Flavia Palombo, Ben Pode-Shakked, Maria S. Shmelkova, Tim M. Strom, Francesca Tagliavini, Michal Tzadok, Amelie T. van der Ven, Catherine Vignal-Clermont, Matias Wagner, Ekaterina Y. Zakharova, Nino V. Zhorzholadze, Jean-Michel Rozet, Valerio Carelli, Polina G. Tsygankova, Thomas Klopstock, Ilka Wittig, and Holger Prokisch

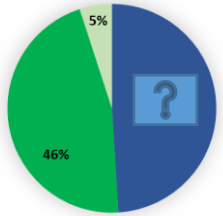

A homozygous *MRPL24* mutation causes a complex movement disorder and affects the mitoribosome assembly

Michela Di Nottia, Maria Marchese, Daniela Verrigni, Christian Daniel Mutti, Alessandra Torraco, Romina Oliva, Erika Fernandez-Vizarra, Federica Morani, Giulia Trani, Teresa Rizza, Daniele Ghezzi, Anna Ardissone, Claudia Nesti, Gessica Vasco, Massimo Zeviani, Michal Minczuk, Enrico Bertini, Filippo Maria Santorelli, Rosalba Carrozzo

WHOLE EXOME SEQUENCING

Trio WES

- New mode of inheritance/de novo AD



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Guide for Authors | About | Explore this Journal
AJHG

Am J Hum Genet. 2016 Oct 6; 99(4): 860–876.
Published online 2016 Sep 29. doi: 10.1016/j.ajhg.2016.08.014
PMCID: PMC5065686
PMID: 27693233

Recurrent De Novo Dominant Mutations in *SLC25A4* Cause Severe Early-Onset Mitochondrial Disease and Loss of Mitochondrial DNA Copy Number

Kyle Thompson,[1,20] Homa Majd,[2,20] Christina Dallabona,[3,20] Karit Reinson,[4,5,20] Martin S. King,[2] Charlotte L. Alston,[1] Langping He,[1] Tiziana Lodi,[3] Simon A. Jones,[6] Aviva Fattal-Valevski,[7] Nitay D. Fraenkel,[8] Ann Saada,[9] Alon Haham,[10] Pirjo Isohanni,[11,12] Roshni Vara,[13] Inês A. Barbosa,[14] Michael A. Simpson,[14] Charu Deshpande,[15] Sanna Puusepp,[4,5] Penelope E. Bonnen,[16] Richard J. Rodenburg,[17] Anu Suomalainen,[11,18] Katrin Õunap,[4,5] Orly Elpeleg,[19] Ileana Ferrero,[3] Robert McFarland,[1] Edmund R.S. Kunji,[2,21] and Robert W. Taylor[1,21,*]

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Mitochondrion
Volume 51, March 2020, Pages 68-78

LONP1 de novo dominant mutation causes mitochondrial encephalopathy with loss of LONP1 chaperone activity and excessive LONP1 proteolytic activity

Arnaud Besse [a], Daniel Brezavar [a], Jennifer Hanson [a], Austin Larson [b], Penelope E. Bonnen [a]

Journal of
Medical Genetics

A novel de novo dominant mutation in *ISCU* associated with mitochondrial myopathy

Andrea Legati [1], Aurelio Reyes [2], Camilla Ceccatelli Berti [3], Oliver Stehling [4], Silvia Marchet [1], Costanza Lamperti [1], Alberto Ferrari [3], Alan J Robinson [2], Ulrich Mühlenhoff [4], Roland Lill [4, 5], Massimo Zeviani [2], Paola Goffrini [3], Daniele Ghezzi [1]

- Incomplete penetrance

ELSEVIER
Molecular Genetics and Metabolism Reports
Volume 5, December 2015, Pages 51-54
MGM Reports

Mitochondrial leukoencephalopathy and complex II deficiency associated with a recessive *SDHB* mutation with reduced penetrance

Anna Ardissone [a], Federica Invernizzi [b], Alessia Nasca [b], Isabella Moroni [a], Laura Farina [c], Daniele Ghezzi [a]

Impaired complex I repair causes recessive Leber's hereditary optic neuropathy

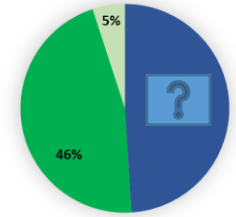
Sarah L. Stenton,[1,2] Natalia L. Sheremet,[3] Claudia B. Catarino,[4] Natalia A. Andreeva,[3] Zahra Assouline,[5] Piero Barboni,[6] Ortal Barel,[7,8,9] Riccardo Berutti,[1,2] Igor Bychkov,[10] Leonardo Caporali,[11] Mariantonietta Capristo,[11] Michele Carbonelli,[11] Maria L. Cascavilla,[6] Peter Charbel Issa,[12,13] Peter Freisinger,[14] Sylvie Gerber,[15] Daniele Ghezzi,[16,17] Elisabeth Graf,[1,2] Juliana Heidler,[18] Maja Hempel,[19] Elise Heon,[20] Yulya S. Itkis,[10] Elisheva Javasky,[7,8,9] Josseline Kaplan,[15] Robert Kopajtich,[1,2] Cornelia Kornblum,[21] Reka Kovacs-Nagy,[1,22] Tatiana D. Krylova,[10] Wolfram S. Kunz,[23] Chiara La Morgia,[11,24] Costanza Lamperti,[16] Christina Ludwig,[25] Pedro F. Malacarne,[26] Alessandra Maresca,[11] Johannes A. Mayr,[27] Jana Meisterknecht,[18] Tatiana A. Nevinitsyna,[3] Flavia Palombo,[11] Ben Pode-Shakked,[8,28,29] Maria S. Shmelkova,[3] Tim M. Strom,[1] Francesca Tagliavini,[11] Michal Tzadok,[8,30] Amelie T. van der Ven,[19] Catherine Vignal-Clermont,[31] Matias Wagner,[1,2] Ekaterina Y. Zakharova,[10] Nino V. Zhorzholadze,[3] Jean-Michel Rozet,[15] Valerio Carelli,[11,24] Polina G. Tsygankova,[10] Thomas Klopstock,[4,32,33] Ilka Wittig,[18,34] and Holger Prokisch[1,2]

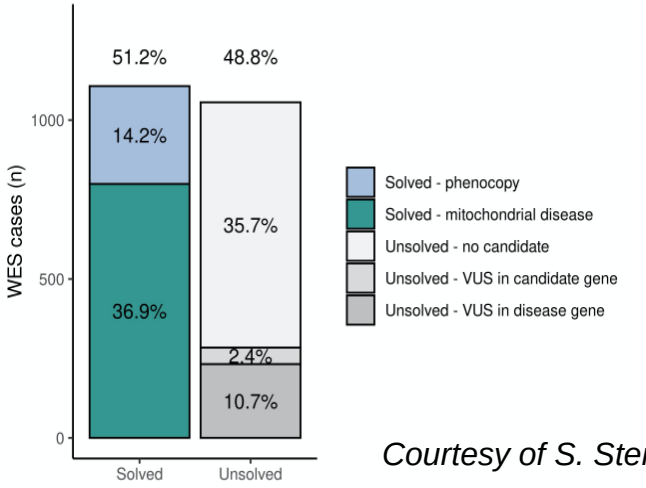
WES: PHENOCOPIES

CANDIDATE VARIANTS → DEAD END → Genes encoding mitochondrial proteins

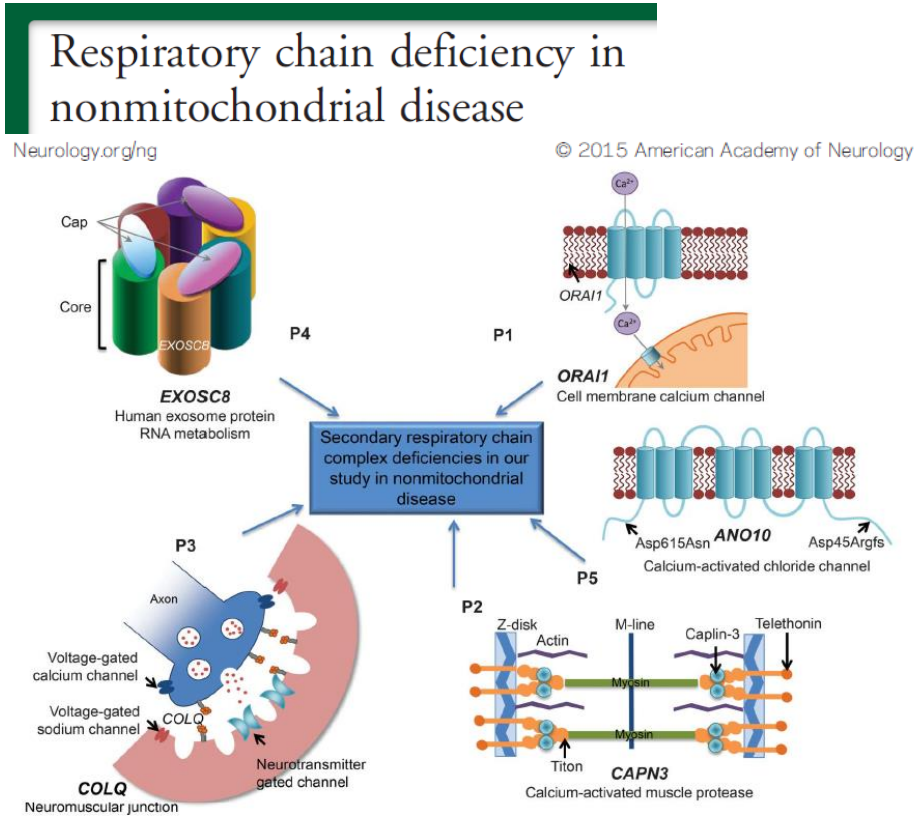
↓

all genes (secondary mitochondrial dysfunction)





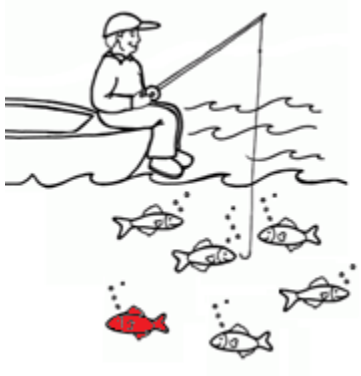
Courtesy of S. Stenton



DIFFERENT NGS APPROACHES

Differences in detection power, output size and data interpretation

Sanger: 1-5 genes, it needs high phenotype/genotype concordance



NGS panel: 2-300 genes, more power, disease mutation easy to find if sequenced

NGS Exome: 20,000 genes, very likely to sequence the disease mutation but hard to find

NGS Genome: entire DNA (~3000000000 bp), almost sure to sequence the disease mutation but extremely hard to find

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Clinicians: Costanza Lamperti, Alessia Catania
Director: Franco Taroni

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Sistema Socio Sanitario Regione Lombardia
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Lab of Neurogenetics and mitochondrial disorders
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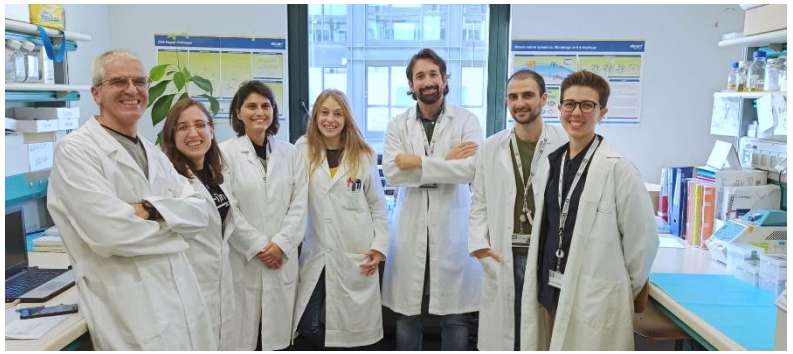
http://www.mitopedia.org
Mariani Foundation Center for the Study of Mitochondrial Pediatric Diseases



THANK YOU!

Ministero della Salute
ERAPerMed
E-Rare CALLS
Fondazione Pierfranco e Luisa Mariani neurologia infantile
Mitocon
TeleThon

La richiesta di competenza neurologica nel prossimo futuro

Sesta edizione

UNIVERSITÀ DEGLI STUDI DI MILANO

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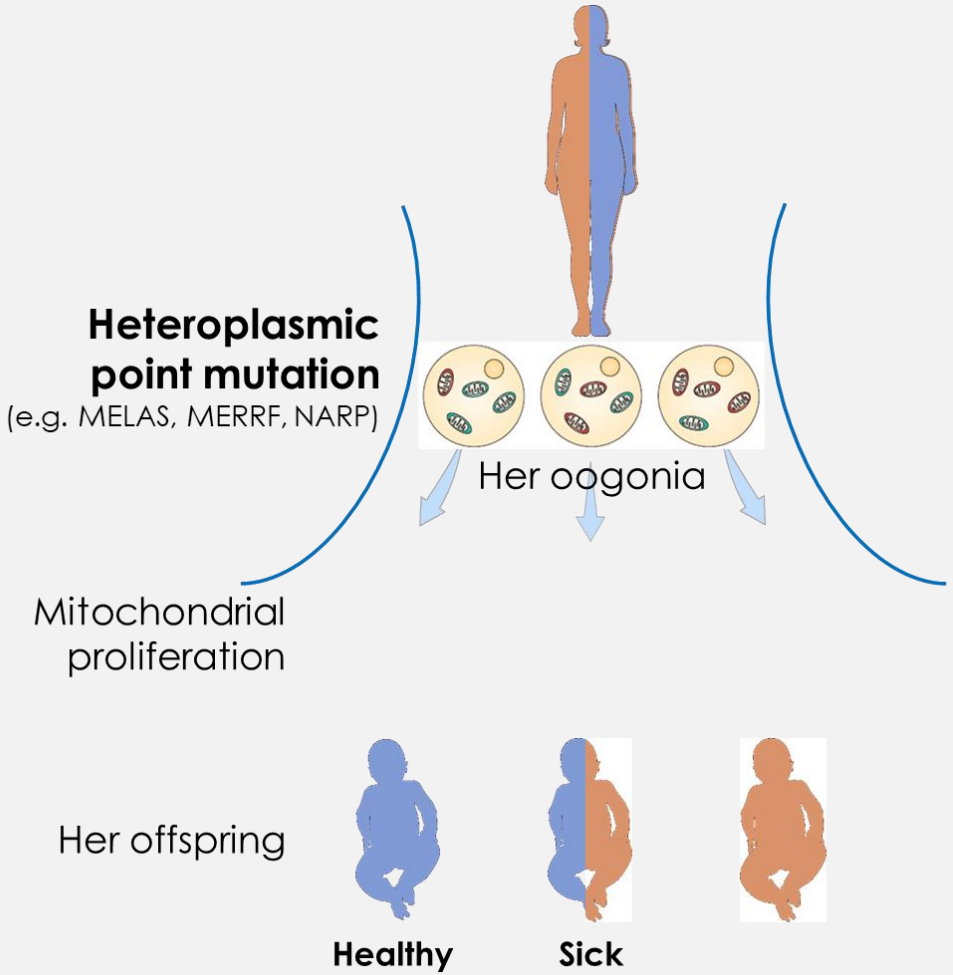
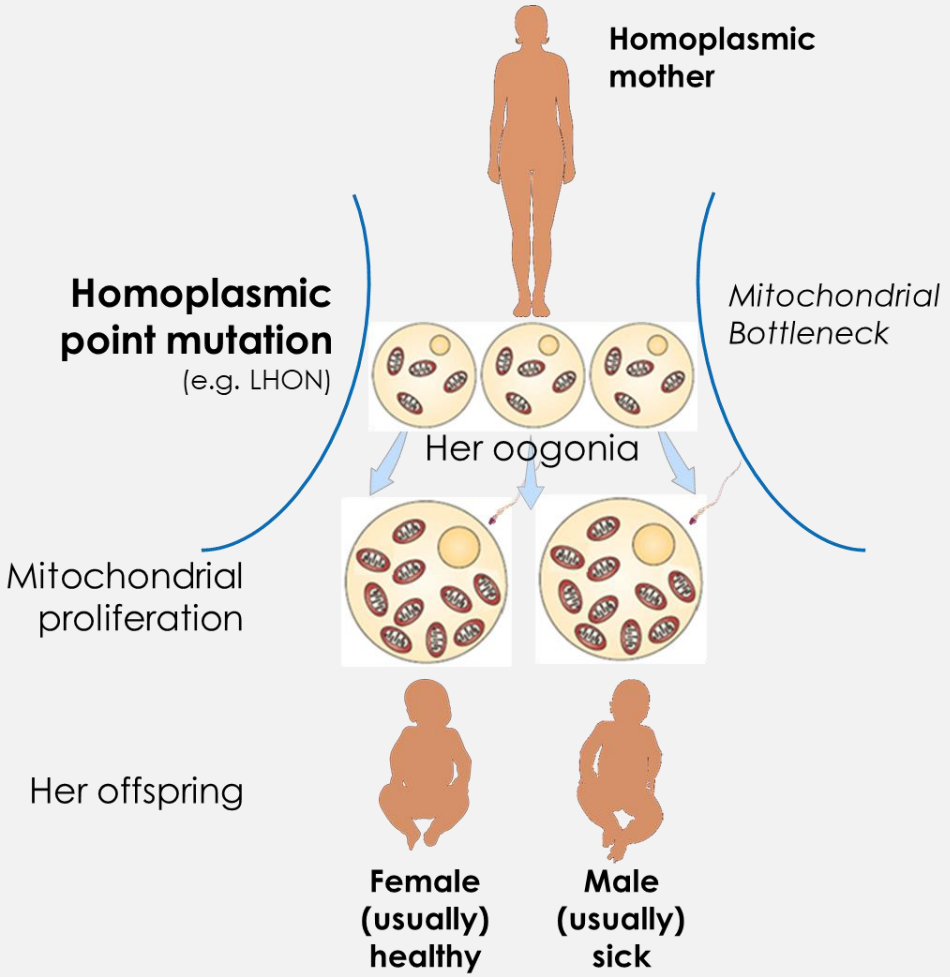
Regione Lombardia

Transmission of mtDNA point mutations





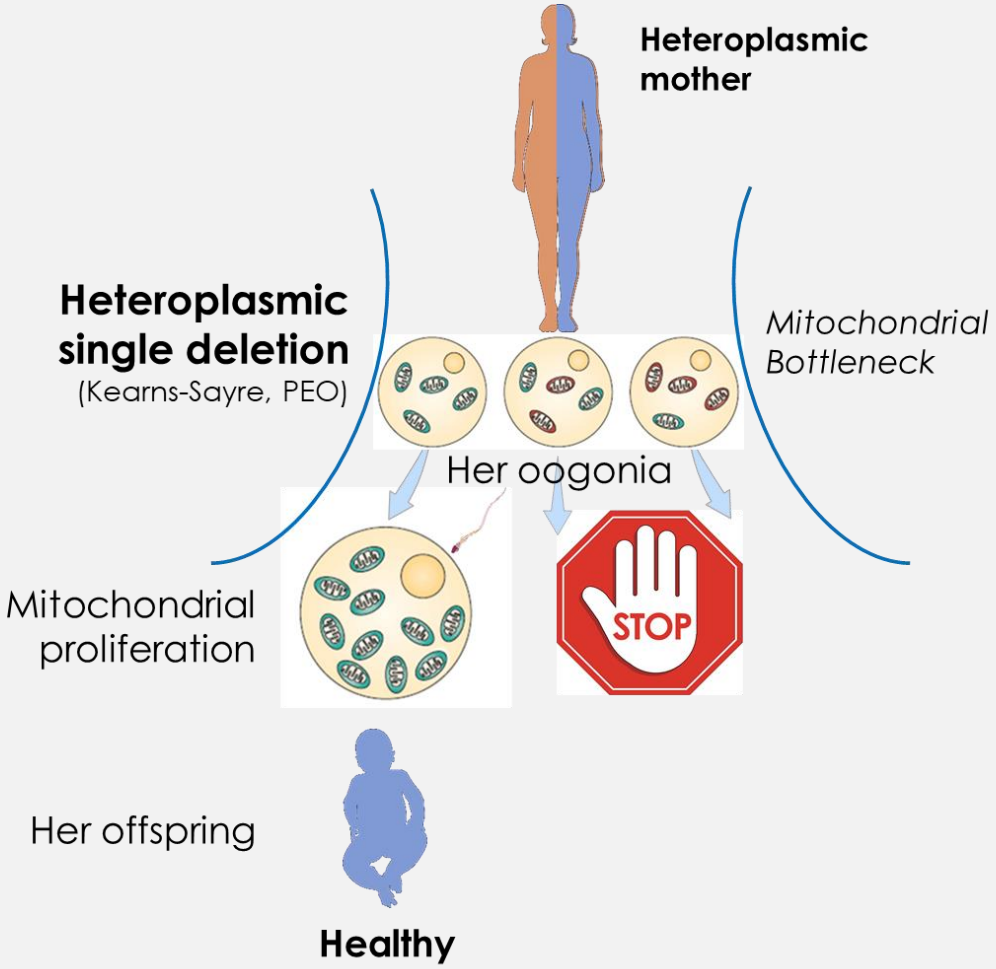
Mutation load can change abruptly in the offspring, due to tight bottleneck in maternal oogonia

Mutation is necessary but not sufficient to produce the disease phenotype

Transmission of mtDNA single deletions

Defects of Mitochondrial DNA

- Protein synthesis genes (rRNAs, tRNAs)
- Protein-encoding OXPHOS subunit genes
- **Large deletions**



Single deletions are usually NOT transmitted

WES AND WGS TO STUDY MTDNA

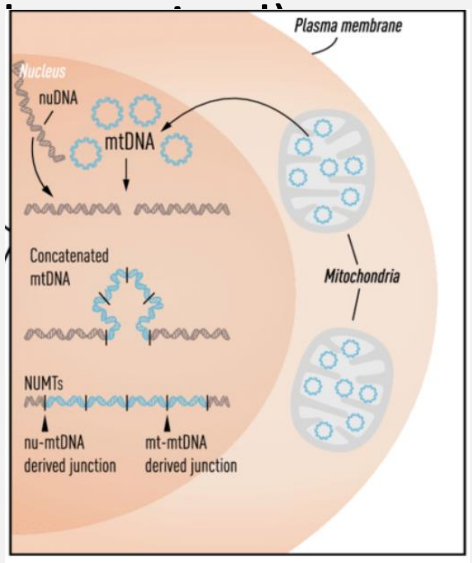
WES
- not all commercial kits for WE capture include probes for mtDNA

WES/WGS
- different analysis pipelines do not annotate mtDNA variants

WES/WGS
- On blood DNA heteroplasmy can be low (high dept

WES/WGS
- **NUMTs** (nuclear DNA of mitochondrial origin)

NUMTs: mitochondrial genome regions can be integrated into the nuclear genome



NUMT Confounding Biases Mitochondrial Heteroplasmy Calls in Favor of the Reference Allele

Molecular Poltergeists: Mitochondrial DNA Copies (*numts*) in Sequenced Nuclear Genomes