mtDNA disease: recurrence risks & reproductive strategies

Sara Seneca
Postgraduate Course in Medical genetics
Centrum Medische Genetica, UZ Brussel

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outline

- mitochondrial disorders & oxidative phosforylation
  - what – where - how ?
- recurrence risks & appropriate genetic counseling
- future prospects & summary

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mitochondrial disorder (1) ?

- single organ or multisystem disease
- dysfunction of oxidative phosphorylation system (OXPHOS)
- clinically very heterogeneous condition, affecting patients
  - at any age (early in infancy or in late(r) adulthood),
  - in any tissue or organ
  - mild or severe phenotype

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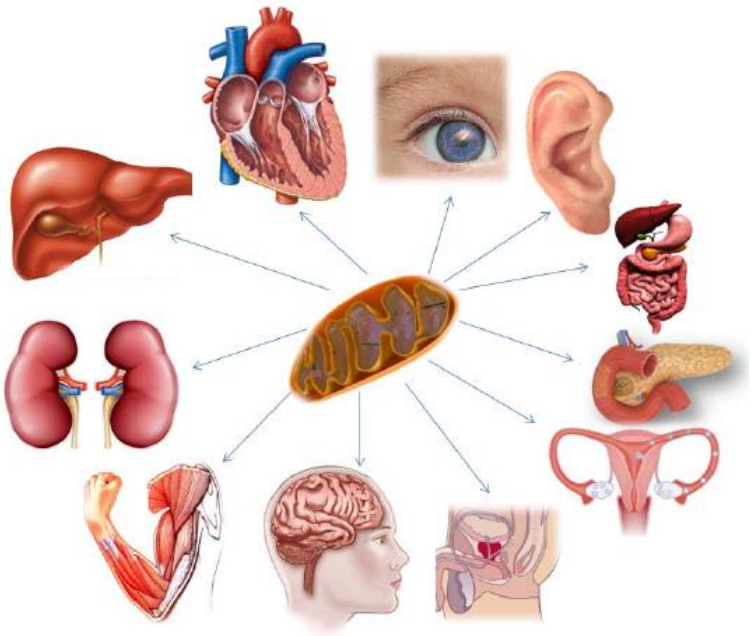
illustration of clinical diversity

might include, but not limited to

- migraine
- deafness
- blindness
- diabetes
- cardiac problems
- epilepsy
- seizures
- dysphagia
- ophthalmoplegia



- respiratory failure
- myopathy
- neuropathy
- gastrointestinal dysmotility
- liver failure
- bone marrow dysfunction
- …..

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mitochondrial disorder (2) ?

- life limiting (or fatal very early in life)
- incidence of mt cytopathies
  - 1/5.000 affected with mt disorder*
  - >1/200 carriership in life births**

* estimated 1/4.000 – 1/6.000

** investigation in UK of 10 frequent mtDNA mutations in cordial blood of neonates

(Gorman 2015, Chinnery & Taylor in 2000 & 2008, Thornburn 2003)

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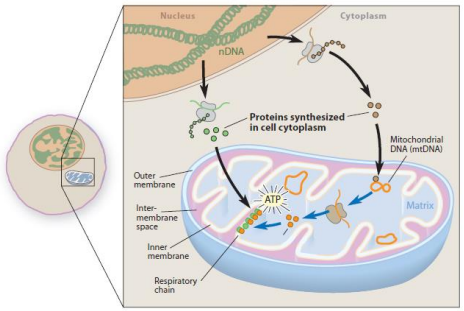
mitochondrial disorder (3) ?

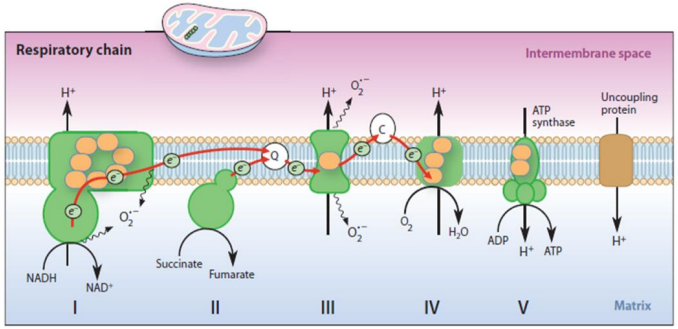
- no cure(s) yet
  - early phase drug trials and clinical studies are (a slow) work in progress
  - idebenone is licensed in Europe for LHON
  - gene therapy for LHON
  - taurine is licensed in Japan for Melas
- other therapies/treatments alleviation (ptosis, cardio, diabetes, epilepsy…) of symptoms

mitochondrial disorder (4) ?

What is the cause of a mitochondrial (mt) disorder ?

a defect of the oxphos system





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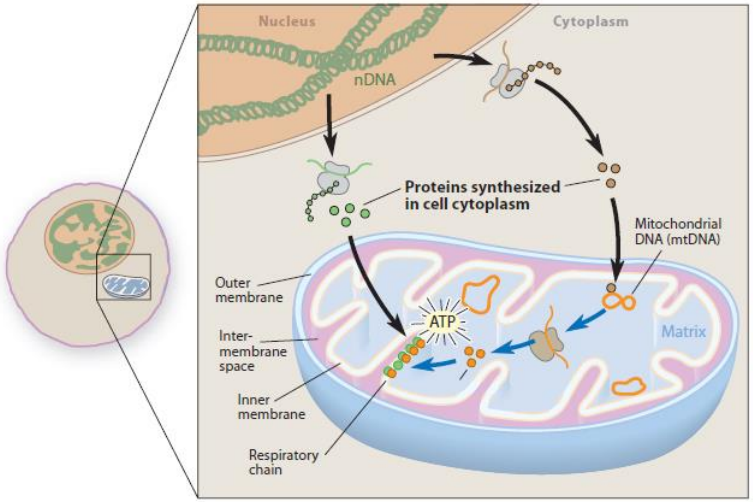
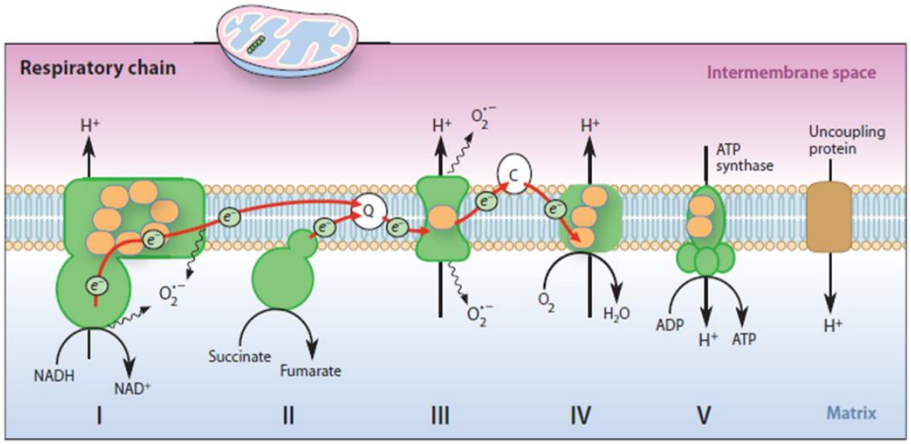
short overview OXPHOS pathway

largest generator of ATP





mitochondria harbour a small genome: **mtDNA**

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mitochondrial disorder (5)

It is not a single disorder but an ‘umbrella’ term for dozen of disorders, in which mitochondria are not able to produce (enough) energy for cells to work properly.

OXPHOS system is under dual genetical control of the n & mt genome

mt disease recurrence risks

- recurrence risk for nuclear encoded gene mutations
  - ➢ Mendelian rules for dominant, recessive and X-linked inheritance

- recurrence risks of **mtDNA mutations**
  - ➢ maternal inheritance

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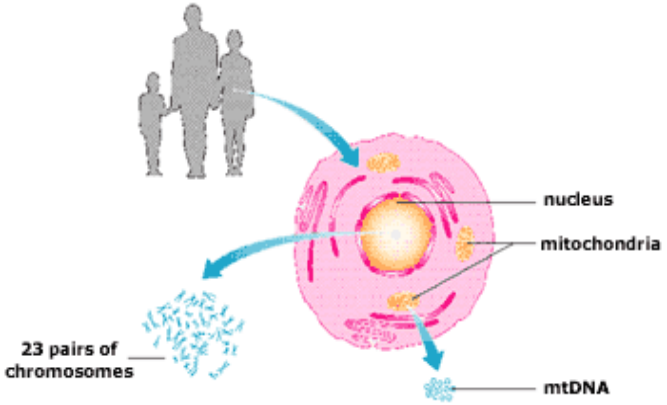
mitochondrial genome (mtDNA)

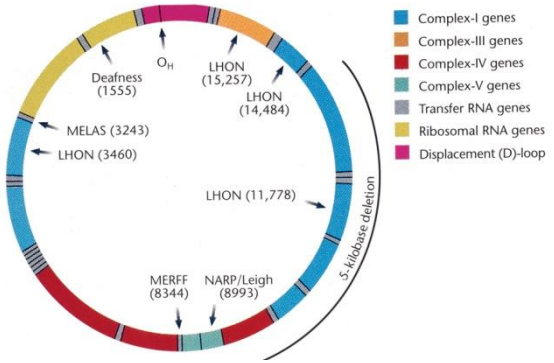
molecular causes of OXPHOS **problems** are **dual** :

- **nuclear DNA**: 3 10^9 bp

versus

- **mtDNA**: 16 569 bp
  - small double stranded molecule
  - only 37 genes
  - essential to OXPHOS
  - compact (no introns)
  - translation system





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unique characteristics of mtDNA

- polyploid genome
- homoplasmic/heteroplasmic
- maternal inheritance
- treshold level
- random mitotic segregation
- high mutation rate (polymorf)
- bottleneck concept

polyploidy : multicopy mt genome

- ⇔ nuclear genome
- multiple mtDNA copies/cell
- # dependent of cell type & energy demand

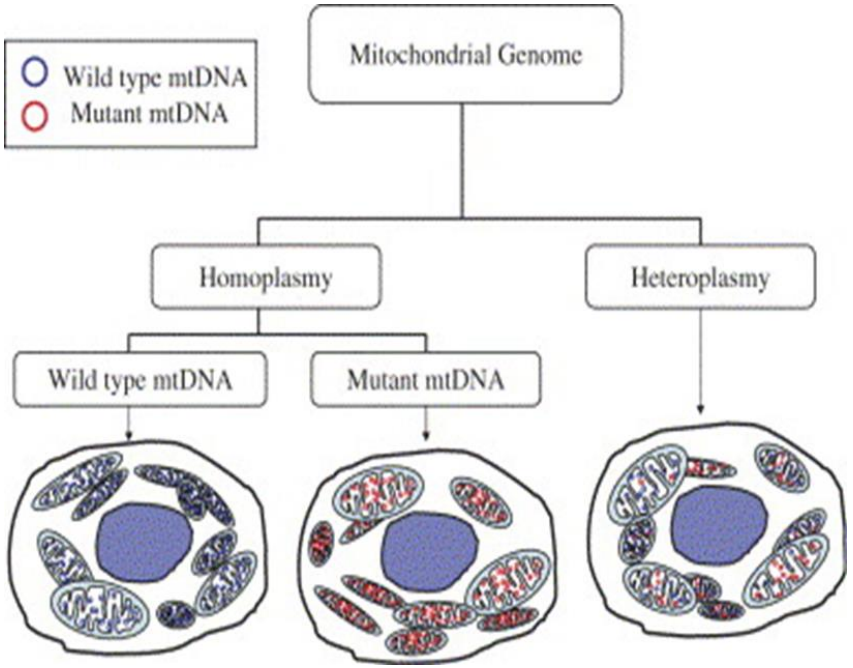
  e.g. sperm cell: ± 10-100 mtDNA molecules

  e.g. oocytes : ±1-3.10^5 mtDNA molecules

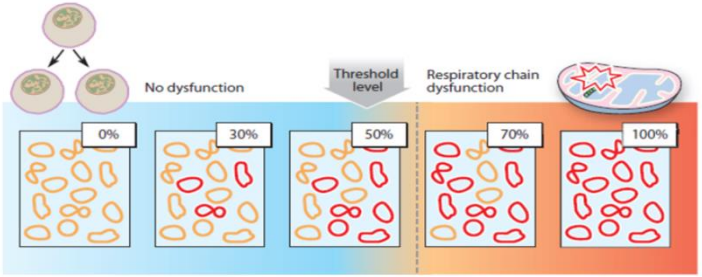
  average cell : ± 10^3-10^4 mtDNA molecules

definition homo-/heteroplasmy

- presence of **identical** mtDNA molecules or **WT** or **variant**
- presence of **different types** (sequence) of mtDNA molecules



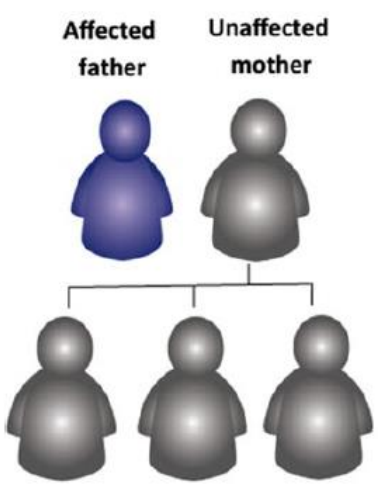
heteroplasmy can vary



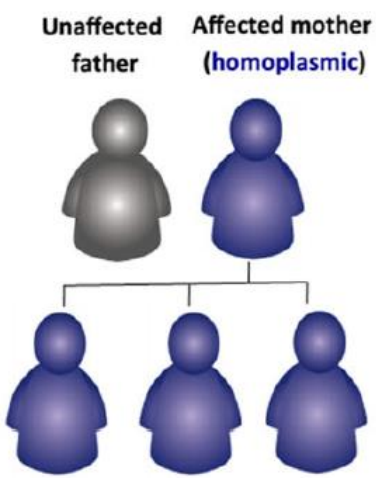
- ≠ **tissues** in 1 individual
- ≠ **cells** of same tissue in 1 individual
- changes with **time** in 1 individual
- (strong) impact on cell fie >> **treshold**
  - **tissue/organ** dependant
  - **age** of patient dependant
  - **variant** dependant
- blood levels (often) << post-mitotic tissues

strict maternal inheritance

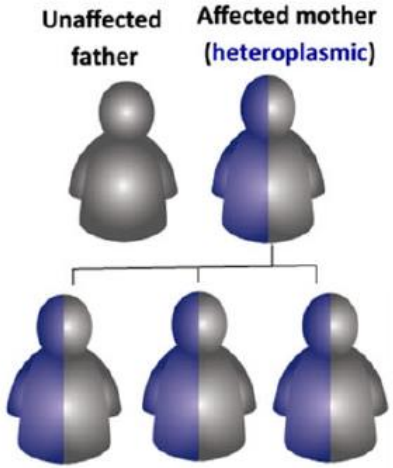
only maternal (from oocyte) contribution,
no paternal (from sperm cell) contribution



No affected children



All affected children (assuming complete penetrance)



Children may be affected or unaffected (depending on level of heteroplasmy, which can vary between children)

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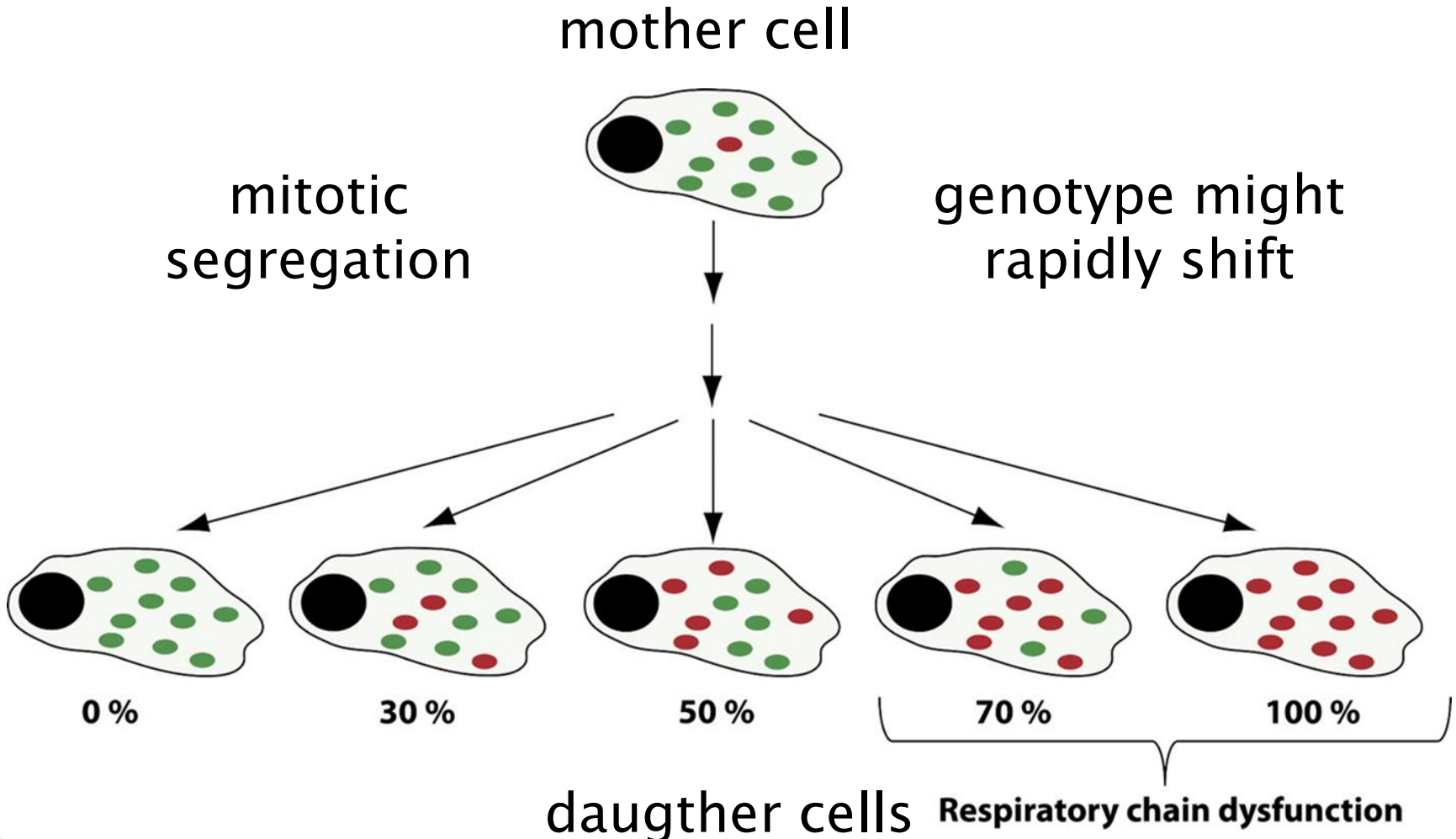
degradation of sperm mtDNA

- active elimination of sperm mtDNA in zygote
  - ubiquitinated & targeted for distruction
  - mitophagy

- paternal transmission
  - extremely rare & results probably from defect
    - Schwartz & Vissing 2002
    - Luo 2018

random distribution of mtDNA



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unique characteristics of mtDNA

- polyploid genome
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- maternal inheritance
- treshold level
- random mitotic segregation
- high mutation rate (polymorf)
- genetic bottleneck concept

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genetic bottleneck concept (1)

- **hypothesis** to explain rapid **shift** in **genotype** in successive generations
- **rapid intergenerational switch** ➢ **no fit** with random genetic drift model
- no exact mechanism in all details
  - ➢ 3 models were proposed

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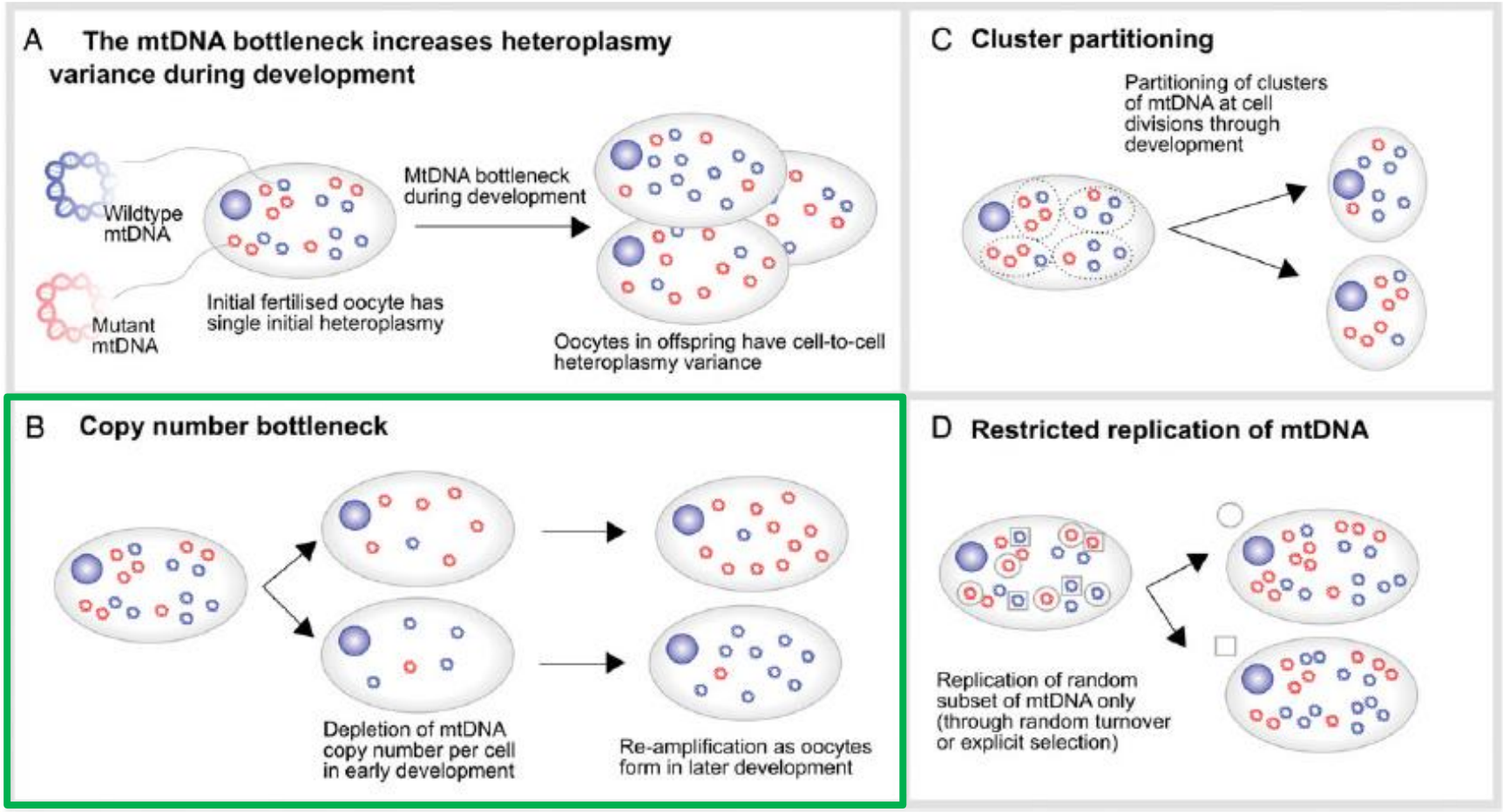
genetic bottleneck concept (2)

- **hypotheses**:
  - Cree et al. 2008: ↓ ↓ in # mtDNAs in oocytes + ↑ ↑ during early embryonic development
  - Cao et al. 2007: no ↓ in # mtDNAs in oocytes
    - random segregation of mtDNA clusters
  - Wai et al. 2008: no ↓ in # mtDNAs
    - replication of subset of mtDNAs

genetic bottleneck concept (3)



from Burgstaller 2015

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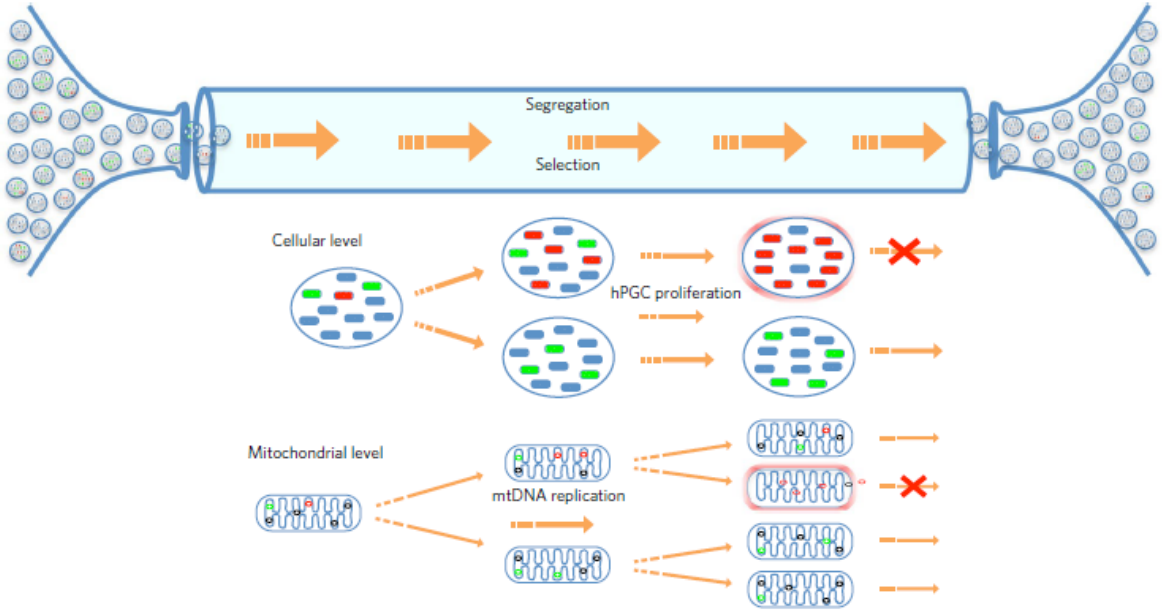
genetic bottleneck concept (4)

copy number bottleneck (Floros et al. 2018)

- human embryos
- reduction ➢ 5 mtDNA molecules / mitochondrion



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mtDNA & disease

defects of mtDNA

- **(large scale) rearrangements**
  - deletions
  - insertions or duplications (occasionaly)
- **pathogenic point variants** (>300)
  - scattered over the **whole genome**
    (protein coding and synthesizing genes)

mtDNA & disease - recurrence risk

mtDNA inheritance

no cure, limited therapy and no effective treatment

⇩

knowledge of **risk assessment and reduction or prevention** of transmission of mtDNA disease is **very important** for **counseling** of these families

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recurrence risk estimation

5 ≠ situations

1. ***de novo*** variant : single large deletions
2. ***homoplasmic*** variant : e.g. LHON
3. ***stable*** variant + ***predictable*** outcome : e.g.m.8993T>G for narp/LS.
4. ***unstable*** variant + ***unpredictable*** outcome : e.g.m.3243A>G melas
5. any private/family-specific variant + ***unknown*** outcome

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1. *de novo* variant

e.g. a single large mtDNA deletion

nearly always sporadic ? …<1% but …? ?

- meta study of 226 families (7 UK centers) (Chinnery 2004)
- unaffected mothers ➢ very unlikely to have another affected child (no case)
- 40 affected mothers ➢ 3/73 children ➢ recurrence risk of ≈ 4%

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2. *homoplasmic* variant

e.g. LHON

(**L**eber **H**ereditary **O**ptic **N**europathy)

- (sub)acute bilateral loss central vision
  - ➢ 15 - 35y (young male adults)
- degeneration retinal ganglion cells
- incidence ➢ ≈12/100.000
- pathogenesis is not clear

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Leber Hereditary Optic Neuropathy

- 3 frequent pathogenic variants (m.11778G>A (*MTND4*), m.3460G>A (*MTND1*), or m.14484T>C (*MTND6*)) : ≈ 95% of cases
- majority patients **homoplasmic**
- strong gender bias ➢ 80% ♂ patients
- major incomplete penetrance (in a family)
  - ➢ secondary factors
  - ➢ w/ 50% of ♂ & 10% ♀ affected

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Leber Hereditary Optic Neuropathy

homoplasmic variant

- all offspring will be homoplasmic
  - PND or PGT is not useful

- incomplete penetrance & gender bias
  - sex selection, an option ?
    - ♀ embryos/fetuses
    - still residual risk

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other situations

3. *stable* variant + *predictable* outcome
4. *unstable* variant + *unpredictable* outcome
5. *private* variant + *unknown* outcome

potential for **PND** ? ➢ **criteria** defined

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criteria for ‘mitochondrial’ PND

- (i) close **correlation** between the level of variant load and disease severity
- (ii) uniform **distribution** of variant mtDNA in all tissues
- (iii) **no change** in variant load with **time**

Poulton & Turnbull 2000

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questions for PND ?

is variant load

- CV sample **representative** other villi ? all fetal tissue ?
- **idem** for amniotic cells ?
- **constant** during development, **now** (fetal) ?
- **constant** during development, **later** (adult) ?

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3. *stable* variant + *predictable* outcome

e.g. **m.8993 T>G mutation**

- Narp (70-80% load)

  neuropathy, ataxia, retinitis pigmentosa (with muscle weakness, seizures, MR, …)
- common in Leigh syndrome (>90% load)
- ‘rapid segregation’ (only 1 generation)
- ‘*de novo*’ families

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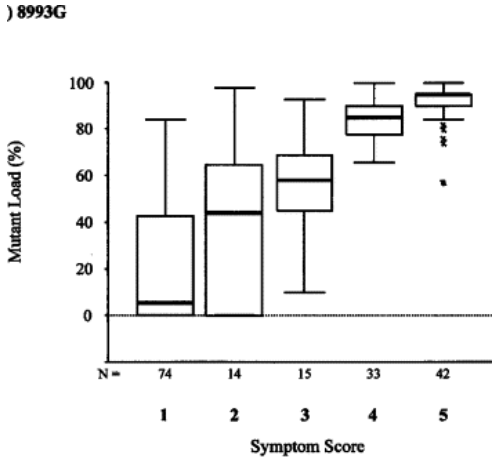
m.8993T>G mutation

check criteria ?

- tissue-dependent variation ↓
- age-dependent variation ↓
- genotype - phenotype correlation

excellent



White et al. 1999

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m.8993T>G mutation : PND ?

- affected fetuses 8 wk & 11 wk & 12 wk
- variety of fetal tissues: placenta, brain, muscle, limb, lung, heart, spinal cord, liver, kidney investigated

results:

equal distribution of variant load & comparable to chorionic villi

A.Harding

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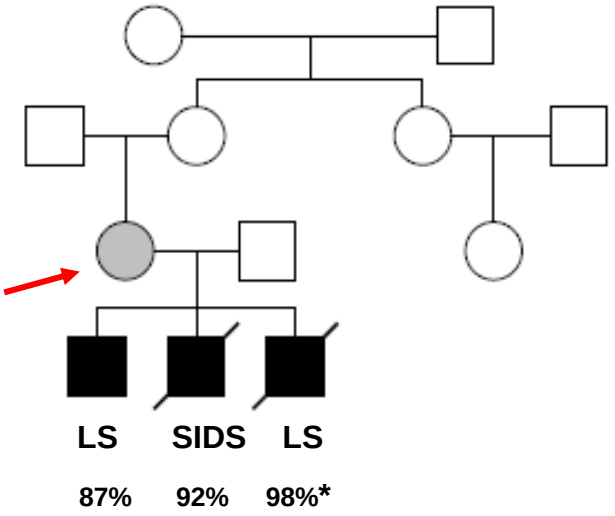
m.8993T>G mutation : oocytes ?

woman 50% m.8993T>G in leukocytes

study of oocytes

- total of 8 oocytes
  - 1 lost for analysis
  - 6 load >95%
  - 1 no mutation detected



Blok et al. 1997

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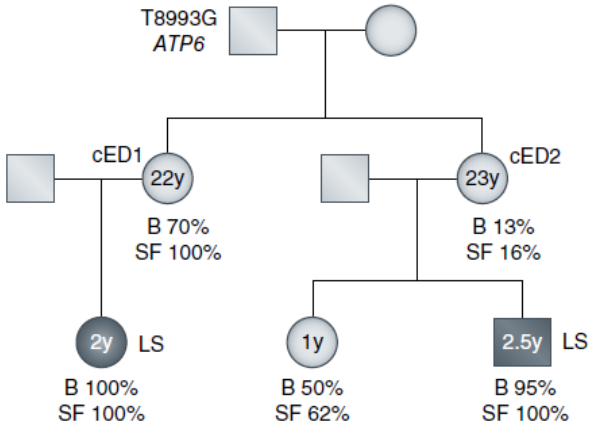
m.8993T>G mutation : oocytes ?



woman 1
B 70%

woman 2
B 13%

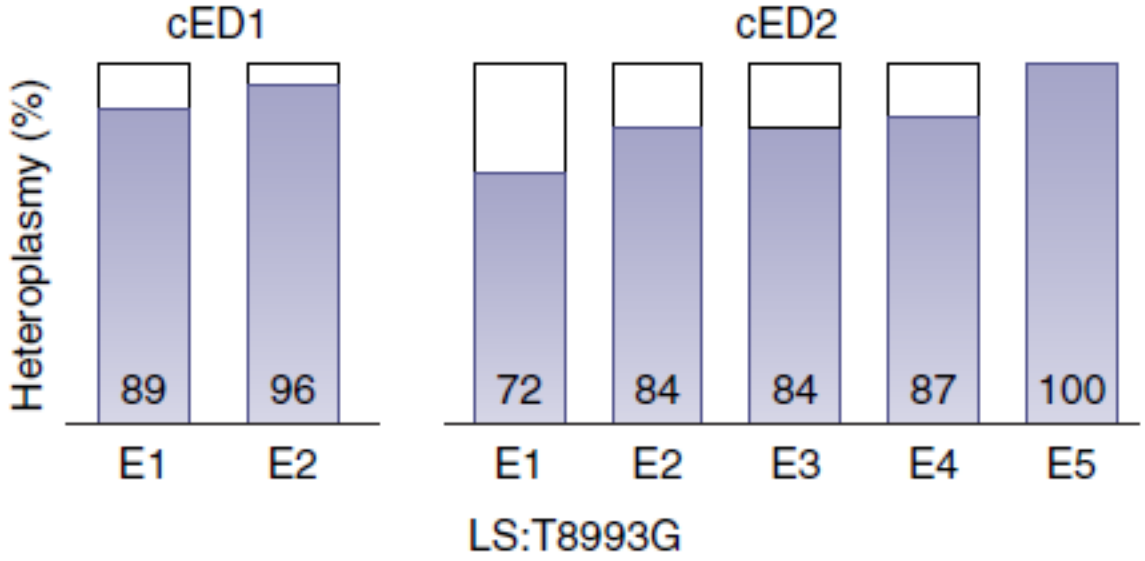


illustration of rapid segregation

Kang et al. 2016

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4. *unstable* variant + *unpredictable*

e.g. **m.3243A>G melas mutation**

check criteria ?

- **poor** geno - phenotype **correlation**
- variant **load differs** among tissues
- variant load **changes in time**

no good

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4. *unstable* variant + *unpredictable*

'unpredictability' of **m.3243A>G** is illustrated:

- load changes in leukocytes over time
- load variability in oocytes
- load variability in placenta samples
- transmission data:
  - mother – child
  - intersiblings

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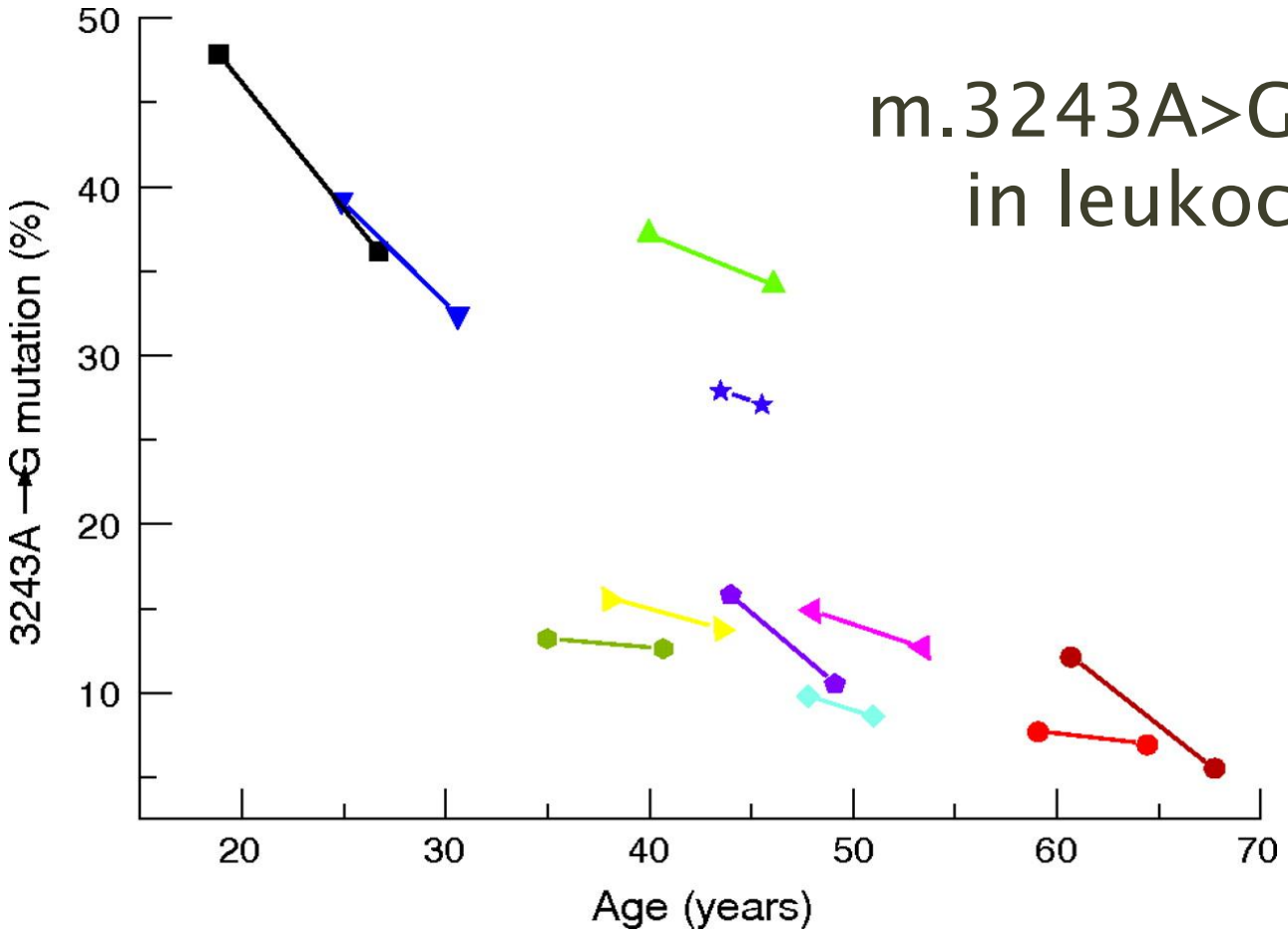
variant load declines with time



m.3243A>G melas in leukocytes

Pyle et al. 2007

Langdahlet al. 2018

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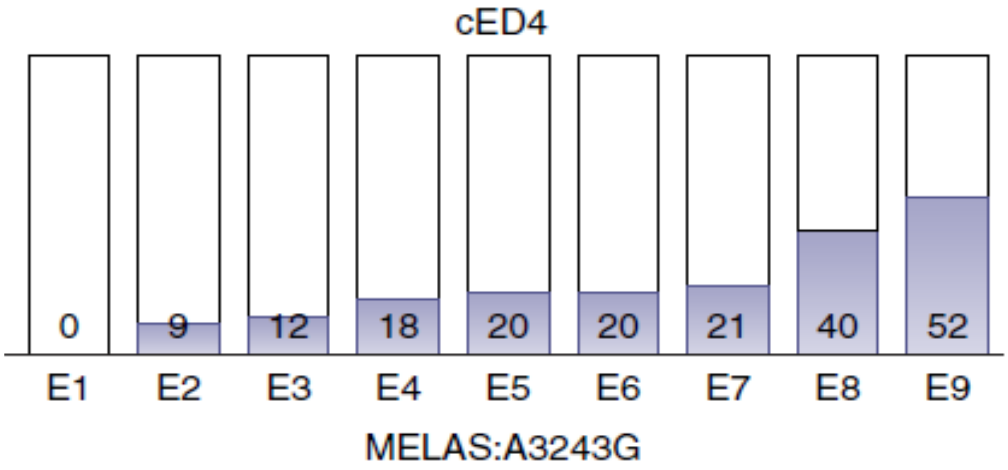
m.3243A>G melas : oocytes

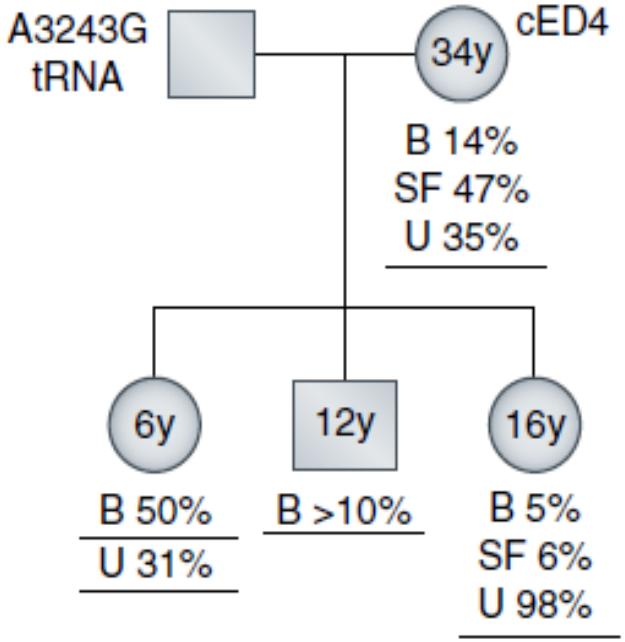
34y old woman

B 14%

U 35%

➢ 9 oocytes investigated





Kang et al. 2016

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inheritance m.3243A>G variant

study of de Laat et al. 2012
analysis of urinary epithelial cells

- 56 **mother-child** relations
  - 3 subgroups (0-5%; 5-25%; 25-50%)
- 63 **intersibling** relations
  - 5 subgroups (0-5%; 5-25%; 25-50%; 50-75%; 75-100%)

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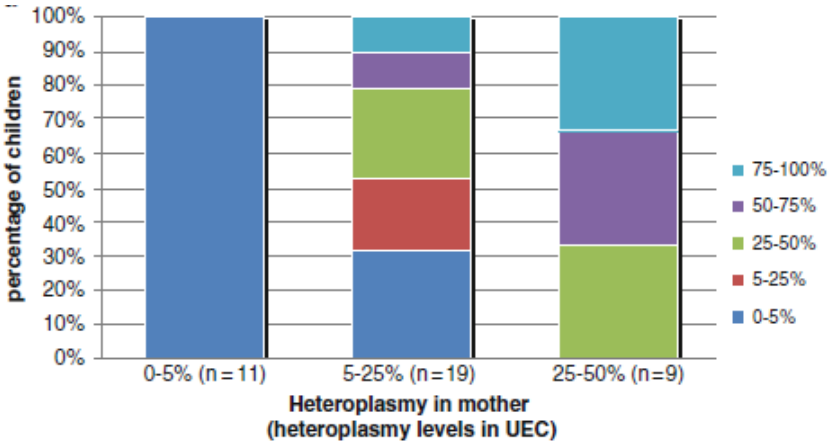
transmission between **mother-child**

- heteroplasmy <5%
  - ➢ no detectable transmission
- heteroplasmy 5-25%
  - ➢ no detectable transmission in 30% offspring
- heteroplasmy >25%
  - ➢ transmission to all offspring



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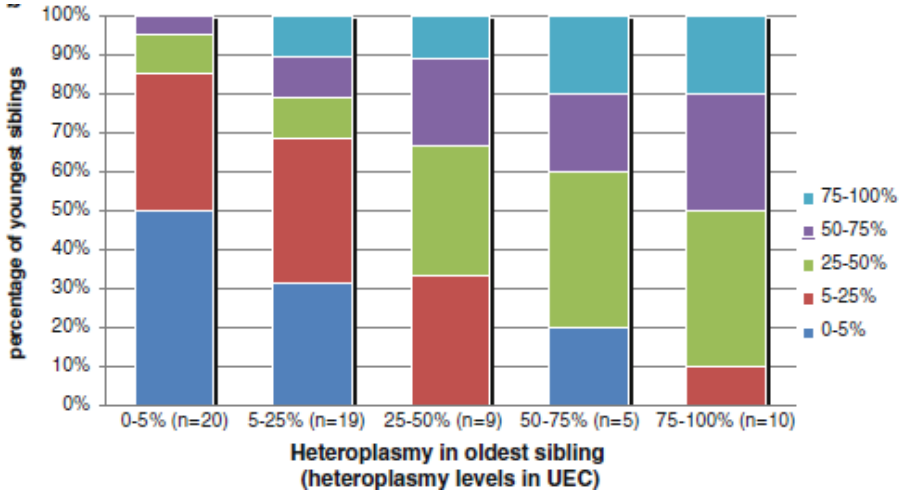
transmission between **siblings**

- in oldest sibling no detectable level >5%
  - < 5% for 50% of youngest sibling
- in oldest sibling level >50%
  - most siblings affected



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5. *unknown* outcome + 'private' variant

- unique/rare variants : few families
- no (or little) specific information
- genotype - phenotype correlation ?
- treshold level ?
- identify potential healthy offspring ?

➢ **insufficient data for conclusion**

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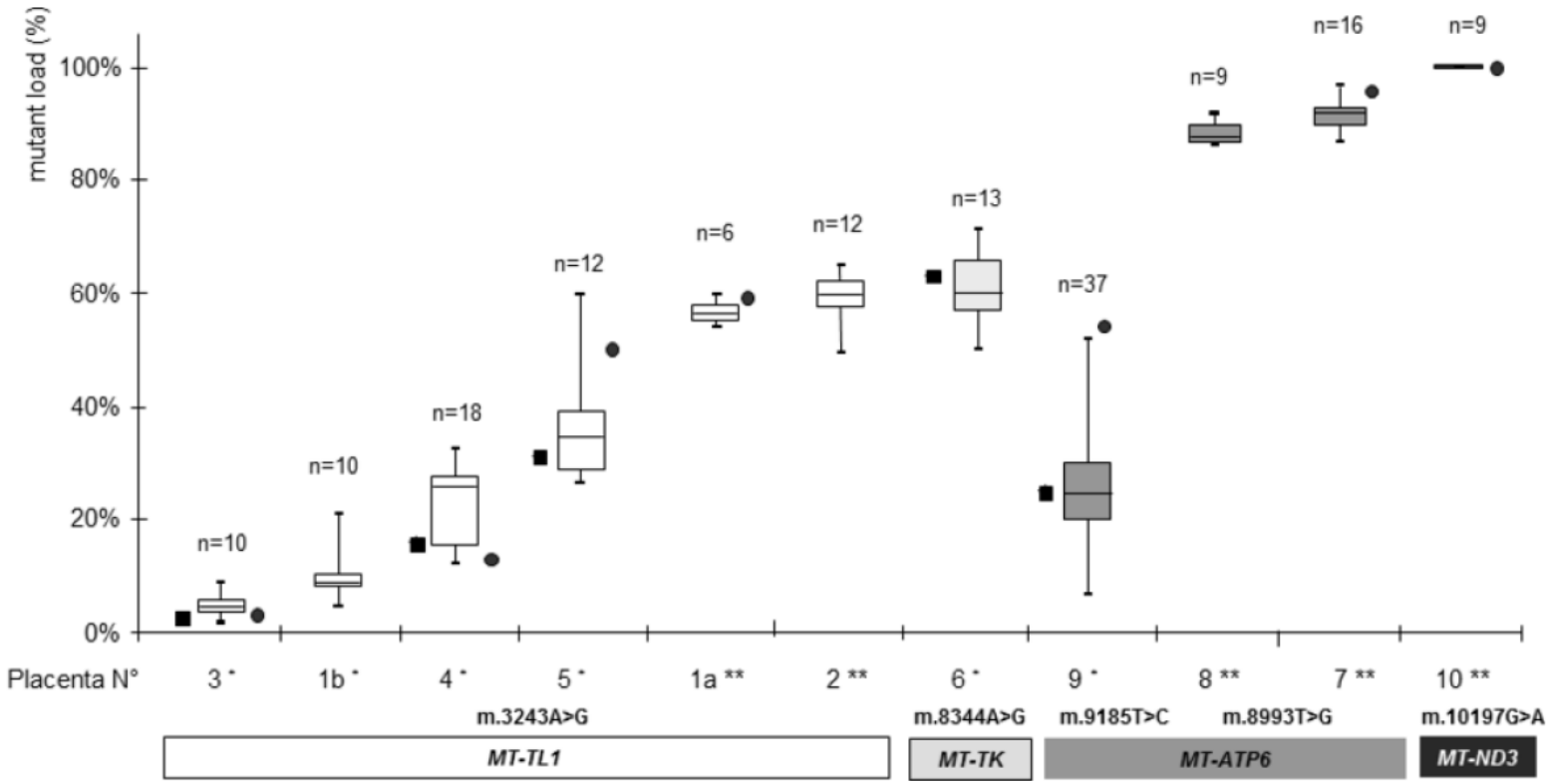
placenta tissue (1)

11 placentas investigated



tRNA gene protein coding gene

Vachin 2017

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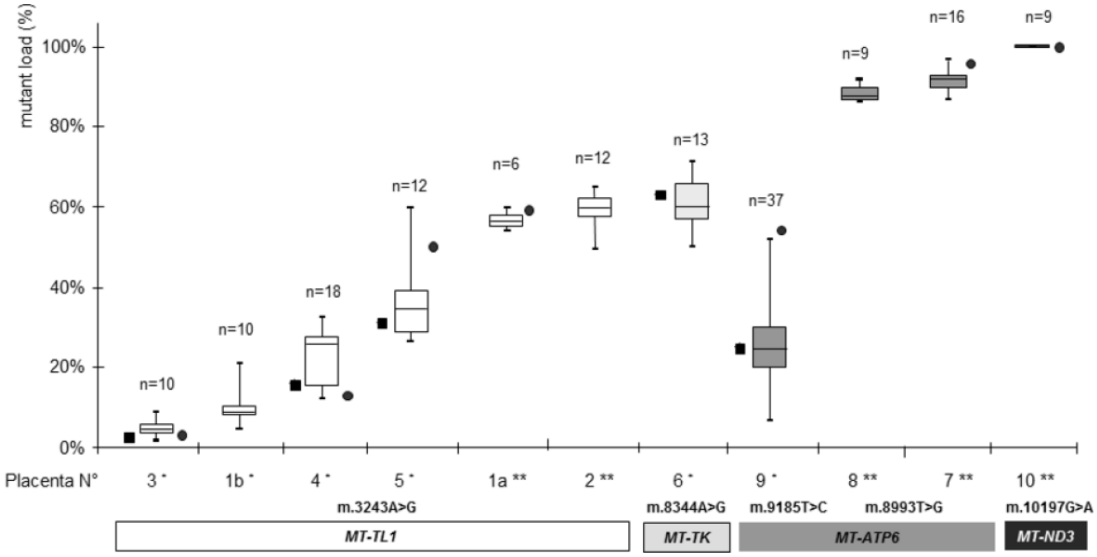
placenta tissue (2)

- 11 placentas investigated (Vachin 2017)
  - 6 full term / 5 ➢ 12-18 weeks gestation

- multiple samples : (n=1 - 37) / placenta
  - 1 placenta homoplasmic (MT-ND3 gene)
  - 6/10: intraplacental variation limited to ≈ 10%
  - 4/10: large intraplacental variation
    - up to 55% in 1 case

placenta tissue (3)



- 1 placenta homoplasmic (MT-ND3 gene)
- 6/10: intraplacental variation limited to ≈ 10%
- 4/10: large intraplacental variation
  - up to 55% in 1 case

CV sample not always appropriate tissue for PND

Vachin 2017

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What about ART ?

Preimplantation **G**enetic **T**est

or

Mitochondrial **D**onation **T**herapy

ART : assisted reproductive technologies

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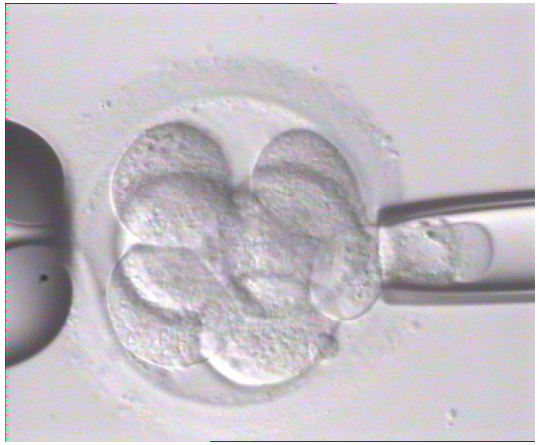
Preimplantation Genetic Test ?

solution ?



aim ?

- **selection of optimal** (‘healthy’ or ‘low risk’) **embryos** to reduce the risk of mt disease transmission

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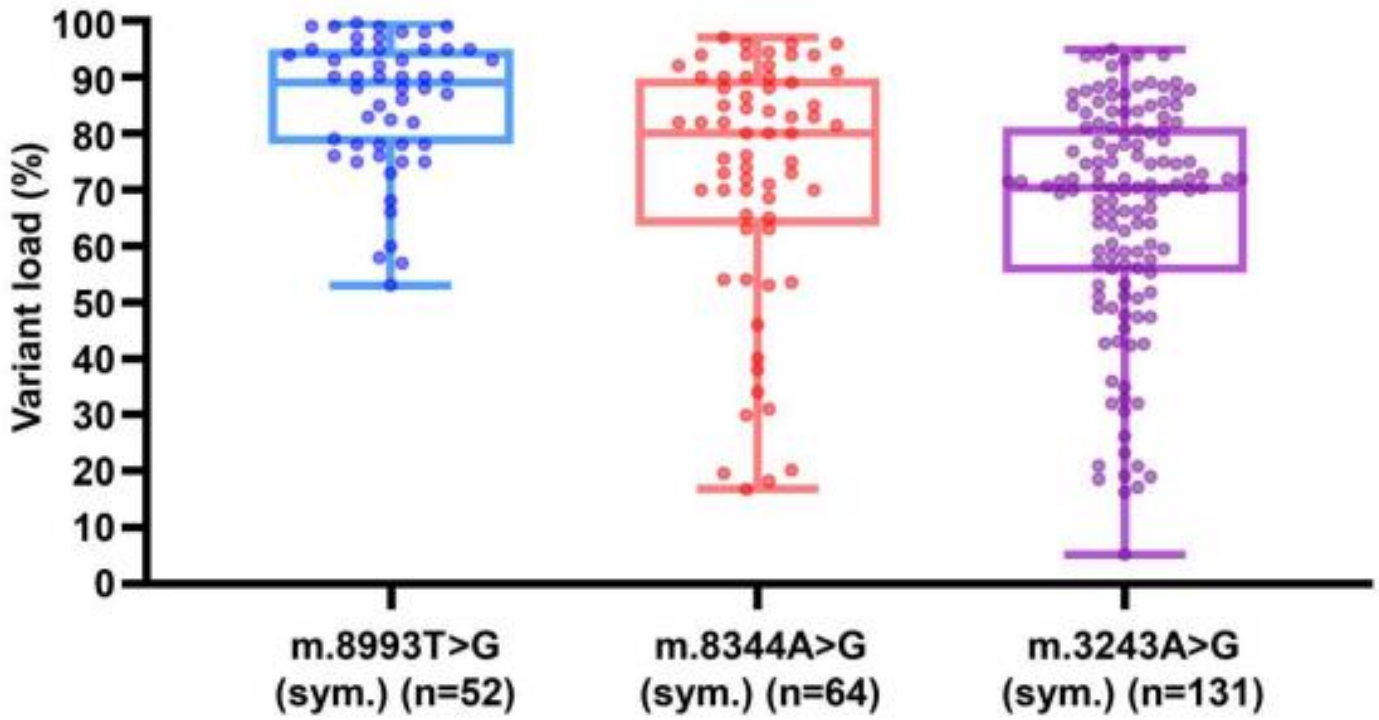
probability treshold for symptoms



meta analysis : distribution of variant load in 455 symptomatic individuals from 187 pedigrees (Ji 2023)

PGT procedure questions ?

- are results of 1 or 2 blastomeres representative for embryo ?
- which embryos are suitable for transfer ?
  - save cut off point ?
  - criteria ?
- results of one family representative for this variant in general ?

mtDNA load in PGT blastomeres

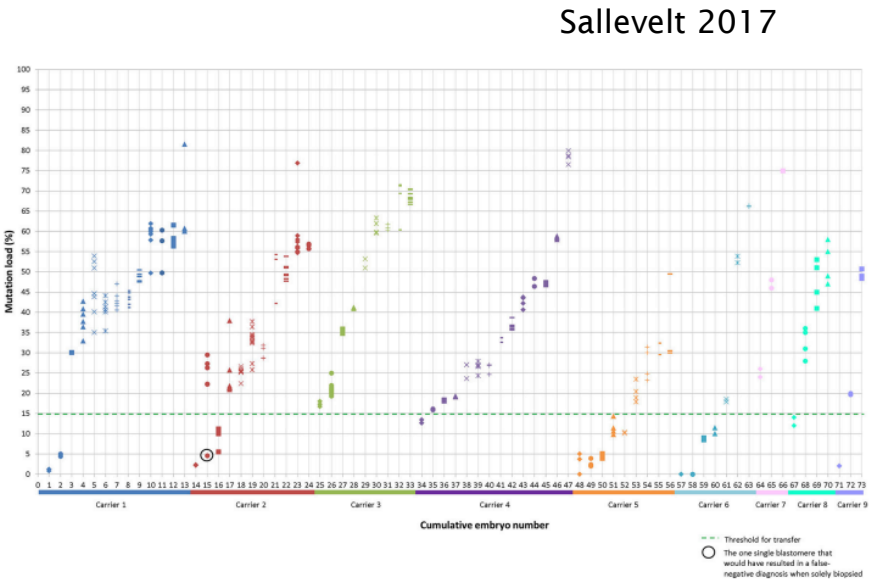
m.3243A>G variant load compared in single blastomeres from the same embryo

- 9 females
- 73 embryos
- 294 blastomeres

  (n= ≥ 2 /embryo)

- threshold 15%

Sallevelt 2017



1 false-negative result (5% versus 22% & 30%)

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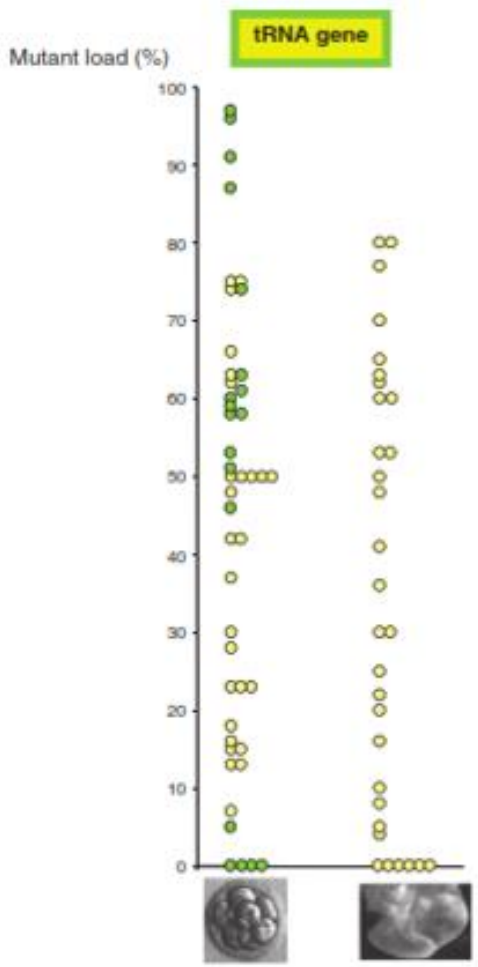
mtDNA load in PGT embryos & fetuses

Steffann J 2015



tRNA genes

- widely, distribution of loads
- also intermediate levels

yellow melas: m.3243A>G (n=27+31)
green merrf: m.8344A>G (n=19)

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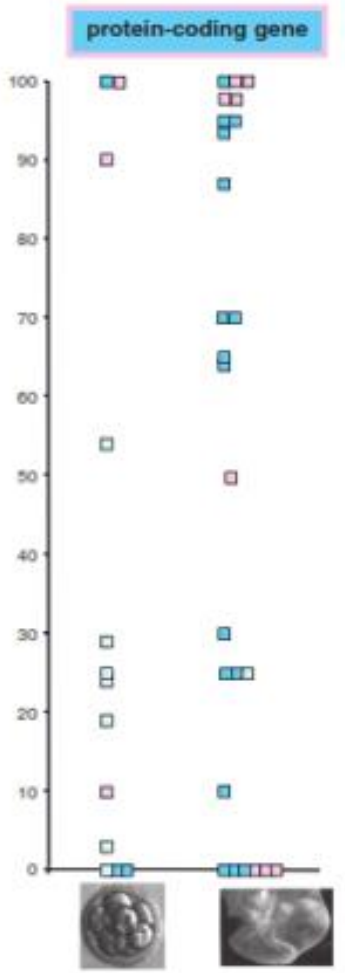
mtDNA load in PGT embryos & fetuses

Steffann J 2015



protein coding genes

- more skewed loads
- ≥ 70%
  ≤ 30%
  with some exceptions

light blue: m.9185T>C (n=3+16)
dark blue: 8993T>G/C (n=7+1)
pink: others (n=3+8)

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Preimplantation Genetic Test Baby

- variant-free embroys are scarcely produced
  - selection of low risk embryos
  - stringent treshold level knowledge important
- robust correlation between blastomeres
  - study Monnot 2011; Sallevelt 2017
- results of TE biopsy migt be a problem
  - heteroplasmy mosaicism risk (Hellebrekers 2023)

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Preimplantation Genetic Test Baby

mutation burden @ biopsy does not always correspond with the one @ birth

Table 2 Mutation levels in live births following PGD for mitochondrial disease

Mutation	At biopsy	At birth	Comments
m.8993T > G	0% & 0%	0%	First report. Two embryos transferred
m.8993T > G	2.5%	4%	3–5% cord blood & placenta; buccal cells 5% at age 4½ years
m.3243A > G	5% & 13%	5%	Two embryos transferred; 15 ± 5% placenta, 5 ± 1% cord blood
m.3243A > G	12%	15%	47% blood, 52% urine at 1½m; 46 & 42% at 18m
m.8993T > G	0%	0%	'Healthy son', no further details
m.8344A > G	53% & 59%	63%	Two embryos transferred; no further details
m.3243A > G	0%	0%	Male; measured in cord blood, urine, saliva
m.36[b]G > A	2%	7%	Female, measured buccal and urine cells
m.83[b]A > G	48%	Not available	Male; <60% generally asymptomatic
m.130[b]T > C	1%	0%	Male; undetectable in blood, buccal and urine cells
m.101[b]*T* > C	1%	1–2%	Male; cord blood

[b] characters hidden to respect confidentiality

Greenfield et al. 2017

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Preimplantation Genetic Test Baby

mutation burden @ biopsy does not always correspond with the one @ birth

Table 1. Clinical PGT cycles carried out for mtDNA disorders.

Reference	mtDNA mutation	Disorder	Number of patients	Mutation load of embryos	Mutation load difference	Mutation load of transferred embryo(s)	Mutation load of fetal/ neonatal tissues
(Steffann et al. 2006)	m.8993T→G	NARP/Leigh syndrome	1	0–100%	6% between two cleavage-stage blastomeres and the entire embryo	0%	0% in cord blood
(Sallevelt et al. 2013)	m.8993T→G	NARP/Leigh syndrome	1	30–100%	<10% between cleavage-stage blastomeres	0%	0% in cord blood
(Tajima et al. 2007)	m.8993T→G	NARP/Leigh syndrome	13	4–22%	2–11% between cleavage-stage blastomeres	NA	NA
(Spath et al. 2021b)	m.8993T→G	NARP/Leigh syndrome	2	0–88.7%	NA	0%	0% (not specified in which tissue)
(Sallevelt et al. 2013)	m.3243A→G	MELAS	3	1–70%	<10% (80% of embryos) and >15% (18% of embryos) between cleavage-stage blastomeres	NA	NA
(Monnot et al. 2011)	m.3243A→G	MELAS	2	5–77%	0–6% between cleavage-stage blastomeres 0–2% between cleavage-stage blastomeres and TE cells <7% between cleavage-stage blastomeres and the entire blastocyst	NA	NA
(Treff et al. 2012)	m.3243A→G	MELAS	1	7–90%	0.5–2.9% between cleavage-stage blastomeres2.1–5.0% between TE cells and inner cell mass	12%	0–15% in buccal cells, blood, and urine
(Heindryckx et al. 2014)	m.3243A→G	MELAS	1	0–65%	0–7% between TE cells	0%	0% in saliva, blood cells, and urine
(Spath et al. 2021a)	m.3243A→G	MELAS	1	3.1–29.2%	0–3.2% between halves of cleavage-stage embryos	NA	NA
(Sallevelt SC et al. 2017)	m.14487T→C	Leigh syndrome	1	6–100%	0–10% between cleavage-stage blastomeres	<18%	5% mutation load in cord blood
(Spath et al. 2021a)	m.10191T→G	Leigh syndrome	1	0%	0%	0%	0% in chorionic villi
(Ji et al. 2021)	m.3697G→A	Leigh syndrome	1	15.2%–100%	0–10.3% between cleavage-stage blastomeres	53.9%	47.7–49.7% in fetal tissues and amniotic fluid cells

Lan et al. 2024

Preimplantation Genetic Test Baby

PGT m.3243A>G embryo

- trophoblast : 12%
- 30 days buccal cells : 15%
- 6 weeks
  - blood: 47%
  - urine: 52%
- 18 months
  - blood: 46%
  - urine: 42%

Blastocyst preimplantation genetic diagnosis (PGD) of a mitochondrial DNA disorder

Nathan R. Treff, Ph.D.,[a,b,c] Jessyca Campos, M.S.,[a,b] Xin Tao, M.S.,[a] Brynn Levy, Ph.D.,[d] Kathleen M. Ferry, B.S.,[a] and Richard T. Scott Jr., M.D.[a,b]

[a] Reproductive Medicine Associates of New Jersey, Morristown; [b] Division of Reproductive Endocrinology and Infertility, Department of Obstetrics, Gynecology, and Reproductive Science, University of Medicine and Dentistry of New Jersey, Robert Wood Johnson Medical School, New Brunswick; [c] Department of Genetics, Rutgers University, Piscataway, New Jersey; and [d] Department of Pathology, College of Physicians and Surgeons, Columbia University, New York, New York

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Limitations of Preimplantation Genetic Diagnosis for Mitochondrial DNA Diseases

Shoukhrat Mitalipov[1,2,*], **Paula Amato**[2], **Samuel Parry**[3], and **Marni J. Falk**[4]

[1]Division of Reproductive and Developmental Sciences, Oregon National Primate Research Center, Oregon Health & Science University, Beaverton, OR 97006, USA

[2]Division of Reproductive Endocrinology, Department of Obstetrics and Gynecology, Oregon Health & Science University, Portland, OR 97239, USA

[3]Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA 19104, USA

[4]Divisions of Human Genetics and Metabolic Disease, Department of Pediatrics, The Children's Hospital of Philadelphia and University of Pennsylvania Perelman School of Medicine, Philadelphia, PA 19104, USA

a clinically affected baby

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what about

other options ?

now ?

in the (near) future ?

mitochondrial donation therapy ?

replacement of mt genome of the **affected** woman with that of a **donor** woman

- applied ***before*** **fertilisation**
  - **MST** (**m**aternal **s**pindle **t**ransfer)
  - **GVT (germinal vesicle transfer)**
  - **PBT (polar body transfer)**

- applied after fertilisation
  - **PNT** (**p**ro**n**uclear **t**ransfer)

Herbert & Turnbull et al. 2018

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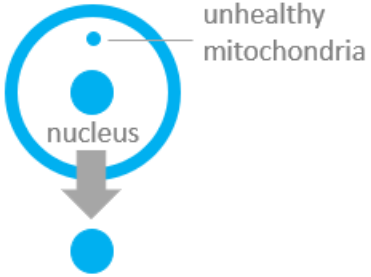
mitochondrial donation therapy ?

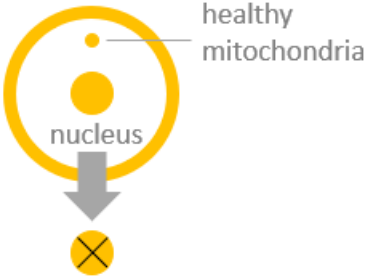
how to make a ‘3 persons’ baby ?

1.
mother’s egg /
single cell embryo



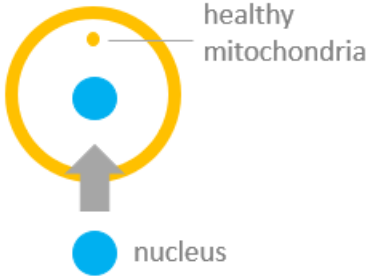
parent’s nuclear material
removed and kept

2.
donor’s egg /
single cell embryo



donor’s nuclear material
removed and destroyed

3.
donor’s egg /
single cell embryo



parent’s nuclear material
placed inside donor egg /
single cell embryo

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Herbert & Turnbull et al. 2018

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mitochondrial donation therapy ?

study of

- proof of principle demonstrated
  - safety
  - efficacy

  - for different strategies & different teams in pilot studies

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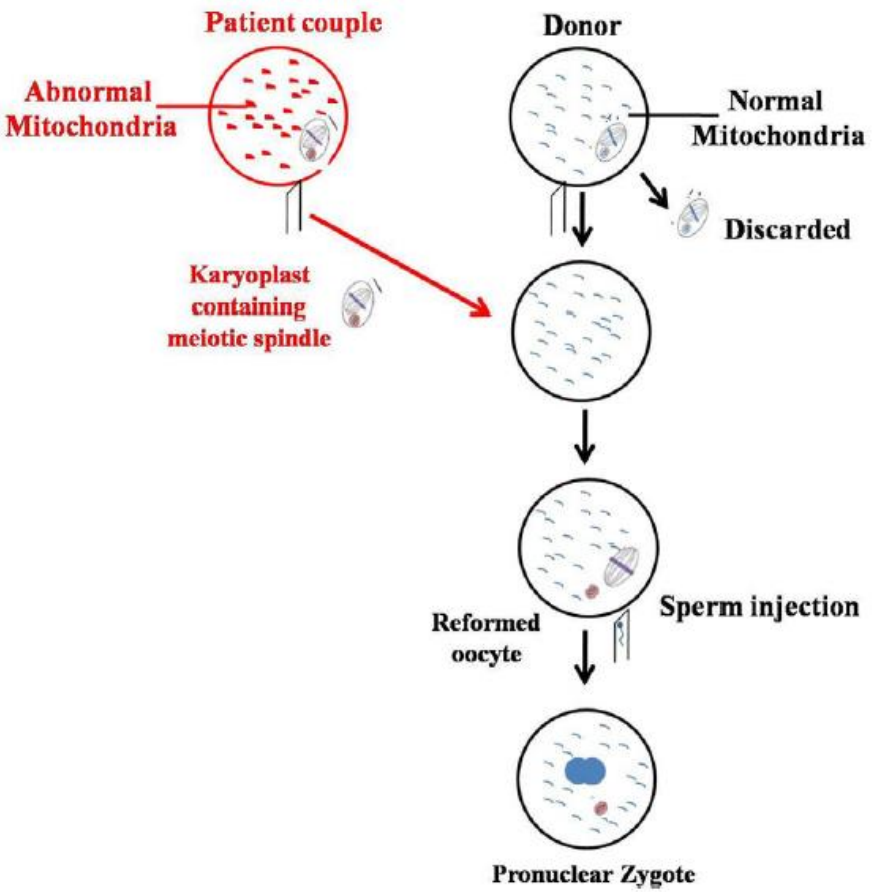
metafase II spindle transfer

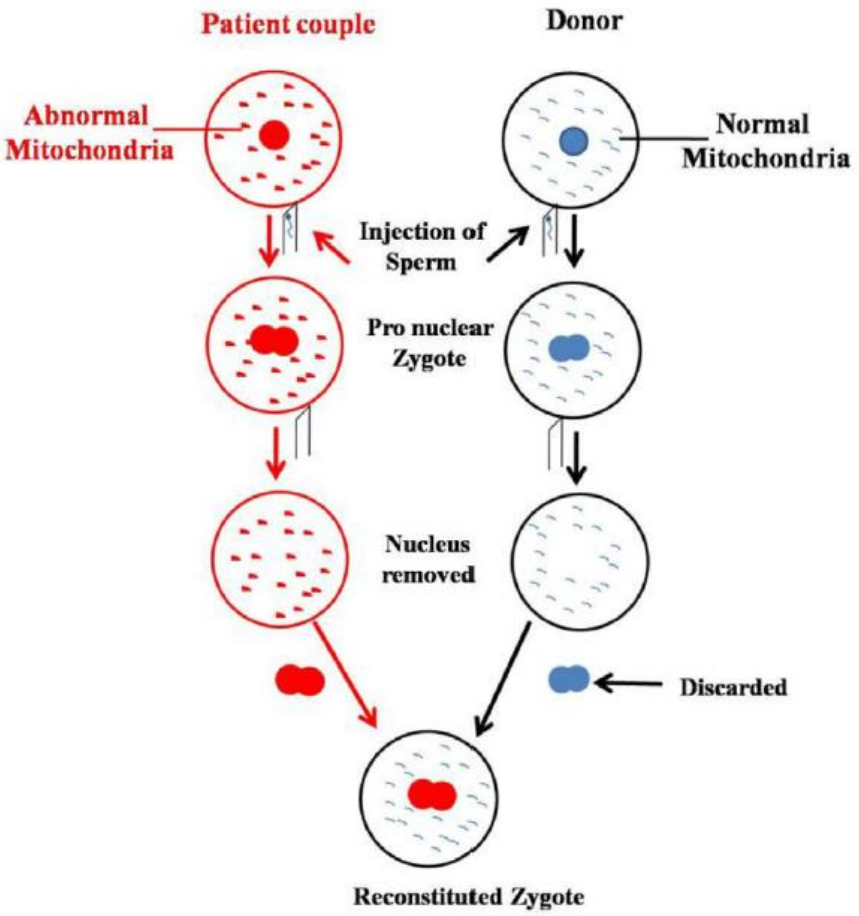


limited hu mtDNA transfer

- in some carry-over ≤ 2.0%

Tachibana 2013

pronuclear transfer



limited hu mtDNA transfer

- carry-over ?
  in 4/9 embryos not detectable;
  in some 0,01 – 2%

Craven 2010

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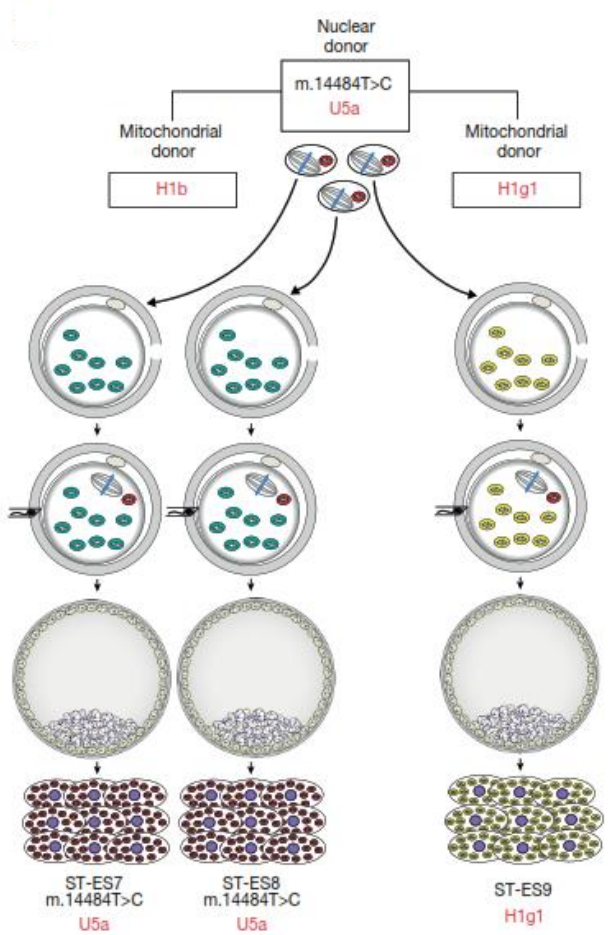
mitochondrial donation therapy ?

promises & perils ?

peril : reversion ? (1)

- 3 ES cell lines derived from MDT embryo
- **in vitro reversion** ➢ original pathogenic LHON mtDNA variant m.14484T>C
- no haplogroup matching
  - ➢ is needed ?
  - ➢ (other) cause ?
  - ➢ what in vivo ?



Hudson 2019

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peril : reversion ? (2)

uncertainties under debate / investigation

- << amounts mtDNA carry over ?
- haplogroup differences ?

- nuclear – mtDNA interaction
  - haplogroup matching needed ?
- mtDNA – mtDNA interaction
  - possible detrimental effect ?
- mtDNA segregation
  - genetic drift & mtDNA reversion ?

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peril : reversion ? (3)

uncertainties under debate

- << amounts mtDNA carry over ?
  - limiting co-transfer = a technical chal
  - genetic drift & mtDNA reversal ?

beneficial effects of MDT **lost over time ?**
Greek pilot study

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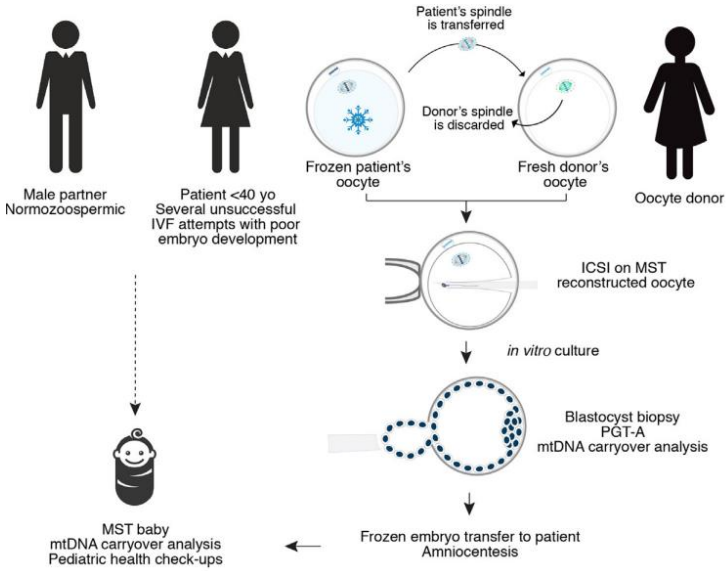
mitochondrial replacement therapy ?

Greek pilot study

- 25 infertile couples
- idiopathic infertility
- repeated IVF failure (n = 3-11)
- no mtDNA disease
- no haplogroup matching of donor
- 28 MST cycli



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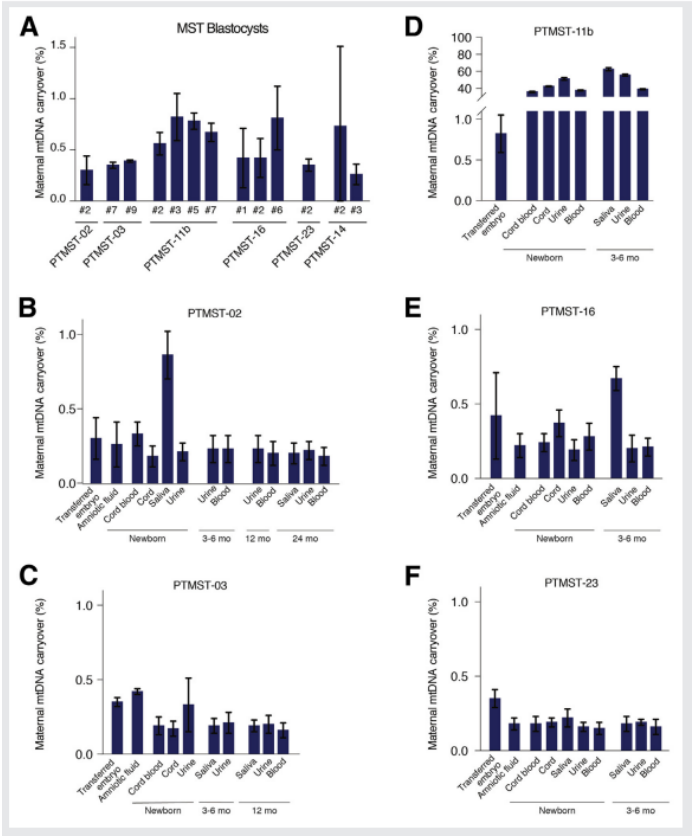
mitochondrial replacement therapy ?

Greek pilot study

- 28 MST cycli
- 6 life births
- << at start amounts mtDNA carry over (<1%) in 5

➢ **HOWEVER ….**



Costa-Borges et al. 2023

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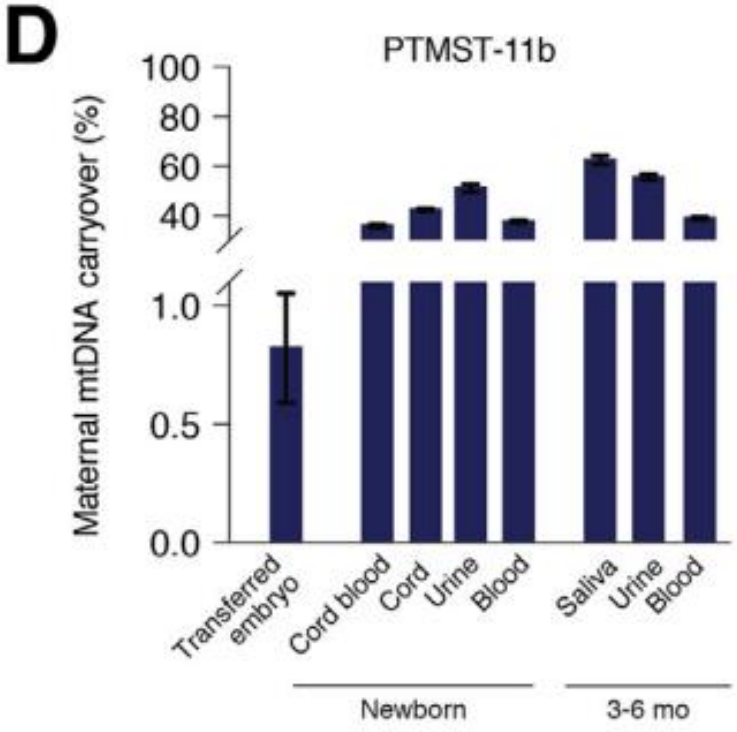
mitochondrial replacement therapy ?

Greek pilot study

HOWEVER

- 1 baby with reversion of 30-60% of maternal mtDNA haplotype in ≠ tissues (cord blood, blood, urine & saliva) at birth



Costa-Borges et al. 2023

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promises ? reduce, not eliminate risk

- 2015 : licenced by UK parliament
- 2017 : HFEA licenced a fertility clinic
  - clinical trials have started in Newcastle Fertility Centre with PNT, on case-by-case basis
- **2023 baby's born** (no further info yet)
- US : FDA ban on clinical trails of MDT

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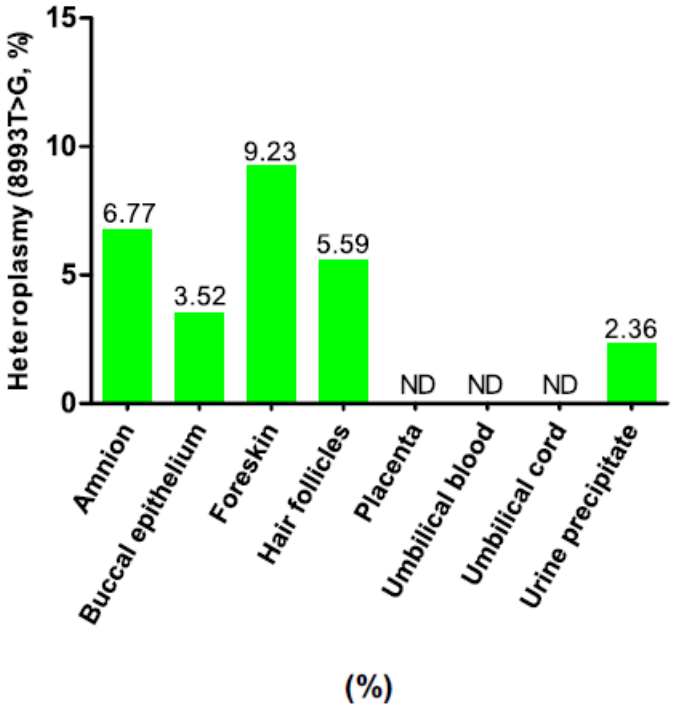
a human baby was born in Mexico

- a ‘spindle transfer baby’ was born in Mexico (Zhang et al. 2017)

- m.8993T>G
  - 5,9% heteroplasmy blastocyst stage
  - 2,36 to 9,23% in various tissues at birth



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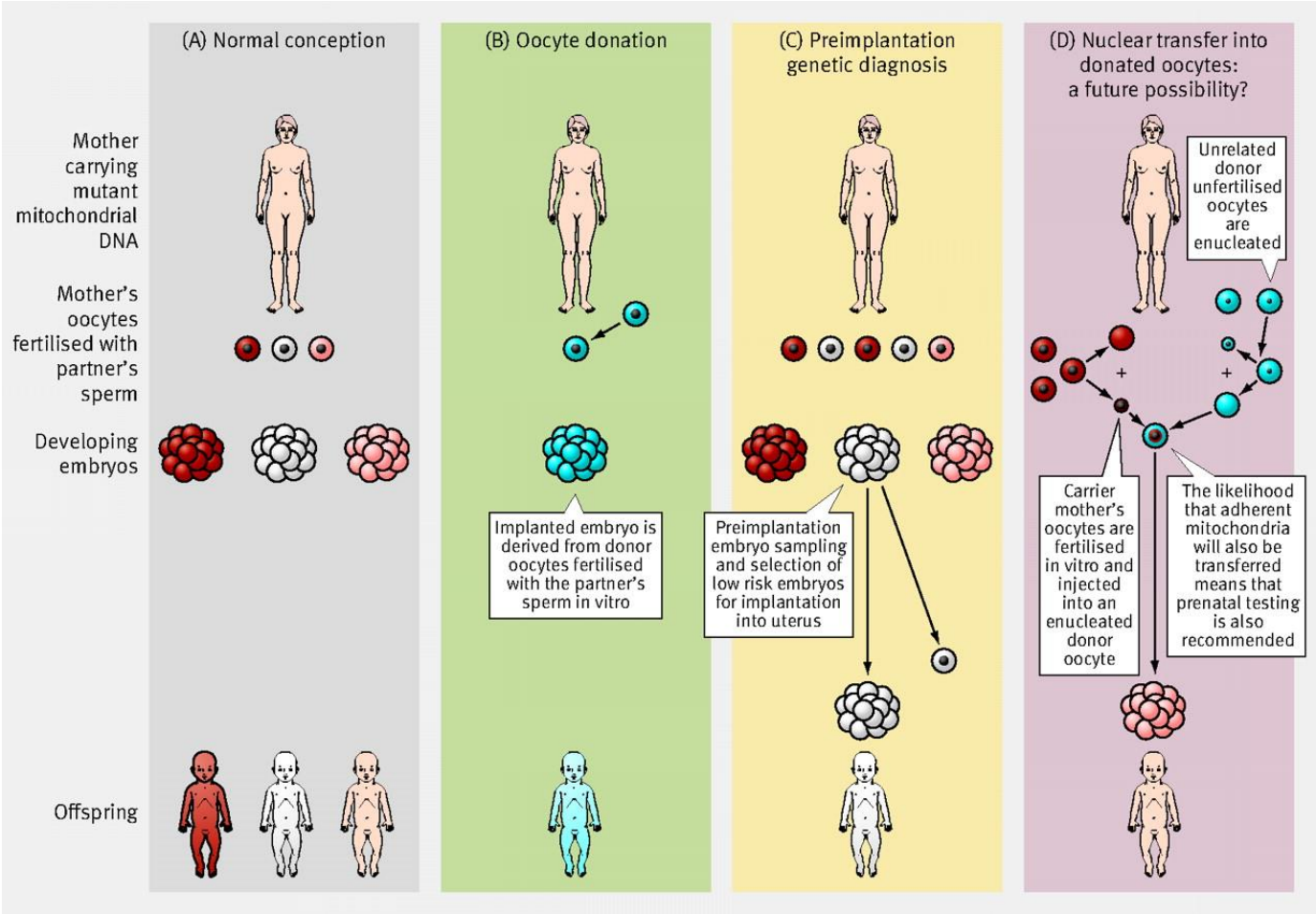
transmitting mtDNA disease



option of oocyte or embryo donation which avoids all riks

key points (1) summary

- mtDNA diseases are frequent in humans
- many different factors interfere in final risk determination
  - heteroplasmy
  - bottleneck
  - variant
- counseling is complex

key points (2) summary

- PNT can be an option
  - low risk situations
- PGT can be an alternative
  - moderate risk situations
  - analysis of oocytes might be directive
- interpretation of test results & ‘grey zone’ could be a problem
  (phenotypic threshold level ?)

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key points (3) summary

- oocyte / embryo donation option
- MDT is still experimental
  - promising track in a near future
  - high risk situations & homoplasmic variants

- **NO one for all solution**

THE END
THANK YOU

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