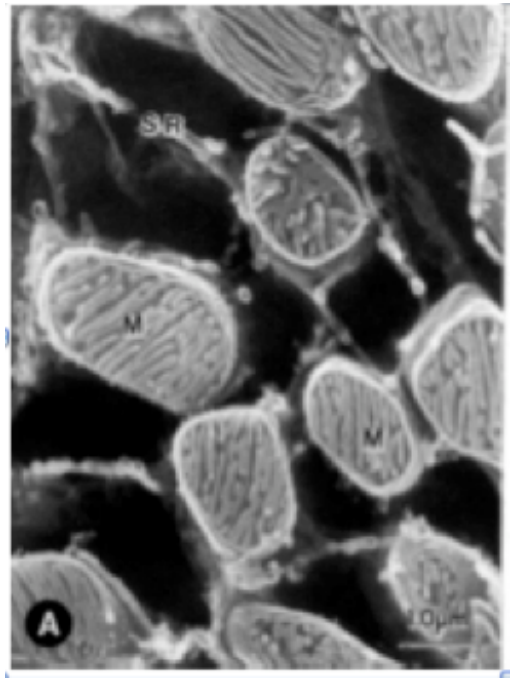
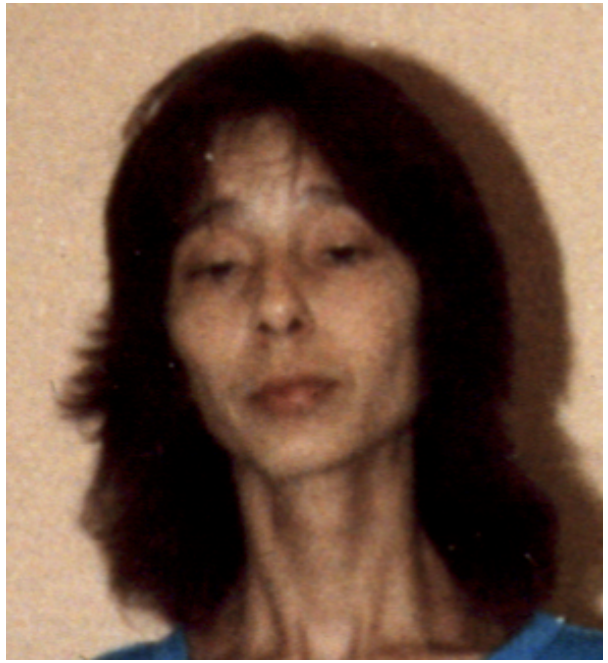
Mitochondrial Replacement Therapy: A Game Changer for the Mitochondrial Disease Community



MitoAction
April 21, 2017

Kristin Engelstad, MS, GC
Clinical Research Coordinator

Michio Hirano, MD
Professor of Neurology
Columbia University Medical Center
New York, NY

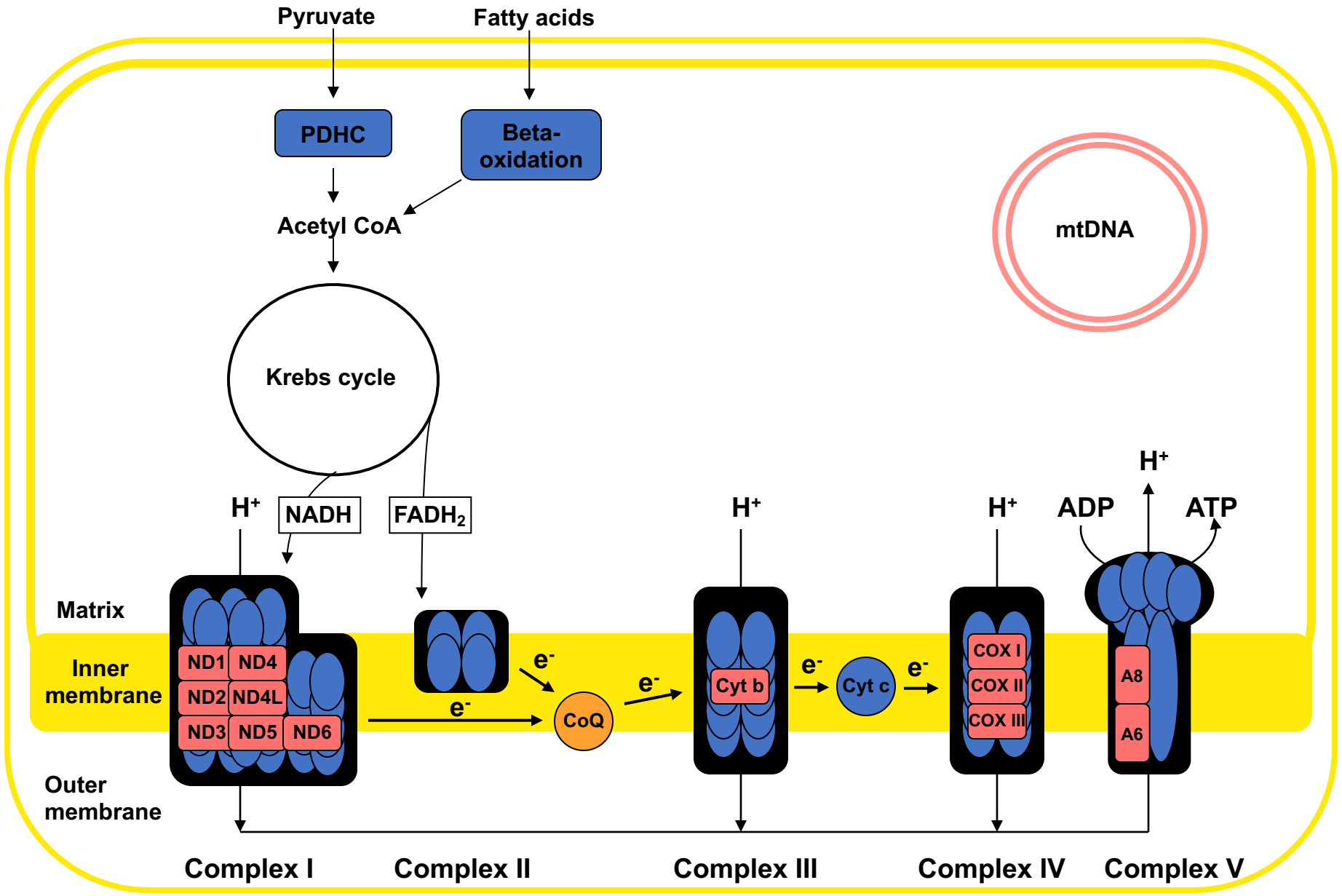


Financial Disclosures

- Consultant for Meves Pharmaceuticals Inc.
- Honoraria from Stealth BioTherapeutics Inc. and Sarepta Therapeutics Inc. for participation in Advisory Board Meetings.

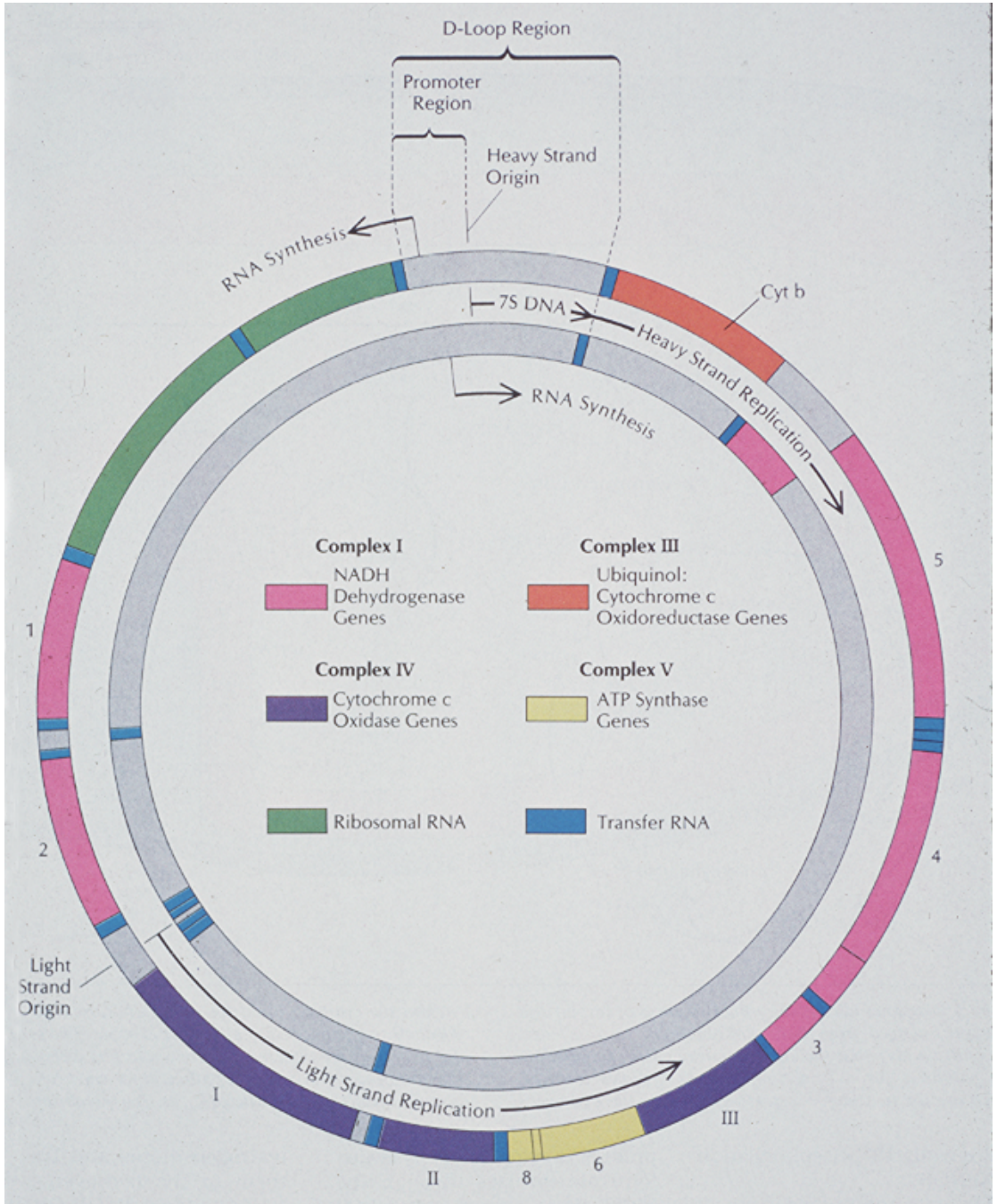
Learning Objectives

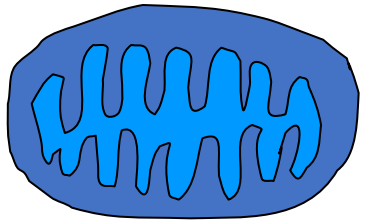
- To be familiar with the rules of mitochondrial DNA (mtDNA) genetics
- To recognize that mtDNA diseases are clinically important
- To be aware of the reproductive options of women who carry mtDNA mutations
- To appreciate the principals of mitochondrial replacement techniques (MRTs) that may prevent transmission of mtDNA diseases
- To understand the current state of clinical MRT in the UK and US



mtDNA-encoded	7	0	1	3	2
nDNA-encoded	39	4	10	10	12

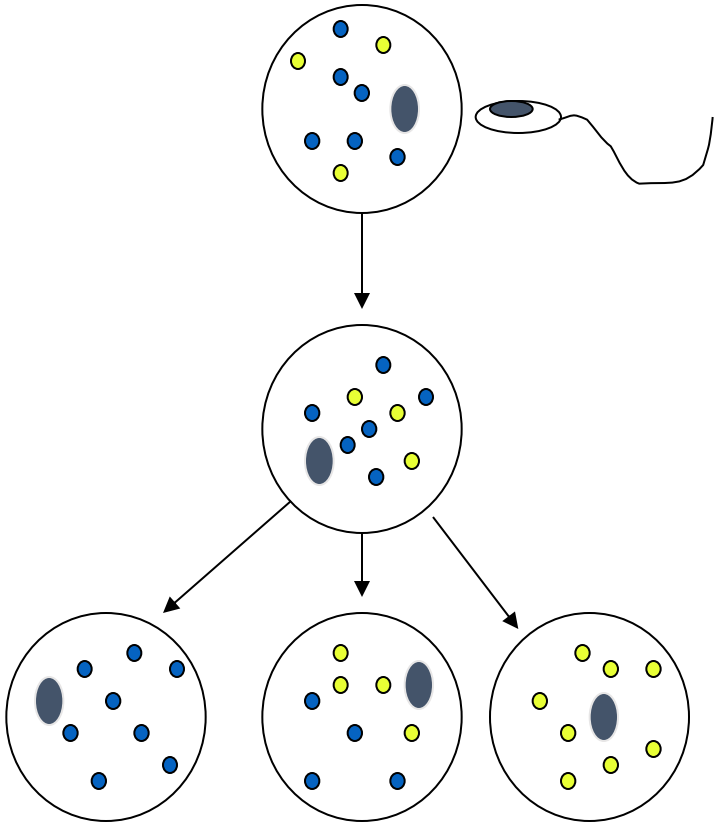
Mitochondrial DNA (mtDNA)

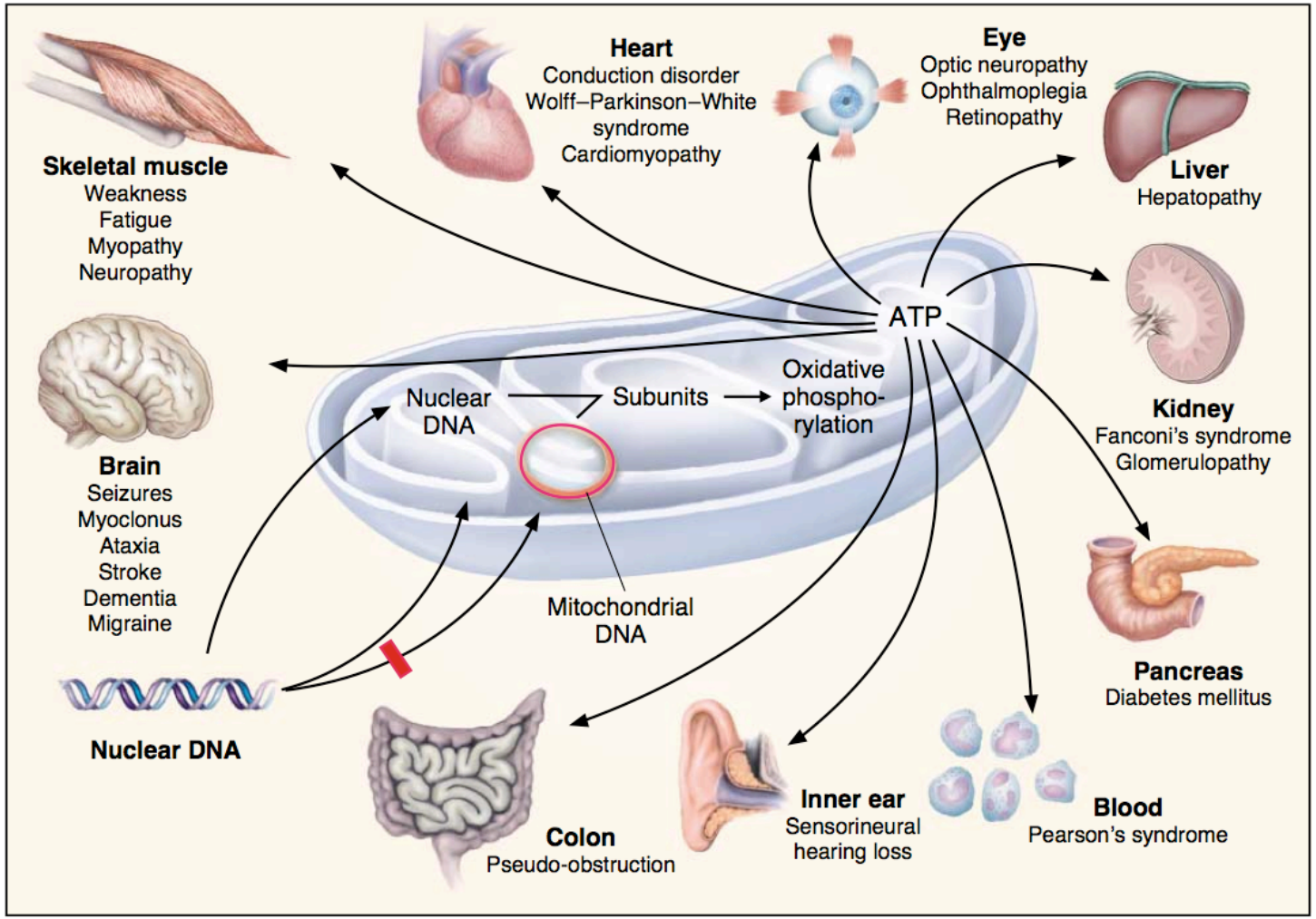




Rules of mtDNA mutations

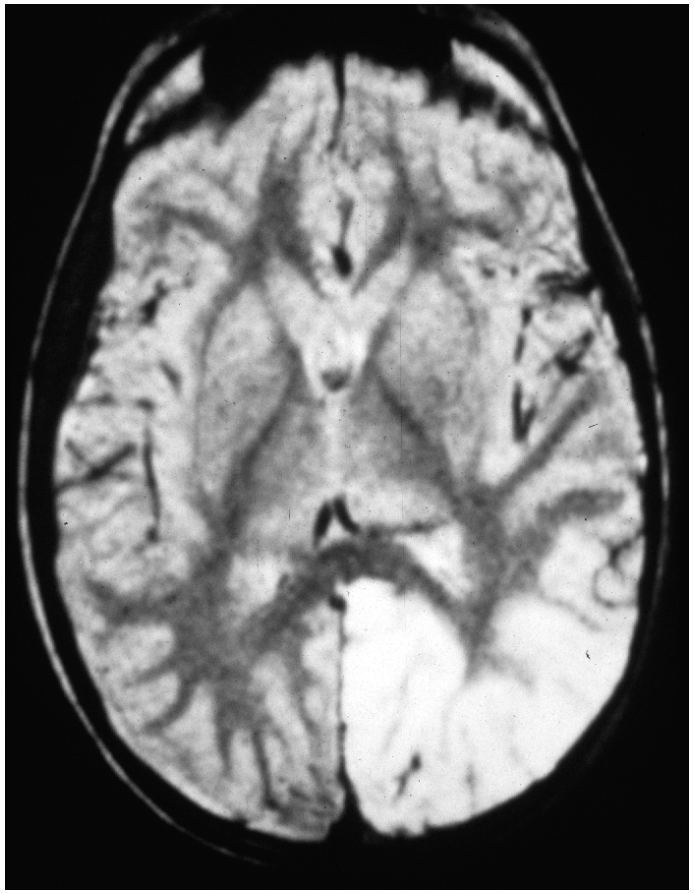
- Maternal inheritance
- Heteroplasmy
- Mitotic Segregation
- Threshold Effect





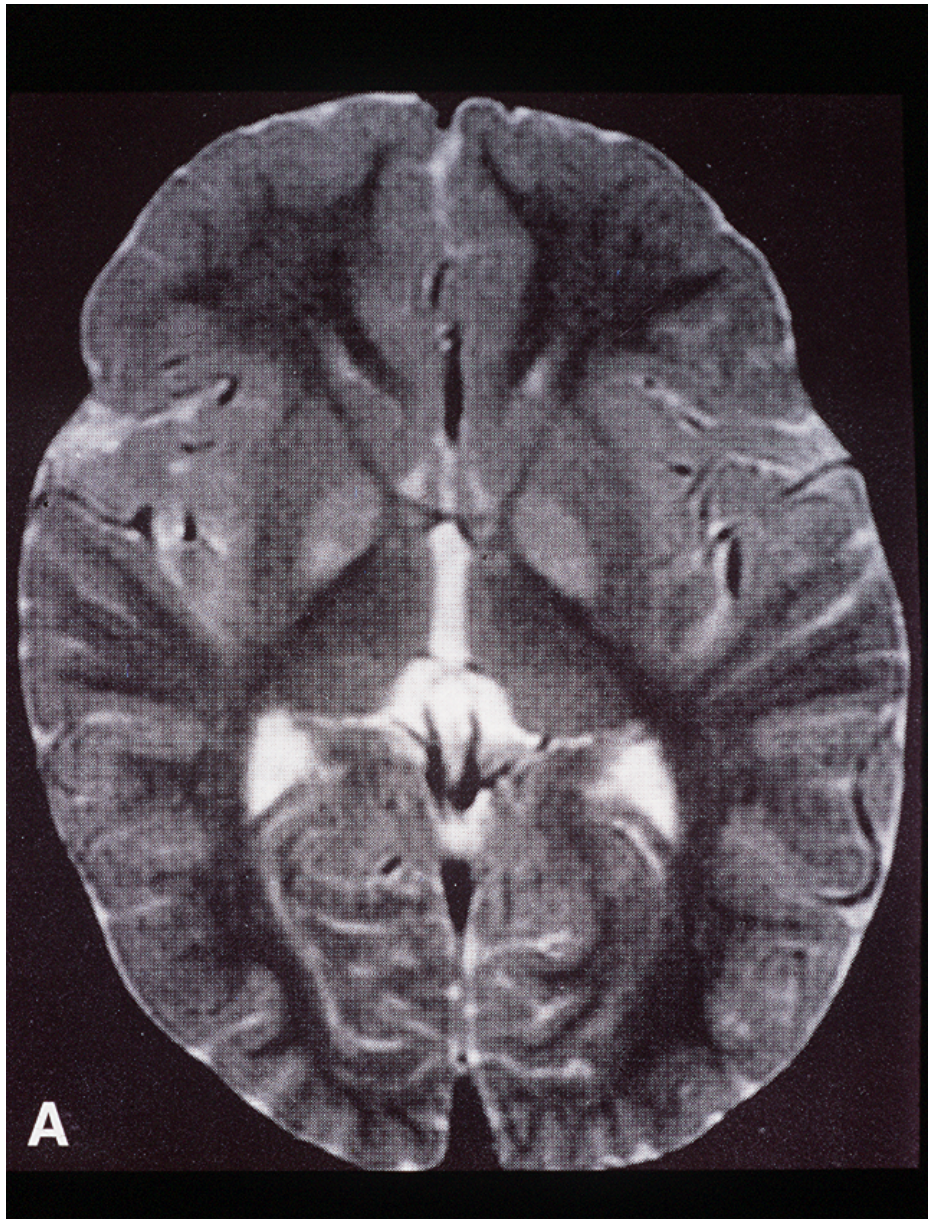
Mitochondrial Encephalopathy, Lactic Acidosis, and Stroke-like episodes (MELAS)

- **Stroke-like episodes at a young age**
- **Encephalopathy manifesting as seizures, dementia, or both**
- **Lactic acidosis, ragged-red fibers, or both**



T2-MRI

Leigh Syndrome



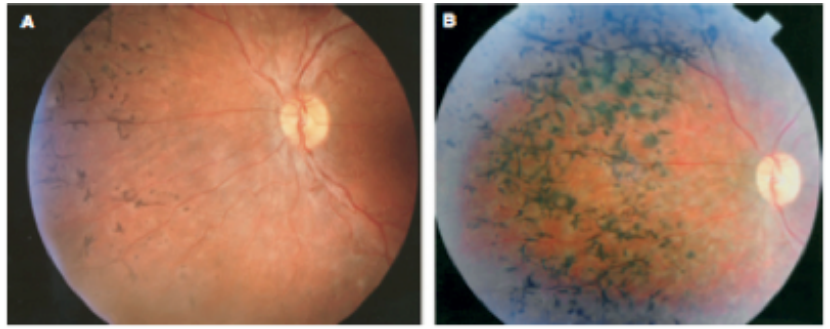
Subacute necrotizing encephalopathy affecting basal ganglia, brainstem, and sparing the mammillary bodies.

Typically begins in infancy with psychomotor regression or retardation.

Other manifestations include: hypotonia, feeding problems, respiratory abnormalities, vision and hearing loss, nystagmus, ataxia, and seizures.

Neuropathy Ataxia Retinitis Pigmentosa (NARP)

- Peripheral neuropathy
- Cerebellar ataxia
- Pigmentary retinopathy
- Maternal inheritance
- Lactic acidosis

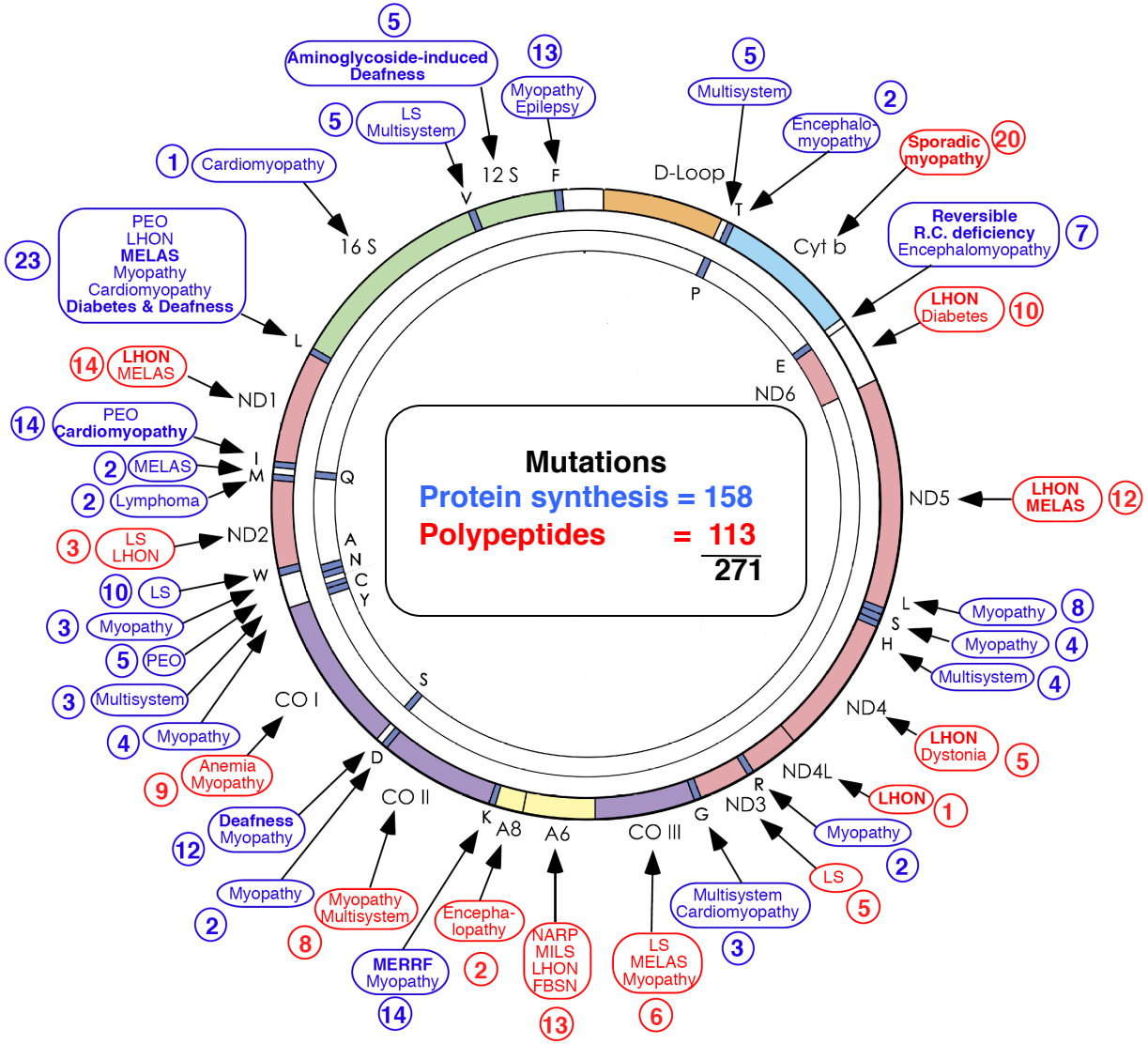


Carelli V, Barboni P, and Sadun AA. "Mitochondrial Ophthalmology" in Mitochondrial Medicine. 2006

Maternally Inherited Leigh Syndrome (MILS)

- **Devastating encephalopathy in infancy or childhood**
- **Psychomotor regression**
- **Other features include: pigmentary retinopathy, seizures, ptosis, ophthalmoplegia, nystagmus, dystonia, tremor, pyramidal tract signs, ataxia, and impaired respiration.**

Mitochondrial morbidity map - 2017



Courtesy of E.A. Schon

Prevalence of Nuclear and Mitochondrial DNA Mutations Related to Adult Mitochondrial Disease

Gráinne S. Gorman, MRCP,[1,2] Andrew M. Schaefer, MRCP,[1,2] Yi Ng, MRCP,[1,2]
Nicholas Gomez,[1,2] Emma L. Blakely, PhD,[1,2] Charlotte L. Alston, PhD,[1,2]
Catherine Feeney,[1,2] Rita Horvath, PhD,[1,3] Patrick Yu-Wai-Man, PhD,[1,3]
Patrick F. Chinnery, PhD,[1,3] Robert W. Taylor, PhD,[1,2]
Douglass M. Turnbull, PhD,[1,2] and Robert McFarland, PhD[1,2]

Ann Neurol, 2015

mtDNA mutations ~1 in 5,000 people
Symptomatic nDNA mutations ~1/34,000

ARTICLE

Pathogenic Mitochondrial DNA Mutations Are Common in the General Population

Hannah R. Elliott,[1] David C. Samuels,[2] James A. Eden,[3] Caroline L. Relton,[3] and Patrick F. Chinnery[1,3,*]

~1 in 200 people carries a mtDNA mutation

Am J Hum Genet, 2008

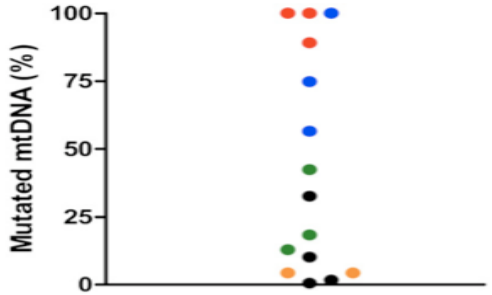
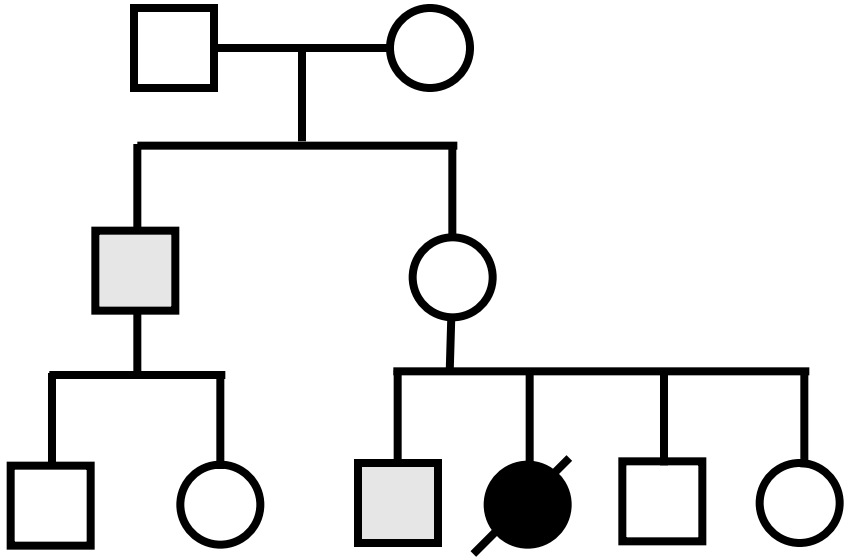


Figure 1. Percentage of Mutated mtDNA in the 15 Mutation-Positive Cases
Red: m.14484T→C; blue: m.11778G→A; green: m.3460G→A; black: m.3243A→G; orange: m.1555A→G.

Pathogenic mtDNA mutations are common in the general population

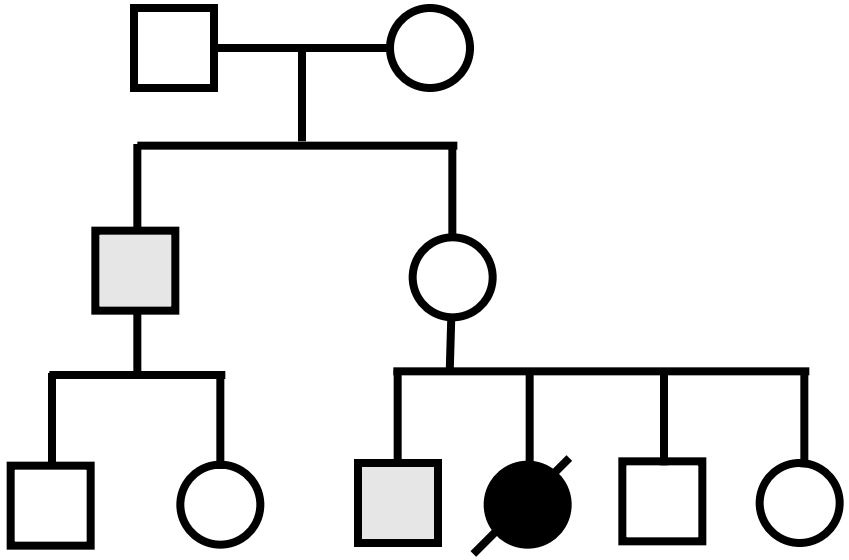
Family A

Phenotype

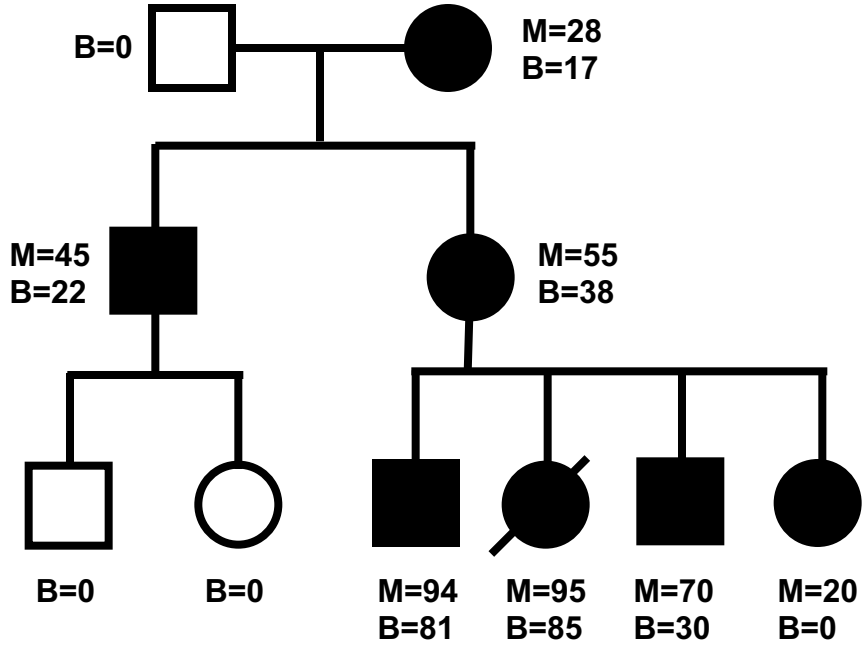


Family A

Phenotype

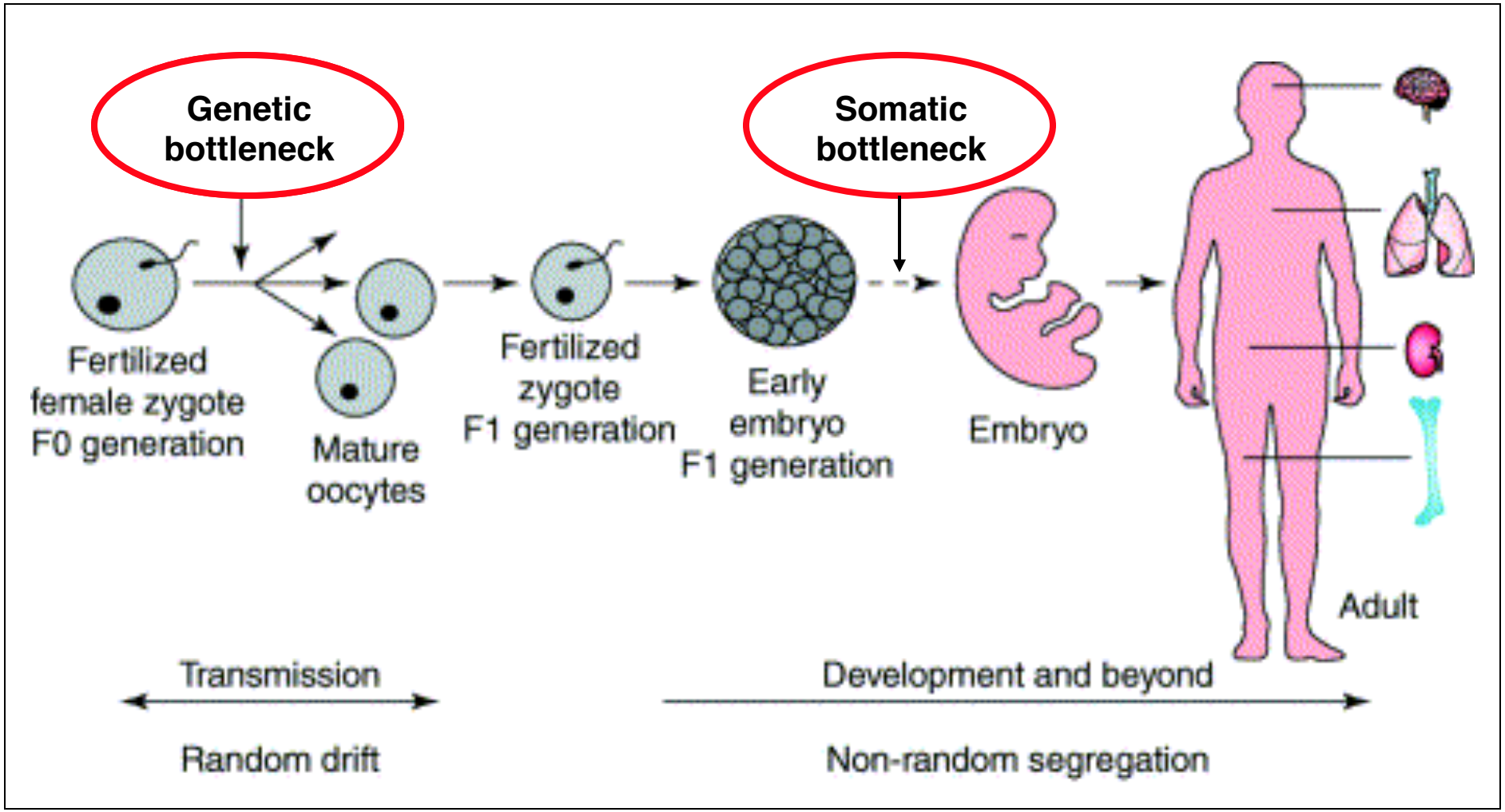


Genotype



Percent mutation
M=Muscle
B=Blood

Mitochondrial segregation during germline development: the "bottleneck"



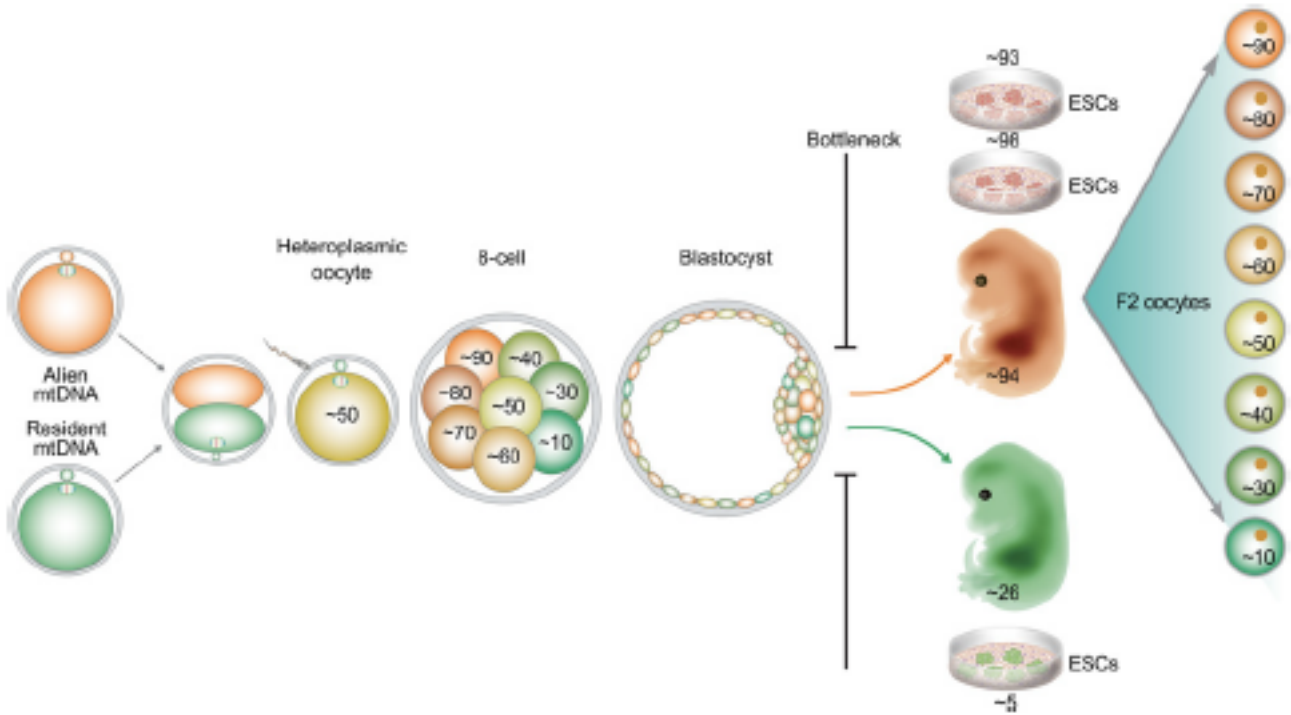
Chinnery (2002) Trends Genet 18:173

Reproductive options of women who carry mtDNA point mutations

- Normal reproduction with associated risks of having a child with mtDNA disease
- Not have children
- Adopt
- IVF with donor eggs
- Preimplantation genetic diagnosis (PGD); however, PGD has limitations…

Rapid Mitochondrial DNA Segregation in Primate Preimplantation Embryos Precedes Somatic and Germline Bottleneck

Hyo-Sang Lee,[1] Hong Ma,[1] Rita Cervera Juanes,[1] Masahito Tachibana,[1] Michelle Sparman,[1] Joy Woodward,[1] Cathy Ramsey,[1] Jing Xu,[1] Eun-Ju Kang,[1] Paula Amato,[2] Georg Mair,[3] Ralf Steinborn,[3] and Shoukhrat Mitalipov[1,2,4,5,*]



Cell Reports 2012;1:506-15

Mitochondrial Replacement Therapy

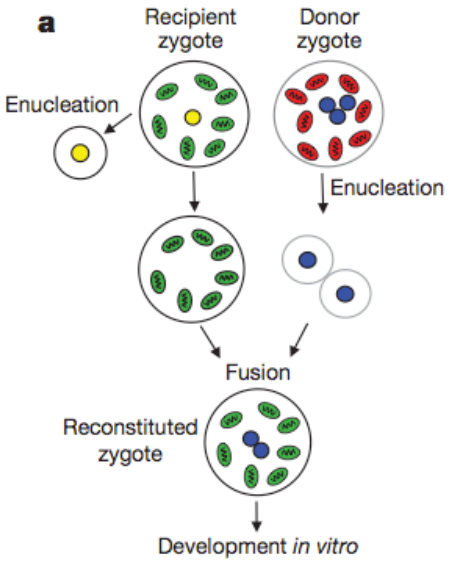
- Three-parent IVF
- Three-person IVF
- Three-parent fertilization
- Mitochondrial replacement
- Mitochondrial gene replacement
- Mitochondrial Replacement Technique
- Pronuclear transfer in human embryos
- Chromosome transfer in mature oocytes
- Nuclear genome transfer in human oocytes

Preventing transmission of mtDNA mutations

Pronuclear transfer in human embryos to prevent transmission of mitochondrial DNA disease

Lyndsey Craven[1], Helen A. Tuppen[1], Gareth D. Greggains[3,4], Stephen J. Harbottle[3], Julie L. Murphy[1], Lynsey M. Cree[1], Alison P. Murdoch[3,5], Patrick F. Chinnery[1], Robert W. Taylor[1], Robert N. Lightowlers[1], Mary Herbert[3,4,5] & Douglass M. Turnbull[1,2,5]

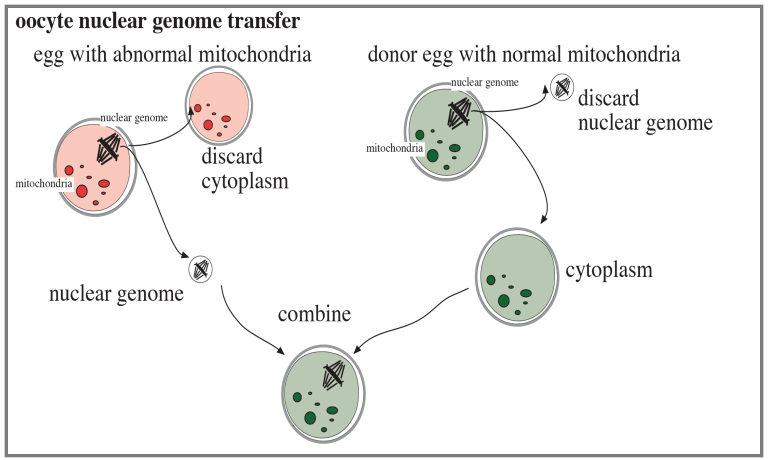
Nature 2010;465:82-85



Nuclear genome transfer in human oocytes eliminates mitochondrial DNA variants

Daniel Paull[1], Valentina Emmanuele[2], Keren A. Weiss[1], Nathan Treff[3], Latoya Stewart[1], Haiqing Hua[1,4], Matthew Zimmer[1], David J. Kahler[1], Robin S. Goland[4], Scott A. Noggle[1], Robert Prosser[5], Michio Hirano[2], Mark V. Sauer[5,6]* & Dieter Egli[1]*

Nature 2013;493:632-7

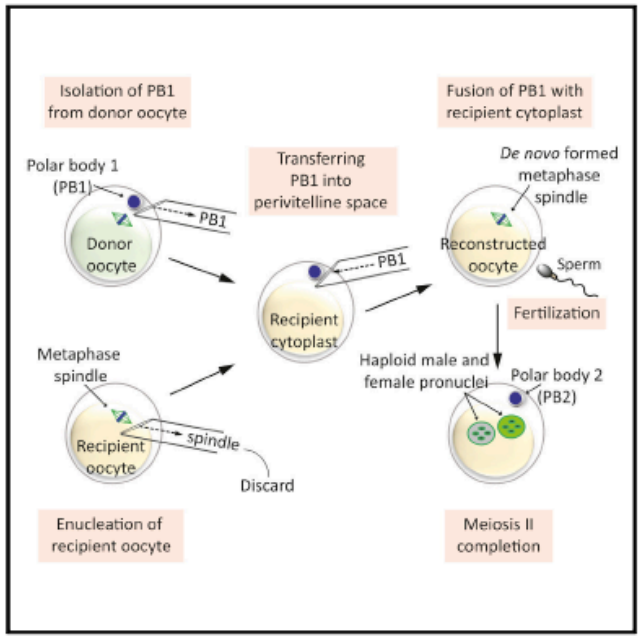


Short Article

Cell Stem Cell

Functional Human Oocytes Generated by Transfer of Polar Body Genomes

Graphical Abstract



Authors

Hong Ma, Ryan C. O'Neil, Nuria Marti Gutierrez, ..., Don P. Wolf, Joseph R. Ecker, Shoukhrat Mitalipov

Correspondence

ecker@salk.edu (J.R.E.), mitalipo@ohsu.edu (S.M.)

In Brief

Ma et al. show regeneration of functional human oocytes through polar body transfer into enucleated oocyte cytoplasts. In addition to providing proof of principle for this process, the approach could be helpful clinically for some forms of infertility and genetic disease.

Highlights

- Metaphase II oocytes can be reconstructed by polar body nuclear transfer (PBNT)
- Reconstructed PBNT oocytes complete meiosis after fertilization with sperm
- PBNT-derived blastocysts can give rise to phenotypically normal hESC lines

Accession Numbers

GSE79310

Cell Stem Cell 2017;20:112-9

Gene therapy for progeny of mito-mice carrying pathogenic mtDNA by nuclear transplantation

Akitsugu Sato*†‡, Tomohiro Kono§, Kazuto Nakada*†¶, Kaori Ishikawa*†, Shin-Ichi Inoue*, Hiromichi Yonekawa‡, and Jun-Ichi Hayashi*‖

Proc Natl Acad Sci 2005

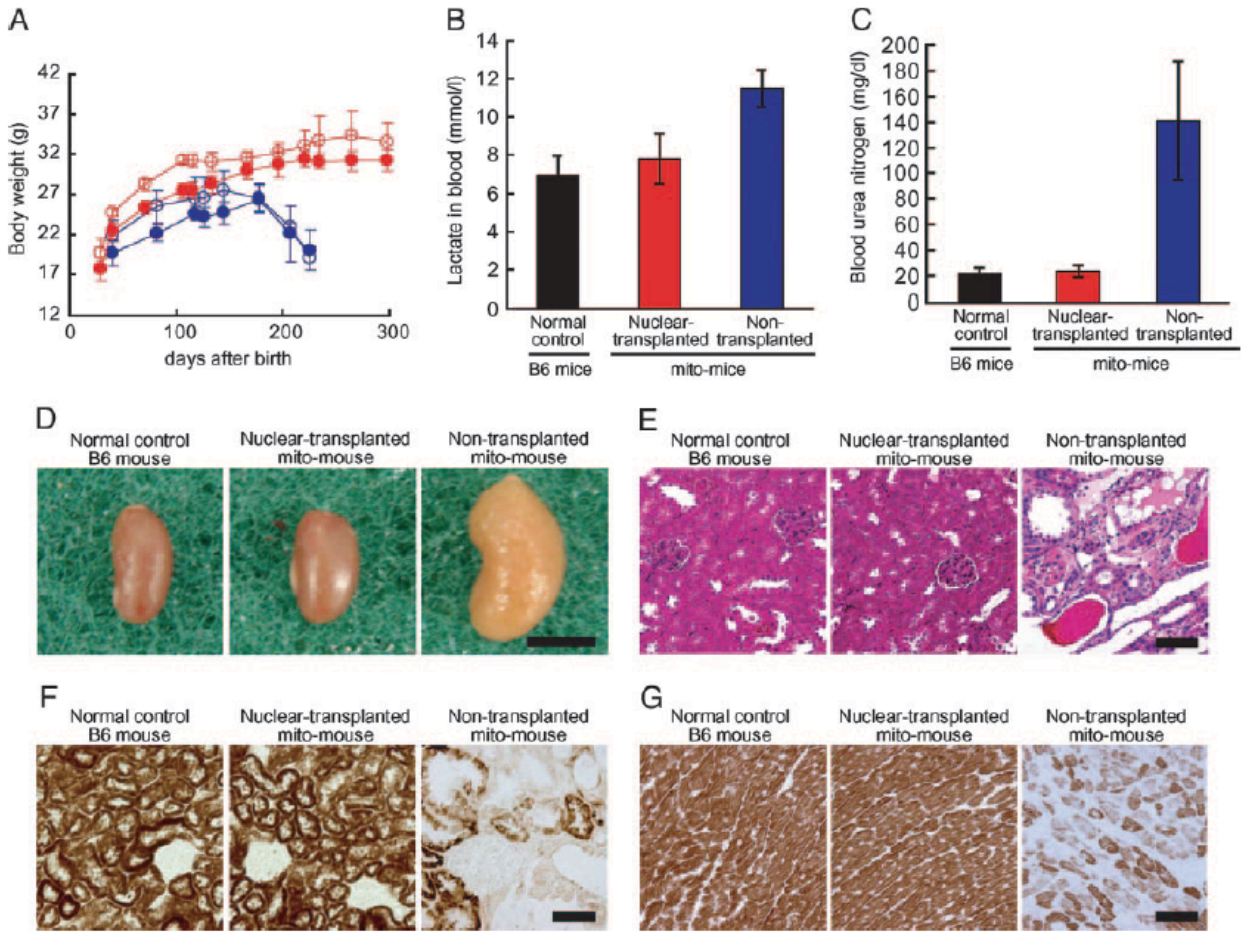


Fig. 4. Effects of nuclear transplantation of mito-mouse zygotes on clinical phenotypes. (*A*) Measurement of body weights. Red and blue circles indicate mito-mice developed from nuclear-transplanted and nontransplanted zygotes, respectively. Open and filled circles indicate males and females, respectively. (*B*) Concentration of blood lactate after glucose loading. (*C*) Concentration of blood urea nitrogen examined 200 days after birth. (*D*) Kidneys. (*E*) Histopathology of kidneys. (*F*) Cytochrome *c* oxidase histochemistry of kidneys. (*G*) Cytochrome *c* oxidase histochemistry of hearts from normal control B6 mouse (*Left*), nuclear-transplanted (*Center*), and nontransplanted mito-mouse (*Right*) killed 210 days after birth. (Scale bar in *D*, 5 mm; scale bars in *E–G*, 50 μm.)

Vol 461|17 September 2009|doi:10.1038/nature08368 nature

ARTICLES

Mitochondrial gene replacement in primate offspring and embryonic stem cells

Masahito Tachibana[1], Michelle Sparman[1], Hathaitip Sritanaudomchai[1], Hong Ma[1], Lisa Clepper[1], Joy Woodward[1], Ying Li[1], Cathy Ramsey[1], Olena Kolotushkina[1] & Shoukhrat Mitalipov[1,2,3]

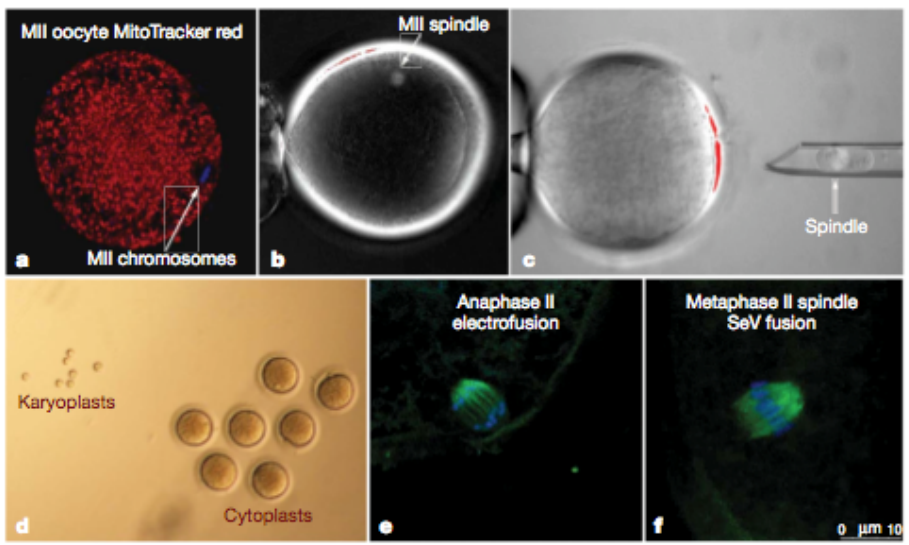


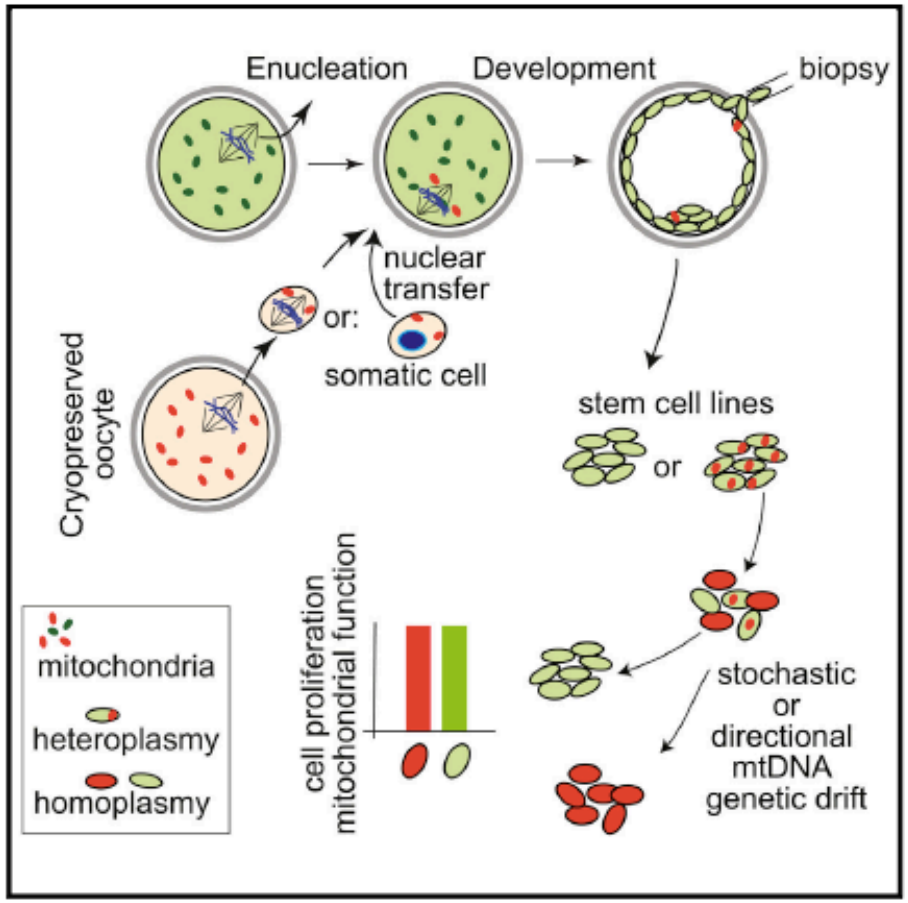
Figure 2 | Mito and Tracker, the first primates to be produced by spindle–chromosomal complex transfer (ST) into enucleated oocytes followed by fertilization and embryo transfer. Twin pregnancy was

Brief Report

Cell Stem Cell

Genetic Drift Can Compromise Mitochondrial Replacement by Nuclear Transfer in Human Oocytes

Graphical Abstract



Authors

Mitsutoshi Yamada, Valentina Emmanuele, Maria J. Sanchez-Quintero, ..., Mark V. Sauer, Michio Hirano, Dieter Egli

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mh29@cumc.columbia.edu (M.H.), d.egli@nyscf.org (D.E.)

In Brief

Yamada et al. show, using human cells, that even small amounts of mtDNA carried over during nuclear transfer for mitochondrial replacement can lead to mtDNA genotype reversion. This situation would need to be avoided for clinical application and stable prevention of mtDNA diseases.

Cell Stem Cell 2016;18:749-54

Mitochondrial Donation — How Many Women Could Benefit?

Grainne S. Gorman, MD; John P. Grady, PhD; Douglas M. Turnbull, MD

New Engl J Med 2015

Women (15-44 years old) at risk for transmitting mtDNA disease

- UK=2,473
- US=12,423

Estimated number of births per year among women
at risk for transmitting mtDNA disease

- UK=152
- US=778

Oocyte MRT Carrier Survey Results

- 100% (92/92) of participants understood that they could transmit the mtDNA mutation to their offspring.
- 78% (35/45) of women of childbearing age had thought about not having children because of transmission risk.
- 73% (37/51) of women who had children prior to knowing they carried (or were “at risk” of carrying) a mtDNA mutation would have thought about not having children had they known of the risk.
- 95% (87/92) said the development of MRT was an important and worthwhile project.

Engelstad et al. Hum Reprod 2016;31:1058-65

Oocyte MRT Carrier Survey Results

Of women considering having children (n=21)

Having biological offspring was considered:

95% - Somewhat or very important

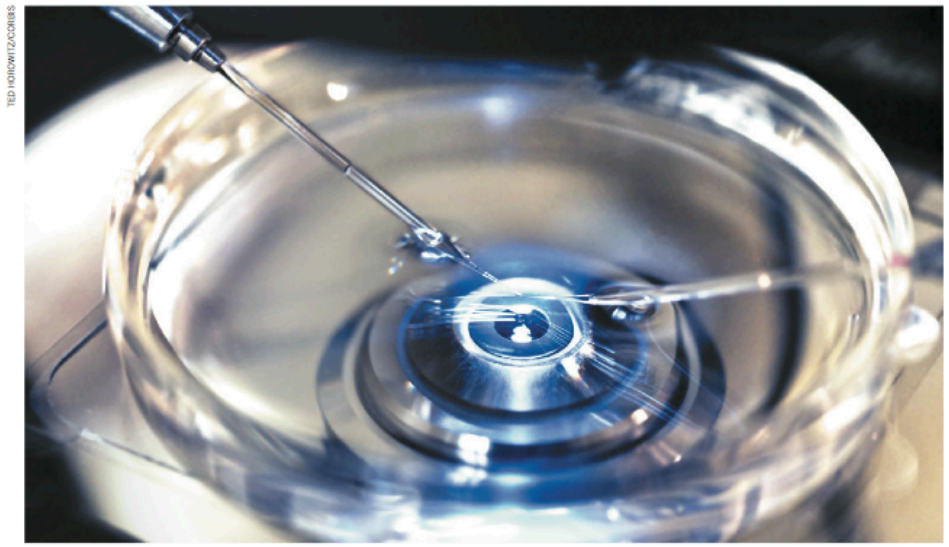
5% - Not important

90% are interested in using MRT to have a child

78% are interested in allowing their eggs to be used for basic laboratory research in the process of developing an implantable zygote.

Engelstad et al. Hum Reprod 2016;31:1058-65

Mitochondrial Replacement Therapy is available in the United Kingdom



TED HOROWITZ/CORBIS

Three-person *in vitro* fertilization prevents women from passing on potentially harmful mutations in mitochondrial DNA.

REPRODUCTIVE BIOLOGY

World hails embryo vote

UK move to allow pioneering fertility technique could spur other countries to relax rules too.

Nature, 2015:518:145-6

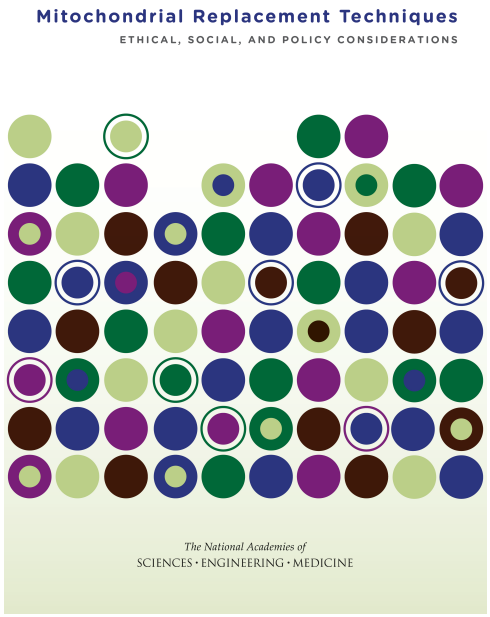
Mitochondrial Donation — Clearing the Final Regulatory Hurdle in the United Kingdom

Mary Herbert, Ph.D., and Doug Turnbull, M.D., Ph.D.

"On December 15, 2016, the Human Fertilisation and Embryology Authority in the United Kingdom approved the use — in certain, specific cases — of a technique that is based on in vitro fertilization (IVF) and involves mitochondrial donation.
Its Science Review Panel stated that 'it is appropriate to offer mitochondrial donation techniques as clinical risk reduction treatment for carefully selected patients.'"

New Engl J Med 2016;376:71-2

In the US, MRT research continues, but is not approved for clinical use



At the request of the FDA, the Institute of Medicine assembled a committee to explore ethical, social, and policy issues related to MRT.

"the committee concluded that it is ethically permissible to conduct clinical investigations of MRT. To ensure that clinical investigations of MRT were performed ethically, however, certain conditions and principles would need to govern the conduct of clinical investigations and potential future implementation of MRT."

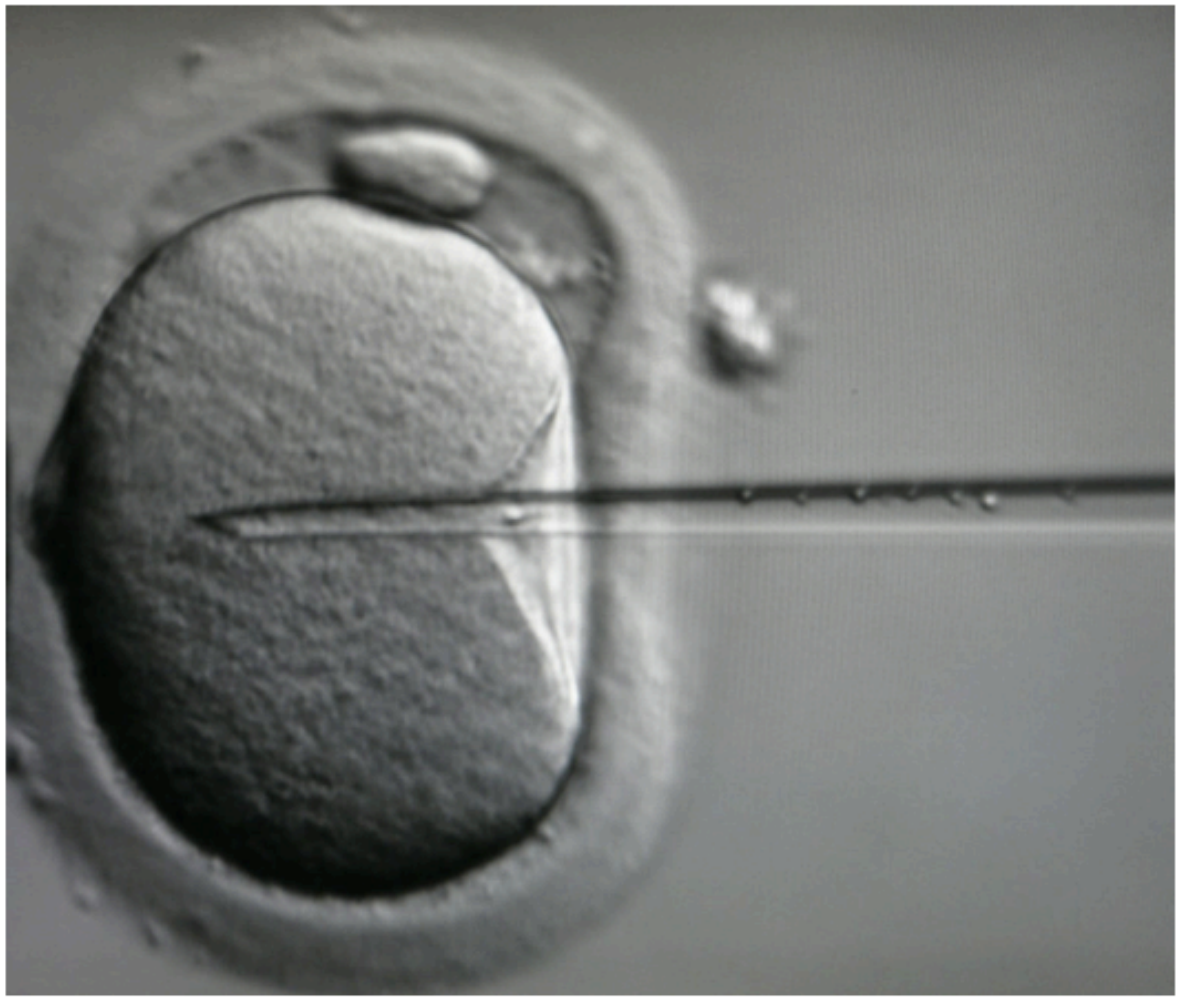
National Academies of Sciences, Engineering, and Medicine. 2016. *Mitochondrial replacement techniques: Ethical, social, and policy considerations.* Washington, DC: The National Academies Press.

Science 2016;353:545-6

HEALTH

Birth of Baby With Three Parents' DNA Marks Success for Banned Technique

By GINA KOLATA SEPT. 27, 2016 The New York Times



A sperm being injected into an egg during an in vitro fertilization procedure.
Jean-Paul Chassenet /Science Source

Asymptomatic Middle Eastern female carrier of a mtDNA mutation had 4 miscarriages and two children who died of Leigh syndrome.

Oocyte mitochondrial replacement technique via spindle transfer was performed and she gave birth to a boy in April, 2016 in Mexico.

At age 3 months, the boy was healthy and reportedly had 1% of his mother's mtDNA in blood.

Research Participation- MRT

Inclusion Criteria Females:

A known carrier of DNA mutation in mitochondrial genome

Maternal relatives- assist with genetic testing

Mutation can cause significant disease

Adult (22-40 years of age)

Male partner/sperm donor

Procedures:

Female: 2-3 outpatient visits, lab tests, hormone treatment, doctor visit, sonogram, oocyte retrieval

Male: 1 outpatient visit, lab tests, sperm donation

Cost: Travel, hotel and procedure cost is provided

Contact: Kris Engelstad 1-212-305-6834 ke4@cumc.columbia.edu

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- Alexandra Sanford, MS
- Richard Buchsbaum
- Seamus Thompson, PhD

University of Florida Gainesville

- Amy Roberts Holbert
- Jeffrey Krischer, PhD

NAMDC
the north american mitochondrial disease consortium

UNITED MITOCHONDRIAL DISEASE FOUNDATION.
HOPE. ENERGY. LIFE.

NIH National Institute of Neurological Disorders and Stroke

NIH Eunice Kennedy Shriver National Institute of Child Health and Human Development

NIH National Institutes of Health Office of Dietary Supplements

RARE CLINICAL DISEASES RESEARCH NETWORK
Initiative of the National Center for Advancing Translational Sciences (NCATS)

Bernard and Anne Spitzer Fund and the New York Stem Cell Foundation (NYSCF)