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

MitoAction
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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
The main components of the electron transport chain include:

- **Pyruvate**
- **Fatty acids**
- **Krebs cycle**
- **PDHC**
- **Acetyl CoA**
- **Beta-oxidation**

The inner membrane of the mitochondria contains:

- **Complex I**
- **Complex II**
- **Complex III**
- **Complex IV**
- **Complex V**

**mtDNA-encoded** components:
- Complex I: 7
- Complex II: 0
- Complex III: 1
- Complex IV: 3
- Complex V: 2

**nDNA-encoded** components:
- Complex I: 39
- Complex II: 4
- Complex III: 10
- Complex IV: 10
- Complex V: 12

The electron transport chain generates

- NADH
- FADH$_2$
- H$^+$

These electrons are transported through the electron carriers CoQ and Cyt b, c, and ultimately to COX, where they are used to pump H$^+$ into the intermembrane space, generating ATP.

**mtDNA** and **nDNA** components are indicated in the diagram.
Mitochondrial DNA (mtDNA)
Rules of mtDNA mutations

- Maternal inheritance
- Heteroplasmy
- Mitotic Segregation
- Threshold Effect
Interaction between Genes Encoded by Nuclear DNA and Those Encoded by Mitochondrial DNA in Oxidative Phosphorylation
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
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

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

Mutations
Protein synthesis = 158
Polypeptides = 113

271

Courtesy of E.A. Schon
Pathogenic Mitochondrial DNA Mutations Are Common in the General Population

Hannah R. Elliott, David C. Samuels, James A. Eden, Caroline L. Relton, and Patrick F. Chinnery

~1 in 200 people carries a mtDNA mutation

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

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

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

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

Pathogenic mtDNA mutations are common in the general population

Am J Hum Genet, 2008
Ann Neurol, 2015
Family A

Phenotype

Genotype

Percent mutation
M=Muscle
B=Blood
Mitochondrial segregation during germline development: the “bottleneck”

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, Hong Ma, Rita Cervera Juanes, Masahito Tachibana, Michelle Sparman, Joy Woodward, Cathy Ramsey, Jing Xu, Eun-Ju Kang, Paula Amato, Georg Mair, Ralf Steinborn, and Shoukhrat Mitalipov

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**

Lynsey Craven, Helen A. Tuppen, Gareth D. Greggain, Stephen J. Harbottle, Julie L. Murphy, Lynsey M. Cree, Alison P. Murdoch, Patrick F. Chinnery, Robert W. Taylor, Robert N. Lightowlers, Mary Herbert & Douglass M. Turnbull

Nature 2010;465:82-85

**Nuclear genome transfer in human oocytes eliminates mitochondrial DNA variants**

Daniel Paulif, Valentina Emmanuelli, Keren A. Weitz, Nathan Treiff, Lotaya Stewa, Haiqing Hua, Matthew Zimmer, David J. Kalbac, Robin S. Goland, Scott A. Noggle, Robert Prosser, Michio Harano, Mark V. Sauer & Dieter Egli

Nature 2013;493:632-7
Functional Human Oocytes Generated by Transfer of Polar Body Genomes

Graphical Abstract

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

Authors
Hong Ma, Ryan C. O’Neill, Nuria Marti Gutierrez, ..., Don P. Wolf, Joseph F. Ecker, Shoukry Mitalipov

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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.

Accession Numbers
GSE79310
Gene therapy for progeny of mito-mice carrying pathogenic mtDNA by nuclear transplantation

Akitsu Sato*†, Tomohiro Kono‡, Kazuto Nakada*†, Kaori Ishikawa*†, Shin-Ichi Inoue*, Hiromichi Yonekawa+, and Jun-Ichi Hayashi*†

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.)
Mitochondrial gene replacement in primate offspring and embryonic stem cells

Masahito Tachibana¹, Michelle Sparman¹, Hathaitip Sritanudomchai¹, Hong Ma¹, Lisa Clepper¹, Joy Woodward¹, Ying Li¹, Cathy Ramsey¹, Olena Koletushkina¹ & Shoukhrat Mitalipov¹,²,³
Genetic Drift Can Compromise Mitochondrial Replacement by Nuclear Transfer in Human Oocytes

Graphical Abstract

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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.
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.
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.
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.’

Mitochondrial Replacement Therapy is available in the United Kingdom

World hails embryo vote
UK move to allow pioneering fertility technique could spur other countries to relax rules too.


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.’

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.”
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

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