Mitochondrial disease – Are 3-parent embryos the answer?

Lynsey Cree
Department of Obstetrics and Gynaecology
University of Auckland
Embryo selection: Implications for mitochondrial disease

• What are mitochondria and what do they do?
• What are mitochondrial diseases & are they important?
• The mitochondrial bottleneck
• What do we know about the inheritance of mtDNA?
• PGD for mitochondrial disease
• Can we prevent transmission? Ethical issues?
What are mitochondria and what do they do?
mitochondrion

CARBOHYDRATES
PROTEINS
FATS

ATP

nucleus
mitochondrion

mtDNA

Complex subunits

Maternal Inheritance

Mendelian Inheritance

ATP

Complex subunits

nucleus
Pathogenic mutations

Deletions

Pathogenic mutations
Heteroplasmy

Homoplasmic wild-type

Homoplasmic mutant

Heteroplasmic
Threshold effect

Wild-type phenotype

Mutant phenotype
How common are mtDNA mutations?

Cumbria Community Genetics Project
- 3168 live births - mum and child
- Analysed 10 mitochondrial point mutations

Pathogenic mutations found in 1/250
De-novo mutation rate 107/100,000 live births
How common are mtDNA mutations?

- Pathogenic mitochondrial mutations detected in 15 offspring (Approx 1 in 250 live births)
- The m.3243 A>G mutation was the most common
- 12 subjects were heteroplasmic and 3 homoplasmic
- Matched maternal samples available in 8/15 cases
- De novo mutation rate for mtDNA defects at 107/100,000 live births.

Elliott et al. AJHG 2008
Non-Neurological

- Respiratory Failure
- Cardiomyopathy
- Liver Failure
- Short Stature
- Marrow Failure
- Diabetes
- Thyroid Disease

Neurological

- Optic Atrophy / Retinitis Pigmentosa
- CVA / Seizures / Developmental delay
- Deafness
- Peripheral Neuropathy
- Myopathy
- Neurological Non-Neurological

http://www.cochrane.org/

THERE IS NO TREATMENT FOR MITOCHONDRIAL DISEASE
Summary 1

- Own genome
- Maternally inherited
- Multiple mitochondria per cell
- Threshold effect
- Severe disorders
- Lack treatment
Cruel Family Curse

Nine get same killer disease

By COREENA FORD

Pregnant Lesley Purvis has more reason than most to be nervous about the birth of her third child.

For a dark cloud has cast a shadow over the young woman's joy ... in the form of a mysterious family curse.

Genetic counselling

Genetic Counselling

I just don't know what the future holds for my children — LESLEY PURVIS

Generations of Suffering

MELAS FACTFILE:

MELAS - Mitochondrial Encephalomyopathy, Lactic Acidosis and Strokes (syndrome) — is a rare killer disease. It is caused by mutations in the DNA and means the body can't make energy.

Symptoms include loss of hearing and sight, diabetes, epilepsy, and other symptoms. Mitochondrial dysfunction causes cell damage, muscle disease, epilepsy, and strokes. The disease is also associated with sensorineural hearing loss, sudden deafness, and speech and language disorders.

Prayers

"We've had a very tough year but we've been supported by friends and family. It's been very tough, but we're getting through it," Lesley said. She added: "We're very lucky to have such wonderful friends and family."

"I just don't know what the future holds for my children," said another family member. "It's been very tough, but we're getting through it."

North patients have a genetic counsellor who provides support and guidance on the disease. The counsellor is Lesley's husband, John, who is also affected by the disease. He has been in contact with the family and has been a great support to them.

Facts about MELAS:

- MELAS is a genetic disease that affects the mitochondria, which are the organelles that produce energy in cells.
- It is characterized by a variety of symptoms, including cognitive impairment, seizures, stroke-like episodes, and changes in muscle function.
- The disease is caused by mutations in the DNA that affect the production of energy in cells.
- There is no cure for MELAS, but treatments can help manage symptoms and improve quality of life.

Lesley's family has been through a lot, but they remain strong and supportive of each other. They continue to fight for a better future and hope for a cure for MELAS.
Preventing transmission of mtDNA disease

Genetic counselling

Risk of developing a mitochondrial DNA deletion disorder

Summary
Background Pathogenic mitochondrial DNA (mtDNA) mutations are found in at least one in 8000 individuals. No effective treatment for mtDNA disorders is available, making disease prevention important. Many patients with mtDNA disease harbour a single pathogenic mtDNA deletion, but the risk factors for new cases and disease recurrence are not known.

Methods We did a multicentre study of 226 families in which a single mtDNA deletion had been identified in the proband, including patients with chronic progressive external ophthalmoplegia, Kearns Sayre syndrome, or Pearson's syndrome. We studied the relation between maternal age and the risk of unaffected mothers having an affected child, and determined the recurrence risks among the siblings and offspring of affected individuals.

Findings We noted no relation between maternal age and the risk of unaffected mothers having children with an mtDNA deletion disorder. None of the 251 siblings of the index cases developed clinical features of mtDNA disease. Risk of recurrence among the offspring of affected women was 4.11% (95% CI 0.86-11.54, or one in 117 to one in nine births). Only one of the mothers who had an affected child had a duplication of mtDNA in skeletal muscle.

Interpretation Unlike nuclear chromosomal rearrangements, incidence of mtDNA deletion disorders does not increase with maternal age, and unaffected mothers are unlikely to have more than one affected child. Affected women were previously thought to have a negligible chance of having clinically affected offspring, but the actual risk is, on average, about one in 24 births.

226 families, recurrence ~4%

Lancet 2004;364:592-6
Genetic counselling in families carrying heteroplasmic mtDNA mutations

- I:1: female
- II:1: female (3rd TM, 33 yrs)
- II:2: female (2nd TM, <2hrs)
- II:3: female (27 yrs)
- II:4: female (3rd TM)
- II:5
- II:6: female (31 yrs)
- II:7: male (33 yrs)

Inheritance chart with images of a heart and a brain scan.
Mutation load vs. phenotype in patients
Mutation load vs. phenotype in patients

Chinnery et al. Brain 1998;121:1889-1894
Mitochondrial bottleneck

Mother

mtDNA GENETIC

BOTTLENECK

Offspring
Genetic counselling in families carrying heteroplasmic mtDNA mutations

Of limited use for heteroplasmic mutations
• Variability in symptoms within families

• Correlation between mutant load and disease progression

• High recurrence risk

• Mitochondrial bottleneck

• Genetic counselling problematic
Prevention of Transmission - Genetic testing?

Pre-implantation genetic diagnosis

Pre-natal diagnosis

Pre-symptomatic genetic testing
Prevention of Transmission - PGD

8-cell embryo, on Day 3 after IVF, held in place with holding pipette

Zona drilling pipette is used to drill a hole through the zona

The embryo biopsy pipette removes a single cell with suction

The cell is removed

Genetic analysis performed
Prevention of Transmission - PGD

- Not suitable for homoplasmic disorders
- Suitability for mothers with high mutant load?
- Which stage to sample? (first polar body, blastomere biopsy or blastocyst biopsy)
- Whether/what stage a cut off point should be established?
- Embryo quality versus mutant load
- Gender selection?
PGD- ENMC (2010)

- PGD data on 9 families
  - 5 different mutations
  - Embryo range 0-50%
  - 15 cycles of ovarian stimulation
  - 103 embryos, 7 embryo transfers and 3 live births
  - Heteroplasmy studied in 200 individual blastomeres
  - Agreement to sample 2 blastomeres
  - More data required
PGD- A case study

- PGD data on 1 couple with the MELAS mutation
  - 30yrs old

*JMG 2013;50:125-132*
PGD- A case study

• 2 rounds of IVF-PGD
• 15% threshold chosen
• First round –
  • 9 eggs
  • 1 unfertilised egg load 65.1%
  • 2 fert - arrested 23.3-65%
  • 5 embryos (range 2.3%- 51.3%
  • 1 embryo suitable for transfer 2.3%
  • No pregnancy
PGD- A case study

- Second round –
  - 6 eggs
  - 1 fert egg- arrested 33%
  - 5 embryos (range 21-56%)
  - No embryos suitable for transfer
  - No pregnancy

However in 11% embryos analysed the mutation load varied >15% between blastomeres of same embryo
Prevention of Transmission - Pronuclear Transfer
Pronuclear stage embryo

Just after fertilisation

Male and female pronuclei which contain nuclear genes
Pronuclear stage embryo

Just after fertilisation

Embryo with abnormal mitochondrial DNA

Can we transfer the pronuclei to an embryo with normal mitochondria?
Pronuclear transfer

Fertilisation (IVF) → Pronuclear stage → Enucleation

Mitochondrial Donor Zygote

Karyoplast → Fusion

Mary Herbert Newcastle University, UK
Mitochondrial DNA Disorders

- HFEA granted a research licence to allow the development of techniques for pronuclear transfer in human embryos.
Is it technically feasible?

Abnormal pronuclear embryos

Monopronucleate

Tripronucleate
Analysis of mtDNA

97% donor mtDNA  2.8% recipient mtDNA

Craven et al. Nature 2010 465: 82-85
Is pronuclear transfer compatible with onward development?

- Abnormally fertilised embryos have decreased potential to develop to blastocyst stage (17%)
- ~ 22% zygotes developed to 8 cell stage
  - Similar number if transferred one or two pronuclei
- 8.3% developed to blastocyst stage after transfer of two pronuclei
- Decrease due to absence of either maternal or paternal genome?

Craven et al. Nature 2010 465: 82-85
Positive headlines?

DAILY EXPRESS
Is this a grotesque Frankenstein experiment?
THE BABY WITH TWO MOTHERS
Three-parent babies a step closer after watchdog gives research go-ahead despite 'life meddling' fears

By DAVID DERBYSHIRE
UPDATED: 11:23 GMT, 29 March 2013

Fertility watchdogs yesterday backed a medical breakthrough which leads to babies having three biological parents.

The controversial IVF technique uses DNA from two women and one man to overcome faults which can lead to incurable, deadly genetic illnesses.

Any babies born would inherit two per cent of their DNA from an egg donor with the rest coming from their father and his female partner.
Three-parent IVF babies a step closer

Published: 6:09AM Thursday March 21, 2013 Source: AAP

UK fertility regulators have paved the way for the government to legalise the creation of IVF babies with three genetic parents.

In advice to ministers, the Human Fertilisation and Embryology Authority (HFEA) set out safeguards for controversial mitochondrial replacement techniques that could affect future generations.

But the HFEA did not explicitly argue for a change of the law that would allow children to be conceived with the help of DNA donated by a second "mother".

Instead, it was left to ministers to decide whether they should ask Parliament to consent to the procedures.
Prevention of Transmission – Meiotic spindle transfer

Metaphase II egg
Just before fertilisation

Mito and Tracker
Spindle transfer
Disadvantages of the technology

- Two embryos required for pronuclear transfer
- Egg donors required for Spindle transfer
- Is it ok to mix nuclei and cytoplasm of different individuals?
- Would the child have a genetic connection to all 3 parents?
- Carryover of mitochondria
- Are we harming the embryos with the manipulations?
Summary 3

- Donor eggs suitable for mitochondrial disease
- PGD may be a suitable approach for some families
- Pronuclear and meiotic spindle transfer may prevent transmission in the future
- Ethical consideration?