Mitochondrial Donation to Prevent Transmission of Mitochondrial DNA Disease

Ewelina Musial* , b2011790, evelina_m_@hotmail.co.uk

Dr. Lyndsey Butterworth

Professor Doug Turnbull

Introduction

Pathogenic mutations in mitochondrial DNA (mtDNA) are highly prevalent in the population and can result in a range of disorders affecting both adults and children collectively known as mtDNA disease. The mitochondrial genome is strictly maternally inherited and so a woman with an mtDNA mutation is at significant risk of transmitting this to her children. There are currently no effective treatments for mtDNA disease and so a major priority for many women is to reduce the risk of having a severely affected child. Mitochondrial donation is a novel IVF-based technique that we have developed to prevent transmission of mtDNA disease (Figure 1). Experiments are ongoing to address both the safety and efficacy of the technique before it can be offered as a clinical treatment.

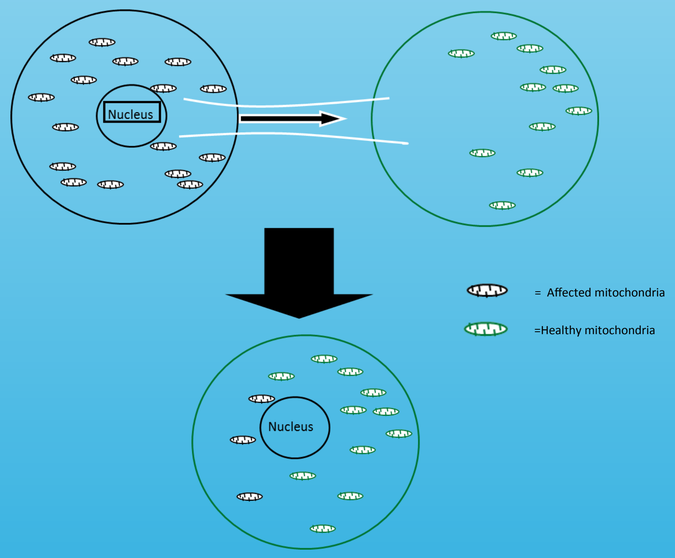


Figure 1 Diagram to show the technique of mitochondrial donation. This involves removing the nuclear DNA from an affected woman's egg (blue cell on the top left) and transferring it to an egg from a healthy donor that has also had its nuclear DNA removed (green cell on the top right). This will result in an egg that contains the nuclear DNA from the parents but normally functioning mitochondria from the donor (reconstituted egg on the bottom with mitochondria from the donor egg and nucleus from the affected egg). The reconstituted egg will potentially contain some mitochondria from the affected egg which has been transferred along with the nucleus . It is important to determine the level of mitochondrial carryover as this must be minimal to reduce the risk of mtDNA disease in the offspring. It may also be necessary to determine the mtDNA sequence of the affected and healthy mitochondria (known as haplotyping) and this will contribute to the safety data.

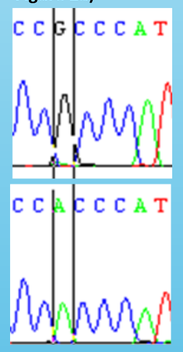
Wellcome trust Centre for Mitochondrial Research
Institute of Neuroscience
Newcastle University

Aims

- To validate a pyrosequencing assay that can be used to investigate the potential carryover of mitochondria following mitochondrial donation
- To sequence specific regions of the mitochondrial genome and identify polymorphisms that can be used to assign haplotypes to the egg donors used in the study

Results

Figure 2a)



2b)

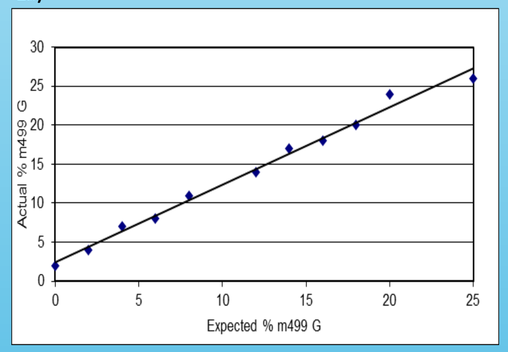
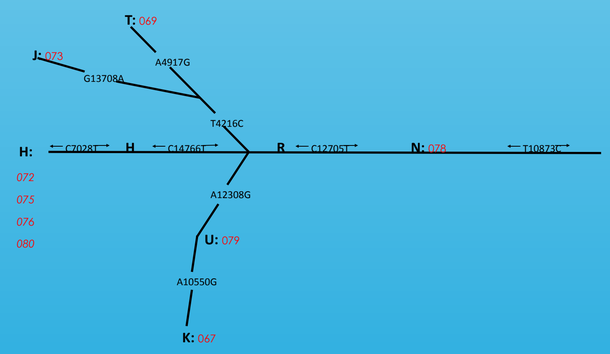


Figure 2a) Sanger sequencing identified a polymorphism at position m499 of the mitochondrial genome that was present in the egg donating the nucleus (m499G) but not in the egg receiving the nucleus (m499A). This polymorphism can be used to determine the amount of mitochondria that has been transferred with the nucleus following mitochondrial donation. **2b)** A pyrosequencing assay was designed to quantitate the level of the m499 G nucleotide. To validate this assay, control samples were generated containing different percentages of the m499 G nucleotide (0-25%). The control samples were amplified using PCR, checked on an agarose gel and then analysed on a pyrosequencer. The graph shows that the expected level of m499 G nucleotide correlates well with the actual level of m499 G nucleotide as determined by pyrosequencing.

Figure 3a)



3b)

PNT	Cytoplast Donor	Karyoplast donor	Haplogroup of cytoplast donor	Haplogroup of karyoplast donor
F3	79	67	U	K
F4	79	67	U	K
F1	81	80	H	H
F2	81	78	H	N

Figure 3a) The flow chart shows the defining polymorphism for different haplogroups. Sanger sequencing was used to identify polymorphisms present in the mitochondrial genome of each egg donor which allowed a haplotype to be assigned. The research code of the egg donor (shown in red) is next to the assigned haplogroup. **3b)** The table shows the combination of haplogroups present in embryos following mitochondrial donation.

Conclusion

- The m499 pyrosequencing assay has been validated and can now be used to determine the level of mitochondrial carryover in embryos following mitochondrial donation.
- Haplotyping revealed that mitochondrial donation embryos may contain mitochondria from the same haplogroup or different haplogroups.