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Clinical and Ethical Implications of Mitochondrial Gene Transfer

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Inherited diseases caused by mitochondrial gene (mtDNA) mutations affect at least 1 in 5,000–10,000 children and are associated with severe clinical symptoms. Novel reproductive techniques designed to replace mutated mtDNA in oocytes or early embryos have been proposed to prevent transmission of disease from parents to their children. Here we review the efficacy and safety of these approaches and their associated ethical and regulatory issues.

Mitochondrial gene disorders

These are distinct genetic diseases attributable to mutations in the cytoplasmic DNA residing in the cell's mitochondria, organelles involved in cellular respiration and energy production. Each cell contains thousands of circular DNA molecules encoding for 37 genes (13 proteins, 22 tRNAs and 2 rRNAs) critical for energy production by oxidative phosphorylation (OXPHOS). The mitochondrial genome is inherited maternally as only the egg's mtDNA is found in offspring. Mutations in mtDNA may arise in oocytes causing maternally inherited mitochondrial disease in children or they may occur in somatic tissues during development and accumulate with aging. Inherited diseases caused by mtDNA mutations were first described in 1988 (Wallace, Singh et al. 1988) and now more than 250 point mutations or large deletions have been identified as causative. The clinical symptoms are diverse but often affect vital organs heavily relying on energy production by OXPHOS such as the brain, heart, kidney liver, pancreas and muscle with the type and severity of disease dependent on the specific mutation type and heteroplasmy level, i.e. the ratio of mutated to healthy mtDNA molecules present in each cell. The transmission of mtDNA mutations that potentially may cause disease has been estimated to be as high as one in every 200 newborns (Elliott, Samuels et al. 2008).

Techniques for mtDNA replacement

A promising recent advancement in assisted reproductive technologies (ARTs) involves the efficient replacement of mutant mtDNA in unfertilized oocytes or zygotes with normal donor mitochondria, thereby allowing women carrying mtDNA mutations to circumvent passage of the condition to their children (Tachibana, Sparman et al. 2009; Craven, Tuppen et al. 2010). Two microsurgical nuclear transfer procedures, termed spindle transfer (ST) and pronuclear transfer (PNT), have been developed. The first approach is conducted at the mature oocyte stage when the nuclear DNA material is assembled into metaphase

chromosomes forming a meiotic spindle (Fig. 1A). The spindle is simply isolated and transplanted into the “empty” cytoplasm of a donated unfertilized oocyte that, itself, has been enucleated. The reconstructed oocyte, now free of mutated mtDNA, can be fertilized and subsequently transplanted to the patient. In modeling the procedure in a nonhuman primate, we have produced several live infants possessing exclusively egg-donor derived mtDNA (Tachibana, Sparman et al. 2009). The postnatal growth and development of these animals has been normal, comparable to control animals produced by conventional ART procedures (Tachibana, Amato et al. 2013). The approach appears clinically relevant since human ST oocytes are capable of high fertilization rates similar to controls. Those abnormalities in pronuclear formation observed in ST oocytes post fertilization can apparently be corrected by simple procedural optimization (Paull, Emmanuele et al. 2013; Tachibana, Amato et al. 2013). Preimplantation development to blastocysts and embryonic stem cell (ESC) isolation rates in human ST embryos were also comparable to controls. Finally, comprehensive genetic and cytogenetic analyses of human ESCs provided assurance that ST procedures are not associated with chromosomal abnormalities.

Alternatively, mtDNA replacement can be carried out by PNT, conducted at the one-celled embryo or zygote stage when pronuclei are visibly prominent (Fig. 1B). PNT was initially accomplished in a mouse model (McGrath and Solter 1983) including proof-of-concept that PNT is effective in preventing transmission of mtDNA mutations (Sato, Kono et al. 2005). The feasibility of PNT has also been tested with abnormally fertilized human zygotes where manipulated embryos were compatible with onward development to blastocysts (Craven, Tuppen et al. 2010).

Safety and efficacy of mtDNA replacement

The safety and efficacy of the ARTs has been established over many years of clinical application without much attention or dependency on animal based research, let alone controlled clinical trials. Clearly, establishing safety and efficacy for any new technology, especially when involving germline gene therapy, represents a major challenge. Several relevant observations are cited below.

In the case of mitochondrial gene replacement, the possibility that the procedure might fail secondary to the co-transfer of mutated mtDNA bound to pronuclei or spindles during nuclear transfer has been evaluated in ST monkey offspring, human ST ESCs and human embryos post PNT (Tachibana, Sparman et al. 2009; Craven, Tuppen et al. 2010; Tachibana, Amato et al. 2013). The mtDNA carryover was either technically undetectable or below 2% and since most mtDNA diseases clinically manifest only when the mutated mtDNA threshold is 60% or higher, it is unlikely that low levels would cause disease in children. Safety concerns have also been raised that children born after mitochondrial DNA replacement may still display abnormalities due to incompatibilities between “unmatched” nuclear and mitochondria genomes. To address these questions, rhesus macaque ST offspring were generated by combining distant nuclear and mtDNA haplotypes. Long-term studies failed to define any abnormalities in health or development (Tachibana, Amato et al. 2013).

In an effort to evaluate safety and efficacy of mtDNA transfer, the Human Fertilisation and Embryology Authority (HFEA) in England, has launched a scientific review of published results (<http://www.hfea.gov.uk/6372.html>) concluding that both ST and PNT are potentially useful for a specific and defined group of patients whose offspring may have severe or lethal mtDNA disease, and who have no other option of having their own genetic child. The panel also concluded that the currently available evidence does not suggest that the techniques are unsafe. However, the first clinical applications should be conducted at specialized mitochondrial disease clinics involving clinical trials. Families enrolling in such trials should be offered full information and support and their participation in the trials should be contingent on consent for follow up observations of children.

Regulatory, legal and ethical questions

The techniques of ST and PNT described herein induce permanent changes to mtDNA that would be transmitted through generations, thus qualifying as germline gene therapy. As such, in the USA, reproductive applications of mtDNA transfer fall under the jurisdiction of the Food and Drug Administration (FDA) requiring that first applications for patient treatment must be done as part of clinical trials. In the UK, current regulations set by the Human Fertilisation and Embryology Act (HFEA) 1990 “only permit eggs and embryos that have not had their nuclear or mitochondrial DNA altered to be used for treatment”. However, the Act allows for additional regulations to be passed by Parliament that would allow DNA modifications of an egg or embryo to prevent the transmission of serious mitochondrial disease. To pass these regulations, the scientific procedures involved should be considered effective and safe, an ongoing process described above.

Ethical issues concerning mtDNA transfer have also been extensively examined by the Nuffield Council on Bioethics in the UK and summarized in a report concluding that “Due to the health and social benefits to individuals and families of living free from mitochondrial disorders, ... we believe that if these novel techniques are adequately proven to be acceptably safe and effective as treatments, it would be ethical for families to use them” (<http://www.nuffieldbioethics.org/mitochondrial-dna-disorders>).

Several other ethical and legal issues were also highlighted including the fact that children born after such therapies would have a genetic connection to three parents: mother, father and the mtDNA donor. However, it would be misleading to describe children born following ST or PNT as having biologically or legally, “three parents” or “two mothers”. Indeed, the genetic contribution from the mtDNA donor is small constituting just 0.1% of the total DNA. Moreover, the sequence variation between different mitochondrial haplotypes in the human population is small translating to just a few amino acid substitutions. In addition, it is possible to further reduce mtDNA differences by using mitochondrial donors from the same haplotype.

The report recommended wider discussions on the ethics of future germline gene therapies that involve the nuclear genome. Similar to mtDNA, many nuclear gene defects cause severely debilitating and life-threatening conditions in children and it might be considered

unethical to deny germline gene therapies for these nuclear DNA diseases if concerns about safety and efficacy were addressed adequately.

In summary, reproductive options involving mutant mtDNA replacement therapy have been described that have the potential to prevent mtDNA disease transmission. Replacing mutated mtDNA by ST or PNT does not involve risky genome modifications with recombinant DNA vectors and has relevance to all mtDNA mutation types. The current challenge is to chart a course that translates these preclinical and clinical studies into clinical trials evaluating efficacy and safety in affected couples. It is also important to continue ethical and regulatory discussions related not only to germline mtDNA replacement but to germline gene therapy in general.

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Figure 1. Mitochondrial genome replacement techniques