Preventing transmission of maternally inherited mitochondrial DNA diseases

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Maternally inherited mutations of mitochondrial DNA can be asymptomatic or cause illnesses such as developmental regression, deafness, blindness, neuropathy, diabetes, cardiomyopathy, and liver failure. Families who have lost a child from a mitochondrial DNA disease (fig 1) often seek genetic counselling before trying to conceive again. They may also need support from their general practitioner when deciding between treatments that could increase their chances of having a healthy baby, but some doctors have limited knowledge of current genetic advances. In February 2008, an important step towards nuclear transfer in humans was reported.1 The nucleus of a fertilised human egg was transplanted into an enucleated recipient egg (oocyte) from an unrelated donor, resulting in an embryo with “three genetic parents.” In future, this technique might help to prevent transmission of maternally inherited mitochondrial DNA diseases, a range of potentially devastating illnesses caused by defects in mitochondrial function. Meanwhile, other in vitro fertilisation related interventions have recently become available that should be offered more widely. Here, we explain why the management of mitochondrial DNA diseases has lagged behind the genetics revolution, and how current advances may now lend hope to affected families.

How common are maternally inherited mitochondrial DNA diseases?

Population screening shows that about one in 400 people has a maternally inherited pathogenic mitochondrial DNA mutation,1 which may cause mitochondria to function abnormally. Because mitochondria generate energy, tissues that use the most energy—such as muscle, liver, and nerve—are particularly susceptible. Mitochondrial DNA mutations can give rise at any age to a range of severe to mild illnesses, including deafness, diabetes, and neuropathy. Affected children may develop liver failure in the neonatal period or die in infancy with developmental regression, lactic acidosis, and cardiomyopathy (fig 1). Case-control studies have implicated severe mitochondrial DNA mutations in 10% of type 2 diabetes2 and 17% of cases of idiopathic dilated cardiomyopathy,3 the major cause of heart transplantation in young people. A less common mitochondrial DNA disease is Leber’s optic atrophy—affected people become blind in one eye over days, weeks, or months, and then blind in the other eye usually within six months. Hence, adults and children with mitochondrial DNA diseases are seen by a wide range of hospital specialists including diabetologists, cardiologists, neurologists, ophthalmologists, paediatricians, hepatologists, and obstetricians.

How is mitochondrial DNA disease inherited and can inheritance be predicted?

Mitochondrial DNA is maternally inherited in humans because mitochondria are located in the cell cytoplasm and the sperm contributes almost no cytoplasm to the fertilised egg. Thousands of copies of mitochondrial DNA are present in every nucleated cell, and in normal people virtually all copies of their mitochondrial DNA are identical. However, in many mitochondrial DNA diseases, both normal and pathogenic, mutant mitochondrial DNA is present in every nucleated cell, and in normal people virtually all copies of their mitochondrial DNA are identical. Hence, adults and children with mitochondrial DNA diseases are seen by a wide range of hospital specialists including diabetologists, cardiologists, neurologists, ophthalmologists, paediatricians, hepatologists, and obstetricians.

METHODS

We used our personal archive of references, Medline searches, and consultation with other experts in the field to produce this review. It is derived from 22 years of research and 11 years of genetic counselling in mitochondrial DNA diseases.
How can mitochondrial DNA disease be predicted or prevented?

Four reproductive approaches are currently available—oocyte donation combined with in vitro fertilisation; oocyte sampling to estimate recurrence risk; chorionic villus sampling to genotype the fetus; and preimplantation genetic diagnosis of embryos produced by in vitro fertilisation so that those with undetectable or low amounts of mutant mitochondrial DNA can be selected for transfer to the uterus. In addition, nuclear transfer may be available in future (fig 2). Although all these approaches can have ethical and practical problems, doctors and patients should be aware of the range of possible new reproductive interventions and their potential benefits.

Oocyte donation

In this approach, the partner’s sperm is used for in vitro fertilisation of donated oocytes and the resultant embryo is transferred into the affected woman’s uterus. Because most mitochondrial DNA diseases are maternally inherited, oocyte donation should virtually eliminate the risk of recurrence. However, some couples find oocyte donation unacceptable, and oocyte donors are in short supply. Maternal inheritance rules naturally will contain a low or undetectable amount of mutant mitochondrial DNA. If the risk of recurrence is estimated to be low (below 5%) the couple may decide to conceive again naturally. The disadvantages of oocyte sampling are that patients must undergo the stress of ovarian stimulation with gonadotrophins (as used for in vitro fertilisation). Each oocyte is assessed for mutant mitochondrial DNA load to see what proportion of oocytes contains dangerously high amounts. If the risk of recurrence is estimated to be low (below 5%) the couple may decide to conceive again naturally. The disadvantages of oocyte sampling are that patients must undergo the stress of ovarian stimulation. Secondly, a significant minority of women produce a small number of oocytes, which precludes a meaningful statistical evaluation of risk of recurrence. Finally, the oocytes retrieved are destroyed by testing and cannot be used to establish a pregnancy. Consequently, although the risk of recurrence can be estimated in most cases, there is no guarantee that an oocyte from a woman who subsequently conceives naturally will contain a low or undetectable amount of mutant mitochondrial DNA.

Oocyte sampling to predict risk of recurrence

This strategy enables women with an affected child to assess the risk of recurrence. It can be used if the load of mutant mitochondrial DNA correlates well with disease severity. Multiple oocytes are retrieved after a single cycle of ovarian stimulation with gonadotrophins (as used for in vitro fertilisation). Each oocyte is assessed for mutant mitochondrial DNA load to see how can mitochondrial DNA disease be predicted or prevented?

A PATIENT’S PERSPECTIVE

Angus was a gorgeous child who was loved by everyone he met. When he was 18 months old and I was six weeks pregnant he was diagnosed with a mitochondrial disease (9176 T→C). When we searched the internet we found a terrifying array of possible symptoms. Angus deteriorated at 2 years when he developed a high temperature. He lost all his skills, including control of his eyes. He deteriorated again with a second infection when he was 3½ years old. After seven weeks in hospital we decided to turn off the respirator.

His death left a terrible hole in our family, but we kept going for Holly who was 14 months old. We desperately wanted to have another child but felt we couldn’t take the risk of having one with mitochondrial disease.

The laboratory in Oxford sampled my eggs to estimate the risk. By checking a chorionic villus sample when I was pregnant they were able to tell us that the baby would not have mitochondrial disease. Having Max (now 5) has helped us heal. He is a robust and happy little boy; it is a joy just to watch him running around, reassured that he will never have mitochondrial disease.

Julia McLellan, mother of Angus (fig 1), Lechlade

Chorionic villus sampling

Prenatal diagnosis using chorionic villus sampling is available for mitochondrial diseases caused by mutations in nuclear genes that are inherited as regular dominant or recessive traits. However, chorionic villus sampling is not widely used for maternally inherited mitochondrial DNA diseases because the clinical severity of the disease in the child cannot be reliably predicted. This is partly because the proportion of mutant mitochondrial DNA varies in different cells and tissues. High or low amounts of mutant mitochondrial DNA in the chorionic villi indicate that the fetus is at high or low risk. An intermediate value is
unhelpful, however, because it is difficult to estimate how the mutant mitochondrial DNA load will change over time.\(^7\)\(^8\) This makes it difficult to decide whether to continue or terminate the pregnancy. Thus, chorionic villus sampling is generally used only for maternally transmitted mitochondrial DNA diseases in which mutant mitochondrial DNA dose and disease severity correlate well. The exceptions are conceptions after oocyte sampling or preimplantation genetic diagnosis. Here, chorionic villus sampling is used to confirm that the fetus is unaffected or that the negligible or low mutant mitochondrial DNA values seen in the preimplantation embryo have not increased.

**Preimplantation genetic diagnosis**

Preimplantation genetic diagnosis (fig 3) is the best management option at present for many mitochondrial DNA diseases.\(^22\) Using this approach, multiple embryos produced by in vitro fertilisation are analysed, and only those with low or undetectable amounts of mutant mitochondrial DNA are transferred to the uterus. This approach cannot guarantee that a fetus or child will be unaffected, but it dramatically improves the probability of an unaffected pregnancy. Although the “take home baby” rate after preimplantation genetic diagnosis is lower than for natural conception,\(^25\) pregnancy rates are improving, and some centres now report pregnancies in more than 45% of cycles.\(^27\) In addition, recent research indicates that, for some mitochondrial DNA mutations, amounts of mutant mitochondrial DNA are on average lower in succeeding generations,\(^28\)\(^29\) so that children may be

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**Fig 2** Three possible ways to reduce the risk of transmitting mitochondrial DNA disease: oocyte donation, preimplantation genetic diagnosis, and nuclear transfer. Red represents mutant mitochondrial DNA, pink and white represent successively higher proportions of normal mitochondrial DNA. Blue represents genetic material from an unrelated donor. (A) No intervention: offspring’s mutant mitochondrial DNA load will vary greatly. (B) Oocyte donation: currently available in the United Kingdom but limited by the availability of oocyte donors. (C) Preimplantation genetic diagnosis: will soon be available in the UK for some mitochondrial DNA diseases. (D) Nuclear transfer: being developed but not yet available.
QUESTIONs FOR FUTURE RESEARCH

What is the minimum size of chorionic villus sampling or minimum number of blastomeres in preimplantation genetic diagnosis needed for accurate prenatal diagnosis?

At what stages of oocyte development is the load of mutant mitochondrial DNA determined for an individual (the mitochondrial bottleneck)?

How many mitochondrial DNA molecules are there per cell at each stage of human oocyte development? How are these organised and does their organisation provide the physical basis for the bottleneck?

Could nuclear transfer become a viable alternative to standard genetic management?

Dolly the cloned sheep was created by transferring an adult somatic cell nucleus into an enucleated recipient oocyte, and her mitochondrial DNA was inherited from the recipient enucleated oocyte and not the somatic cell. This implied that nuclear transfer could be used to manipulate the mitochondrial content of a human embryo. One step in this direction was recently taken in humans.1 The nucleus (containing both maternal and paternal DNA with a few adherent mitochondria) from a severely abnormal human embryo was transplanted into a donated oocyte with its nuclear DNA removed. The resulting embryo developed normally for the first six days, at which time it had to be discarded for legal reasons.

Will nuclear transfer be a viable alternative to standard genetic management? It is a simple technique that seems to be effective in mice.31 Donated mitochondria injected into human embryos might improve fetal viability, but there are many unknowns. Firstly, a single cell embryo is derived from the mother’s oocyte fertilised with the father’s sperm. Before the cell divides, adherent mitochondria are stripped from the nucleus by treating them with drugs such as cytochalasin D, which are potentially toxic.33 Despite this treatment, some mutant mitochondrial DNA that is carried over with the nucleus may increase to clinically relevant amounts.32 Secondly, because mitochondrial DNA is compact, the nucleus encodes most mitochondrial proteins, and many important interactions occur between the nucleus and the mitochondria. In embryos derived by nuclear transfer, the DNA originates from three unrelated parents—two provide the nucleus from the fertilised egg and one the mitochondrial DNA in the oocyte. Subtle differences in maturation between the transferred nucleus and the recipient enucleated oocyte could lead to incompatibility and miscommunication. These complex nucleo-mitochondrial interactions, the subtle effects of which might have been overlooked in the mouse experiment,32 have not yet been fully investigated in humans. Other challenges also exist. Nuclear transfer is inefficient—more than 100 oocytes may be needed to create one baby, but oocyte donors are scarce and it is unclear how such large numbers of eggs could be obtained. The efficiency of nuclear transfer will need to improve considerably before this approach becomes practical. The long term effect of nuclear transfer on patterns of gene expression that influence inherited characteristics (for example, so called “genomic imprinting”32) is also a concern. Finally, preimplantation genetic diagnosis may still be needed after nuclear transfer to check that all nuclear material has been removed from the cytoplasm of the enucleated donated oocyte.

Conclusion

Reducing the transmission of mitochondrial DNA diseases has met with many ethical,23 scientific, and pragmatic problems. New approaches are being developed, however, that could help affected families increase their chances of having healthy children.

ADDITIONAL EDUCATIONAL RESOURCES

Resources for healthcare professionals

Dubowitz Neuromuscular Centre (www.ich.ucl.ac.uk/gosh/clinicalservices/neuromuscular_services/Homepage)—Major London based diagnostic service for paediatric muscle disease

Clinical Molecular Genetics Society (http://cmgsweb.shared.hosting.zen.co.uk/BPGs/Best_Practice_Guidelines.htm)—Guidelines on molecular approaches to diagnosing mitochondrial disease

UK Genetic Testing Network (www.ukgtn.nhs.uk/gtn/Search/Search_by_Disease)—Website listing the availability of mitochondrial DNA tests in regional laboratories

Neuromuscular (http://neuromuscular.wustl.edu/mitosyn.html)—Basic background information on mitochondrial disease

Nijmegen Centre for Mitochondrial Diseases (http://ncmd.ruhosting.nl/)—European based diagnostic laboratory specialising in complex1 deficiency

Resources for patients

United Mitochondrial Disease Foundation (www.umdf.org/site/c.dnjEKLQhFoG/b.3042169/)—US based patient information website

National Information Centre for Metabolic Diseases (www.climb.org.uk/)—Website of a patient organisation called CLIMB (Children Living with Inherited Metabolic Diseases)

Mitochondrial Diseases (www.kathleensworld.com/mitochon.htm)—One family’s story

Orphanet (www.orpha.net/consor/cgi-bin/index.php?lng=EN)—Covers a wide range of metabolic diseases and provides information for families and professionals internationally. It includes contact details for patient organisations for different metabolic diseases by country

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SUMMARY POINTS

About 1 in 400 people have a maternally inherited pathogenic mutation of mitochondrial DNA
Mutations may be asymptomatic or cause illnesses such as developmental regression, deafness, blindness, neuropathy, diabetes, cardiomyopathy, and liver failure
Patients may present at any age
Families with affected children often seek genetic counselling
Risk of recurrence is difficult to estimate because both mutant and normal mitochondrial DNA is present
New approaches using preimplantation genetic diagnosis, oocyte donation, or oocyte sampling may now give hope to affected families

Although nuclear transfer may be promising for the future, oocyte donation, oocyte sampling34 and preimplantation genetic diagnosis92—backed by chorionic villus sampling21—are available today.

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Patient consent obtained.
Oxford currently offers oocyte donation and oocyte sampling for mitochondrial DNA diseases. Preimplantation genetic diagnosis will be available in the near future. JP takes clinical and diagnostic referrals for the Oxford centre in the Rare Mitochondrial Disorders Service for Adults and Children (National Commissioning Group). A referral form is available from her website (www.obs-gyn.ox.ac.uk/research/Poulton).