SUMMARY - Mitochondrial Replacement Therapy
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Mitochondrial Replacement Therapy (MRT) is a game changer for the mitochondrial disease community. Although not yet available in the United States, MRT will, in time, offer a great reproductive option to women carrying mitochondrial gene mutations (slide 1). Financial disclosures are reviewed on slide 2, but none have any bearing on MRT or this discussion.

Learning Objectives (slide 3)

- To be familiar with the rules of mitochondrial DNA (mtDNA) genetics.
- To recognize that mitochondrial DNA diseases are clinically important.
- To be aware of the reproductive options of women who carry mitochondrial DNA mutations.
- To appreciate the principles of mitochondrial replacement technique, or therapy, that may be used to prevent the transmission of mitochondrial DNA diseases.
- To understand the current status of the clinical use of MRT in United Kingdom and the United States.

Mitochondria are tiny organelles within nearly every cell of our body. Originally arising from bacteria, mitochondria carried the capacity to make ATP through the process known as oxidative phosphorylation (slide 4). The enzyme complexes embedded in the yellow bar are enzymes that are important in transferring electrons that are used to drive ATP synthesis. The fuels for the ATP synthesis are the fats and sugars consumed in the diet. That bagel and cream cheese ingested this morning, for example, is being digested and processed by our mitochondria to make ATP energy. The mitochondria have their own DNA, derived from bacterial DNA (red circle in this figure). The mitochondrial DNA is critical for these enzyme complexes to function. The pink rectangles in the figure are the protein components that are encoded by the mitochondrial DNA. When the mitochondrial DNA is mutated, those pink rectangles will be affected and will diminish the capacity to make ATP, the energy needed by the cell.

Mitochondrial DNA is a small circular molecule about 16,500 bases in length, which is tiny compared to the nuclear DNA, which has 3 billion pieces of DNA information (slide 5). MtDNA:

- are critical for energy production;
- are very compact as virtually the whole circle is enclosed;
- forms structures required by the mitochondria.

Mitochondrial functions are affected when there are mutations in any part of the circular mtDNA molecule...

Mitochondrial DNA behaves very differently from the nuclear DNA (slide 6).

- Maternal Inheritance -- mtDNA is exclusively transmitted from mothers to their children, which is true for most organisms. There are few organisms where the father actually transmits mtDNA, namely redwood trees and some mussels, but virtually every other organism has maternal inheritance of mitochondrial DNA. The eggs of women transmit the mitochondrial DNA and when the egg has a mutation, that mutation can be transmitted to future generations.
- Heteroplasmy -- mitochondrial DNA is present in hundreds and thousands of copies per each cell, which is very different from nuclear DNA, which has only two copies per adult cell. Having many copies of mitochondrial DNA can be good because a little tolerance of that mutation is created. For example, humans can tolerate a low-level mutation in the background, but if that mutation expands to a very high-level, generally over 70%, diseases can surface, such as MELAS, MERRF, and Kearns-Sayre syndrome.
- Threshold Effect -- level of mutation, usually 70% or more, needed to cause the disease.
- Mitotic Segregation through mitosis -- the mutation can be split into different cells at different levels, and the division of cells allows that the mutation may be segregated in different levels at different parts of the body.

Mitochondria support many parts of the body (slide 7), but when defective, many organ systems may be affected and symptomatic, especially the brain and muscle, which both require a lot of energy. The heart may be affected as it is a highly energy dependent organ. Eye muscles are full of mitochondria and are frequently affected in mitochondrial disease with symptoms including ptosis (droopy eyelids), blindness, optic nerve atrophy, ophthalmoplegia (inability to move the eye), and more. The liver, the kidneys, and hearing are also frequently affected. A diversity of organs can be affected by mitochondrial diseases, and there are many types of mitochondrial disease.

MELAS -- Mitochondrial Encephalopathy, Lactic Acidosis, Stroke-Like Episodes is one of the more common mitochondrial diseases (slide 8). MELAS often presents with devastating strokes as shown in the MRI image of the brain with the right part of the brain affected by a stroke. MELAS patients have atypical strokes because they often affect young people and children who generally do not get strokes. This type of stroke is not due to clots in the blood vessels (vascular stroke) but is due to mitochondrial dysfunction, although the exact mechanism remains unknown.

Leigh Syndrome -- Subacute necrotizing encephalopathy affects the basal ganglia, brainstem, and spares the mammillary bodies (slide 9). The MRI image is the brain of a child with Leigh Syndrome, the most common presentation of mitochondrial disease in young children. Leigh Syndrome is a devastating brain disease. These kids start out normal in the first couple months of life, but then begin to lose developmental

milestones or stop reaching new milestones. They lose the ability to walk or to speak and progressively get worse throughout early childhood. This disease is often fatal in early childhood. Lesions in the basal ganglia in an MRI are the hallmark of this disease. Leigh's has many genetic causes, including many mitochondrial DNA mutations and sometimes maternally inherited through the mitochondrial DNA mutation.

NARP -- Neuropathy Ataxia Retinitis Pigmentosa (RP) (slide 10) is a disease where the eye and vision are affected (RP) in addition to peripheral neuropathy (numbness and tingling of the hands and feet), cerebral ataxia, and lactic acidosis. The images of the back of the eye were taken 12 years apart. The back of the eye on the left side of the slide has a yellow-orange appearance, which is a normal color, with little black specks. The right panel shows the black specks getting more prominent due to the degeneration of the back of the eyeball (RP). NARP and Leigh's can affect many people in a family. The same family can have both diseases because some who have the milder disease, which is still pretty severe NARP disease, have 70% to 90% mutation, and the family members with over 90% mutation have severe Leigh Syndrome. So the level of mutations, the heteroplasmy, is very important in determining whether a patient has the disease and also the severity of the disease. There may be people in the family who have less than 70% heteroplasmy and may have no symptoms at all.

Mitochondrial Morbidity Map -- Slide 11 shows the mitochondrial circle with various shapes around the circle depicting the sites of different mitochondrial DNA mutations. More than 270 different mitochondrial point DNA mutations have been identified as well as many deletion mutations. In general, the point mutations are maternally transmitted and the deletions are sporadic and generally not transmitted for reasons that are not fully understand. The point mutations are important for this discussion because those mutations are the ones women are going to carry and transmit to their children.

Papers from the North East England's New Castle Group, who have been doing epidemiological studies, show that mitochondrial diseases, although rare, are not very rare (slide 12). One in 5000 people are symptomatic of mitochondrial DNA mutation, meaning that there are over 60,000 people who are probably affected by mitochondrial disease in the USA. The number of people who carry these mitochondrial DNA mutations is even greater with 1 in 200 people carrying a pathogenic (disease causing) mitochondrial DNA mutation.

Genetic counselors often use pedigree drawings similar to the one found on slide 13 (males = squares; females = circles). The black circle represents a girl who has MELAS. She presented with muscle disease, myopathy, difficulty climbing stairs at 5 years of age and then developed stroke-like episodes, cardiomyopathy and, unfortunately, died at age 10 of severe heart disease. That line through her circle indicates that she passed away. Her brother (gray square) was severely affected with mental retardation and some muscle disease but was not as severe as his sister. The mother, who carries the mutation and transmitted it, is asymptomatic. Even though she is ab obligate carrier and transmitted the mutation, she has shown no signs of that mutation. The mom's brother, on the other hand, has some symptoms, such as droopy eyelids and an inability to

move his eyes. In this family, three people are affected and a few others, including the mother and the maternal grandmother, are not affected, yet do carry the mutation.

In slide 14, people who carry the mutation are in the black squares and circles along with the level of the mutation in the muscle (M) and blood (B). The child who is most severely affected has the highest level of mutation, over 90% in muscle and over 80% in blood. Other family members who carry the mutation in variable degrees and the mother, who is not affected, all have lower levels than the affected child. The heteroplasmy makes a huge difference and shifts from one generation to the next. Mitochondrial segregation goes through a genetic bottleneck during germline development, meaning there is a limited number of mitochondrial DNA molecules that are transmitted (slide 15). When unlucky, a large proportion of the mutation is passed to the child, causing severe disease. Imagine a jar full of 500 black and 500 white marbles, but there is a bottleneck and so only 10 marbles are randomly passed to the child. If 9 out of the marbles are black (mutated mitochondrial DNA), the child will end up with severe Leigh Syndrome. If lucky and 9 of the 10 marbles are white, then the child will be OK. Mitochondrial DNA segregate through different parts of the body, which is called a somatic bottleneck, so different parts of the body may end up with different percentages of mitochondrial mutations. Mitochondrial segregation may be a little technical, but leads to the issue at hand: Mitochondrial Replacement Therapy.

Reproductive options for women who carry mtDNA point mutations

- Normal reproduction -- carries the risk of transmitting the mutation to each child with no way of predicting reliably what the mutation level will be in that child.
- Opt not to have children at all.
- Adopt children to avoid the risk of transmitting the DNA mutation.
- IVF with donor eggs from women who do not carry mitochondrial DNA mutations.
- Preimplantation genetic diagnosis (PGD) -- in vitro fertilization, having an embryo or multiple embryos, ideally and biopsying those embryos to see what the level of mutation in that embryo. PGD limitations:
    - No control over the amount of mutated mitochondrial DNA passed to an embryo -- it's simply luck of the draw;
    - After an egg is fertilized, many cells develop as it develops from the blastomere to the blastocyst. When testing the mass of cells for mutation, only one or two cells are sampled, but that mass of cells may have variable levels of mutations in different cells as shown on slide 17. The red cells have high levels of mutations and the green cells have low levels of mutations. When biopsying the cell mass and red cells happen to be chosen, the team may be led to think that the embryo will have a high level of mutation even though it is a mixture. Conversely, if a green cell is chosen, the team may be misled into thinking the embryo has a low level of mutation, which can prove inaccurate.

Mitochondrial Replacement Therapy (MRT)
This technique is referred to by different names, such as three-person IVF, for example (slide 18). Dr. Hirano prefers the term mitochondrial replacement therapy because the process replaces mutated mitochondria with mitochondria from a donor. MRT is analogous to an organ transplant, but instead of an organ being transplanted, an organelle, mitochondria, is transplanted. Hirano finds the name "three-parent IVF/embryo" catchy, but does not think the mitochondrial DNA donor should qualify as a "parent."

- Pronuclear Transfer is the process where IVF is performed, the egg is fertilized, and the nucleus is transferred from one embryo into the cytoplasm of another embryo that lacks the mitochondrial DNA mutation and the embryo is allowed to grow and then implanted.
- Chromosomal Spindle Transfer involves harvesting the egg through in vitro fertilization, the nucleus is plucked out of the cytoplasm containing the mutated mitochondrial DNA, and the nucleus is transferred into the cytoplasm of a donor egg and the IVF is performed. This method is also known as MRT at the oocyte level.
- Pronucleus Transfer involves removing the small pronucleus (which contains nuclear DNA) during the during the division of the egg and putting it in the cytoplasm of a healthy donor egg and then do IVF.

The three methods described all use cytoplasm from a donor woman who does not carry a mitochondrial DNA mutation, basically separating the nucleus from the mutated mitochondrial DNA and putting that nucleus into the cytoplasm that has normal, healthy mitochondrial DNA. Mouse and monkey models have demonstrated successful nuclear transfer in preventing transfer of mitochondrial DNA from the female mouse to its progeny (slides 19-22). Rhesus monkeys do not have mitochondrial DNA mutations but different subspecies of rhesus monkeys have different mitochondrial DNA, so chromosomal spindle transfer was used to produce these young monkeys in 2009. The monkeys, Mito and Tracker, are doing well to this day with no sign of any damage from this manipulation.

When working with colleges at Columbia on nuclear transfer with the oocytes and skin cells, occasionally, maybe 1 out of 8 times, the mitochondrial DNA that was transferred with the nucleus expanded. That transfer and subsequent expansion would not be good when working with oocytes to prevent transmission of a mitochondrial DNA mutation. If the nucleus that is transferred to a healthy egg cytoplasm carries a little bit of mutated mitochondrial DNA (genetic drift), and that mutated mitochondrial DNA expands, disease could develop (slide 23). This flaw in the technology needs further investigation to determine whether this might happen before MRT is used in people.

MRT -- How many women could benefit?
The Mito group in Newcastle, England, determined the number of women who might benefit from this mitochondrial replacement therapy in both the UK and the USA. They estimate that in the Us there are about 12,000 women of childbearing age who carry

mitochondrial DNA mutations, and each year almost 800 women may give birth and potentially transmit their mitochondrial DNA disease to their children (slides 24-25). This research indicates that a lot of women don't even know that they carry the mutation. A lot of women find out, or suspect that they carry the mutation, after someone in their maternal family lines has the mitochondrial mutation. Those women are called "obligant" carriers where they probably do carry, but do not have genetic testing. Since the development of MRT techniques requires time and money, determining the support from the Mito community as well as from healthy donors is important. The team created a survey, and gives thanks to MitoAction for helping to get MitoAction members to complete surveys: 92 surveys were returned from women 18 and older who were either known carriers of mutations in the mitochondrial DNA or obligate carriers.

- Among the group of completed surveys:
- 13 mitochondrial gene mutations were represented; the MELAS mutation was most common.
- 100% knew that they had the possibility of passing on mitochondrial DNA mutation to their offspring.
- 78% of the women of childbearing age had thought about not having children because of this risk of transmitting the mutation.
- 73% of the women who had children prior to knowing they carried the risk would have thought about not having children if they had known of the risk.
- Several women, who were beyond childbearing ages, and who had already had their kids, said had they known that would have been very important for them to think about not having children.
- 95% that the development of MRTs was an important and worthwhile project.
- The results were as expected, but the very high numbers confirmed our feelings regarding the level of concern in the mitochondrial community for transmitting these mutations, not only if the mother is healthy, but also if she has had some symptoms.
- Twenty-one women who were considering having children either currently or in the near future were asked whether or not having biological offspring (using own nuclear DNA) was important to them.
- 95% said Somewhat Important or Very Important
- 5% said Not Important
- 90% of those 21 women were interested in using MRT to have a child
- 78% were interested in allowing their eggs to be used for basic laboratory research in the process of developing an implantable zygote.

Women (112) from the local IVF clinic were also surveyed with 87% willing to donate to MRT if a viable embryo were going to be created. Again, very positive among the mitochondrial community, but also within the oocyte donors. This work is published in

the Journal of Human Reproduction in 2016 (slide 26).

In the UK, IVF and related issues and techniques are regulated by the government, and the British government has approved the use of MRT (pronuclear transfer) for severe mitochondrial DNA diseases. At least one center in Newcastle is using MRT to prevent mitochondrial disease. MRT is heavily regulated by the Human Fertilization and Embryology Authority (slides 27 -28). MRT is not yet approved in many other countries and, in the US, research continues. The FDA asked Institute of Medicine to explore the ethical and social issues of MRT, and the Institute of Medicine, a very prestigious organization, concluded that it would have to be permissible to conduct a clinical investigation of MRT to ensure ethical handling of specimens. Certain principles and conditions must be met before approval. At this point, the FDA is prohibited from allowing MRT to be used clinically as legislated by the US government.

A child was born in Mexico after MRT from a woman who had four miscarriages and two children who died of Leigh Syndrome due to mitochondrial DNA mutation (slide 29). IVF experts from New York, not from Columbia, went to Mexico and performed the MRT technique using oocytes. The woman gave birth to the boy in April of last year, and apparently the boy has a very low level of mutation, and at 3 months of age, he is healthy. After that event, the Mexican government has forbidden further MRT procedures. The only country in the moment that is using MRT is the UK.

Research Participation

Columbia University's IRB (Institutional Review Board) approved a protocol to actually do the MRT technique. The Institutional Review Board is allowing work on MRT because embryos are not being created purely for research purposes but it may be applied clinically. Mothers with mitochondrial DNA mutations and healthy donor eggs from an IVF clinic are undergoing MRT. The nucleus of the egg from a Mito carrier is implanted into the healthy egg of the donor with the nucleus removed. After switching the nucleus from the Mito carrier to the healthy donor egg, the egg is fertilized and, someday, the team hopes to get approval to implant this embryo. The embryos are biopsied to ensure the levels of mutation are low and that the nuclear DNA is intact and normal. This step will make sure that the procedure itself doesn't cause any harm to the embryo and is a necessary step to show the FDA that MRT can be done safely and efficaciously. The fertilized eggs created through MRT are stored in a freezer to be used at a later date, like in a regular IVF clinic. The technique of MRT is conducted in the INF clinic lab that has been using IVF techniques for over 15-20 years. The only new step is the transfer of the nucleus or the spindle from Mito carriers to a healthy oocyte egg. This study is currently offering women, 22-40 years of age, the possibility of having some eggs created using MRT (slide 30)

Inclusion Criteria:

- Known carrier of mitochondrial DNA mutations causing significant disease (a woman wouldn't go through MRT if her mutation did not cause much of a health issue)

- Maternal relatives with disease -- Obligate carriers can get assistance with genetic testing
- Male Partner or Sperm Donor

Procedure includes 2-3 trips to New York City

- First trip
    - Several blood tests
    - See the doctor
    - Visit with the staff
    - Sign consent
- Second and third visits
    - Receive hormone treatments for 10 days (the purpose is to create an egg within your ovaries that IVF staff can actually retrieve)
    - 12 days after the treatments, eggs are retrieved
- Male Partner: One outpatient visit, lab, meet doctors, sign consent, provide sperm donation
- Costs covered by research study (travel, hotel, and procedure)
- Hoping to find 10 women, currently have 3
- Grant ends in December

If you are interested in MRT, contact Dr. Hirano's team right away because the timing of the procedures can take some time as it plans with monthly cycles. The procedure itself will cost between $20,000 to $30,000 when approved and insurance probably will not cover it, but the MRT under this study is free.

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You can contact MitoAction at info@mitoaction.org.

Additional Reading

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