Out of the shadows: Freeing families from mitochondrial inherited disease

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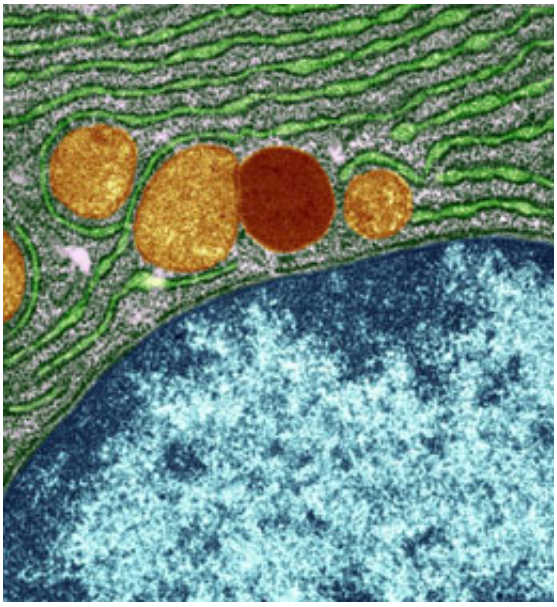

Mitochondria (in orange) in a pancreas cell. Credit: University of Edinburgh, Wellcome Images.

(Medical Xpress) -- Mitochondrial inherited diseases (MIDs) can devastate families, but there is hope in the form of new techniques to prevent them passing from mother to child. Anjana Ahuja speaks to the researchers at the forefront of this research, and a family living with the reality of such a condition, to find out why change is so desperately needed.

Eight weeks after Bella Williams was born, her mother, Jessica, knew

that something was terribly wrong. "At six weeks old, she was still not fitting into newborn clothes," says the 32-year-old primary-school teacher, who'd been told that her first child was simply a fussy feeder. On later advice, Jessica gave up breastfeeding and put Bella on formula milk, but to no avail.

"She was quite floppy and I could feel her spine. She didn't really have a bottom. I was also worried about her eyesight, because she'd try to make eye contact and her eyes would go in different directions." Her GP ordered further investigations; an MRI scan revealed lesions on the brainstem, dating back to early pregnancy.

Then came a barrage of terms that Jessica and her soldier husband, Karl, had never heard before - metabolic disorder, MID and suspected Leigh syndrome - and the sit-down talk that every parent with a sick child fears the most. To have serious symptoms at such a young age, they were told, probably signalled a severe form of MID, which means her cells are not making enough energy to function properly. The prognosis for Bella, now four months old, would be uncertain but she would be unlikely to survive to school age. The Williams family must now live with the chronic uncertainty that is the hallmark of MID, and the painful knowledge that there is probably very little that medical science can do for Bella.

Energy crisis

"I explain to my patients and families that if you have a severe defect in your batteries, it is incompatible with life," explains Doug Turnbull, Professor of Neurology at Newcastle University's Institute for Ageing and Health. He and his colleagues - Professors Robert Lightowlers, Patrick Chinnery, Robert Taylor, Mary Herbert and Zofia Chrzanowska-Lightowlers - have been awarded a total of £5.8 million (£4.4m from the Wellcome Trust and £1.4m from the University) to establish the

Wellcome Trust Centre for Mitochondrial Research.

As well as advancing fundamental understanding of how mitochondria power our bodies - and how they go wrong - the Centre also has a profoundly important social goal in mind: to perfect radical advances in IVF techniques that could free some families like Bella's from the shadow of MID for ever. What this means is that the Newcastle team don't only have to overcome the technical challenges of perfecting these techniques. They also have to make a very different social and ethical argument, to win the support of ministers and MPs for the change in the law that would allow them to start treating patients.

There are hundreds of MIDs, with their origins in malfunctioning mitochondria. Technically, mitochondria are organelles (small, independent subcellular entities) that exist inside our cells and convert carbohydrates and fat into chemical energy, called ATP (adenosine triphosphate). ATP is the fuel of life - without it, cells can't function properly - and any mis-step in its chemical supply chain can disrupt normal cell functioning. Every cell of your body (apart from red blood cells) contains mitochondria; cells in some tissues, such as brain, liver and muscle, harbour more mitochondria than others, reflecting the greater energy requirements of those organs, just as some appliances need more batteries than others.

Each mitochondrion carries multiple copies of mitochondrial DNA (mtDNA). This DNA is quite distinct from the DNA in the cell nucleus, to which mothers and fathers contribute equally. Instead, mtDNA is a genetic heirloom handed down exclusively from mothers to their children, through the generations. The mitochondria that power your cells contain mtDNA that you inherited from your mother, which she inherited from her mother, and so on. While both men and women inherit mtDNA (and half of their nuclear DNA) from their mothers, only women pass it down to their children.

To grasp what's happening right down at the cellular level, imagine being inside one brain cell: that single neuron is powered by a crowd of mitochondria continually moving around, coalescing and pulling apart again. Now, imagine plucking out a single mitochondrion from the crowd; it contains multiple copies of mtDNA, and each copy contains 13 genes (out of a total of 37) that encode for proteins involved in producing ATP. But the recipe to make ATP doesn't just involve mitochondrial proteins: some ingredients are proteins that are coded for by nuclear genes. A normal mitochondrion combines all those protein ingredients to cook up just the right amount of ATP.

In a person with MID, however, something goes wrong in the biochemical kitchen. If only a few mitochondria in the crowd are out of action, then the cell might still be able to produce enough ATP to function. But if most mitochondria in the cell are compromised, then ATP production isn't possible.

Scientists believe that if the proportion of malfunctioning mitochondria in a cell exceeds a threshold - say around 60 per cent - then the cell is plunged into an energy crisis. This is why disease sometimes takes a while to emerge: the division and replication of mitochondria during a person's development allows a critical mass of faulty mitochondria to accumulate and eventually shut down cell metabolism.

Many symptoms, few options

In the clinic, every patient has a different distribution of uncompliant mitochondria, and so symptoms can be as bewilderingly diverse as muscle weakness, diabetes, neurological disorders and blindness - and their severity spans the whole spectrum of disease (see 'Mutations, symptoms and syndromes', below). Muscle weakness that worsens over time is one of the best-known features of MID; the Muscular Dystrophy Campaign has worked very closely with the Newcastle team.

"Some people have no symptoms, while some patients die within the first 48 hours of life," says Doug Turnbull, who, with colleagues in Newcastle, also runs one of three clinics offering nationwide diagnosis and treatment for patients with MID in the UK (the others are in London and Oxford). Interestingly, an estimated one in 200 people carries a pathogenic mtDNA mutation, but the incidence of diagnosed disease is much lower, at one in 10 000 (perhaps because many people with milder impairments never seek medical help).

When it comes to treatment, though, clinicians are frustratingly short of options. Even though a special high-protein feed has restored Bella to a healthy weight for her age, feeding leaves her exhausted and the left ventricle of her heart is enlarged, showing that her little body is having to work hard, even though she spends much of the day cradled in her mother's arms or lying on her play mat. It is fair to say that Bella's condition is being managed, rather than treated.

"There aren't really good treatments out there," Turnbull admits, although transplantation can be an option when disease is confined to a specific organ, such as the heart or kidneys. "There are a few things we can do to help - so if the heart is involved, we can use drugs to reduce the load of the heart. We can sometimes increase the amount of mitochondria through exercise. But we can't correct the genetic defect and there's no tablet yet that makes the mitochondria work better."

Looking for a revolution

Given the limited menu of treatment options, Turnbull believes that one way to make headway is to stop transmission in the first place. Pre-implantation genetic diagnosis has been used to help a few families to minimise the risk of having affected children. It involves a couple undergoing IVF treatment to produce multiple embryos, which can then be tested for the mtDNA mutation. The idea is to rank the embryos

according to their mtDNA mutation level and choose one with a low mutation level. But it doesn't work for everyone.

"Some couples produce embryos that have high levels of mtDNA mutation," Turnbull points out. "If all your embryos have mutation rates of 50 per cent or more, what do you do? One of my patients lost seven children with the same defect, six within the first 48 hours of life and a seventh child at 21. In those circumstances we have to find a new way of helping those families."

This is why researchers are looking at the possibility of mitochondrial transplants. One such technique is pronuclear transfer (PNT). If successful, this could be as revolutionary for MID as IVF was for infertility. PNT is a reproductive technique that has the potential to prevent the transmission of mutated mtDNA, not just to the resulting child but potentially to all future descendants. Since faulty mtDNA is transmitted through the egg (mtDNA resides in the cytoplasm, the jelly-like substance surrounding the nucleus), the idea is to remove the nuclear genetic material from a healthy donor egg (keeping the donor mtDNA) and replace it with the nuclear genetic material from the affected couple's embryo.

The resulting child would still derive half its genes from the mother and half from the father, but would carry the healthy mtDNA of the donor rather than the biological mother.

Turnbull's work with Mary Herbert, from the renowned Newcastle Fertility Centre, has pioneered research in this area. They have shown, using eggs and embryos unsuitable for IVF, that it works in principle. A donor egg can have its nuclear genetic material removed and replaced with the nuclear genetic material from another fertilised egg, and the embryo can develop normally in the lab (before being destroyed, as required by law). The technique has not been without controversy: the

revelation two years ago that scientists were studying PNT led to headlines about the 'three-parent embryo' with its biological mother and father, and its additional 'mitochondrial mum'.

But the law has to change if the technique is ever going to benefit patients. In a legal proviso originally intended to prevent the unethical use of reproductive technologies - such as designer babies or human cloning - the Human Fertilisation and Embryology Act 2008 does not allow the implantation of an embryo whose nuclear DNA or mitochondrial DNA has been altered. Last year, the Human Fertilisation and Embryology Authority (HFEA) stated that the technique deserved backing. The Department of Health has asked the HFEA to seek the public's views on the possible use of this technique for treatment. Working with Sciencewise, the HFEA will begin the consultation later this year. Another technique for transplanting mitochondria also being studied by the Newcastle team and other groups is metaphase spindle transfer, which is essentially the same as PNT except that fertilisation occurs after, rather than before, transfer of the nuclear genetic material.

The ethics and genetics of parenthood

The Nuffield Council on Bioethics ran a recent consultation about the ethical issues raised by these techniques, and has just published its findings.

As well as the obvious safety considerations about minimising harm to both mother and child, this method does not just manipulate the child's mtDNA but - if the child is a girl - could also alter the mtDNA of all her descendants. The long-term effects of altering the germline in this way are unknown, although evidence in mice indicates no ill-effects on subsequent generations. If a girl is born through PNT, then scientists may be placed in the extraordinary position of having to monitor her descendants.

The psychological welfare of children born through PNT is also a consideration. What would it be like, for example, to have a 'mitochondrial mum'? Would a PNT child even be told? Would PNT children want a relationship with their so-called 'third parent'? Should mitochondrial mum be regarded like an egg donor, or would the relationship between an mtDNA donor and a PNT child be more akin to that between a kidney donor and the recipient?

We won't know for sure until a generation of PNT children is able to tell us. But Professor Susan Golombok, who directs the Centre for Family Research at the University of Cambridge, and who has conducted extensive research on the welfare of IVF children, does not foresee a big ethical battle ahead.

Golombok says: "Whether you believe a PNT child has three parents depends on how you define 'parent'. We already have children whom we can describe as having five parents: the egg donor, the sperm donor, the surrogate mother and the couple that brings the child up." While many children conceived using donor sperm or a donor egg want to meet their biological parents, she says, this tends to be motivated by curiosity rather than the desire for a parent-child relationship.

"They often just want to see what the donors look like," she adds. "But then again, some donor-conceived children show no interest at all in finding their biological parents when they grow up. For these individuals, the fact that they are donor-conceived is just not central to their identity. In fact, I suspect that PNT children really won't regard themselves as having three parents, because the amount of mtDNA compared to nuclear DNA is so tiny [mtDNA has 37 genes in total, compared with around 23 000 in nuclear DNA].

"And I imagine that parents of PNT children are less likely than the parents of donor-conceived children to hide what's happened, because

PNT doesn't have the same difficult issues associated with it, such as infertility. They can tell their child a positive story: 'Oh, a kind person helped you to be born free of mitochondrial disease'. But a PNT child might want to meet a mitochondrial donor to say thank you." Golombok, who has a Wellcome Trust Senior Investigator Award, says she is considering researching the issue of PNT children more deeply because she is so frequently asked to comment on it.

Turnbull and colleagues are hopeful that the public - and politicians, who will have to approve - will get behind the use of the new technique in the clinic, something they hope will be possible within the next five years: "I think if the public is informed they will feel positively towards it. We have to convey the seriousness of these diseases and why we want to prevent them in future."

At the new Wellcome Trust Centre, he and his colleagues are working to fulfil four main ambitions. The first is a better grasp of how mtDNA makes proteins, in order to understand how the process goes awry. The second is to prevent the maternal transmission of MIDs by using new IVF techniques. The third goal is to understand how deficient mitochondria cause neurological disease, because many patients present with neurological problems such as strokes and seizures. One plan is to take regular muscle biopsies: because both muscle and brain cells don't divide, Turnbull explains, what's going in muscle tissue could be a reasonable proxy for what's going on in the brain. The idea is to discover what drives deterioration in some patients, in the hope that basic research will feed into clinical practice and vice versa, in a virtuous circle.

The fourth part of the project, Turnbull says, is to examine whether mitochondria have a behind-the-scenes role in driving common diseases. Mitochondrial dysfunction, for example, has been implicated in Parkinson's disease: "Mitochondria play such a key process in the cell

that they are likely to be making a contribution to common diseases such as ageing, cancer and neurodegenerative disease. But the exact contribution is very uncertain. That's why we want to be able to look at it in a bit more detail, perhaps using next-generation sequencing technologies to study disease cohorts." The team has already linked up with Newcastle-based gerontologist Professor Tom Kirkwood, to try to crack the role of mitochondrial dysfunction in ageing.

Giving Bella the best care

Bella, who is a little chubbier than usual because of the steroids she is taking for an infection, is lying on her play mat and has just rolled from her side onto her front. She is unlikely to ever develop the muscle strength to roll from back to front. Jessica says she would sign up for PNT tomorrow if it helped her and Karl to have healthy children. "We definitely want siblings for Bella. We don't want this disease to define our family; if Bella has a short life, we want it to be a normal one."

Leigh syndrome has been ruled out; the couple are awaiting the results of further genetic tests. Some light amid the gloom has been provided by the Royal Hampshire County Hospital in Winchester, which Jessica praises for its care of Bella, and by the Lily Foundation, a charity that provides information about MID, raises money for research and encourages affected families to support each other. The mothers Skype each other; some are already bereaved, while others, like Jessica, live from day to day, constantly watchful for physical changes.

"That's what makes this disease so horrendous: you never know how it's going to manifest itself," says Jessica, who apologises needlessly for briefly losing her composure. "Bella recently went into hospital crying, and now she doesn't cry. The hardest thing about trying to be a strong mum for Bella is watching my four-month-old baby being so ill and yet being helpless to make her better."

More information: Craven L et al. Pronuclear transfer in human embryos to prevent transmission of mitochondrial DNA disease. *Nature* 2010;465:82-5.

Further reading

Tuppen HAL et al. Mitochondrial DNA mutations and human disease. *Biochim Biophys Acta* 2010;1797(2):113-28.

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