The Mitochondrial Disease Report: Progress Towards Overcoming Life’s Energy Crisis
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Foreword

Professor Ed Byrne AO

Professor Ed Byrne is a pioneering neuroscientist who has combined an outstanding clinical career with an extensive contribution to basic neurological research in Australia and the United Kingdom. He is recognised worldwide for research into disorders in the mitochondrial DNA and played a role in the discovery of mitochondrial links to ageing. He was a founding director in 1993 of the Melbourne Neuromuscular Research Unit and the Centre for Neuroscience. Professor Byrne is President and Vice-Chancellor of Melbourne’s Monash University and patron of the Australian Mitochondrial Disease Foundation.

In the course of my career as a neurologist and researcher I have seen major advances in our understanding of this important aspect of medicine. As a young neurologist, I was at the birth in some ways of mitochondrial medicine and in my work in London I had the fortune to do my doctorate with John Morgan-Hughes who was one of the key figures in the earlier elucidation of this area of human illness. At that time the possibility of mitochondrial dysfunction had only been identified in a handful of very rare cases and there was no understanding that this was an important area of human illness. Early understanding evolved from the development of muscle histo chemistry as a way of probing mitochondrial function in muscle biopsies. This was followed by the application of simple technologies looking at biochemical studies again on muscle biopsies and on mitochondria isolated from muscle biopsy using oxygen sensitive electrodes and similar technologies. This resulted in delineation of a series of biochemically identified defects in the human respiratory chain.

The discovery of the polymerase chain reaction opened up the modern DNA story and mitochondria, because of the relatively small size of their intra-mitochondrial genome, represented a great opportunity for early genetic studies. Sequencing of the
mitochondrial genome revealed mutations in a number of areas that underpinned very specific neurological diseases. At the same time it became increasingly apparent that a number of genetic mitochondrial disorders related to defects in the nuclear genome and that led to a search over some years which uncovered an increasing family of nuclear gene mutations underpinning mitochondrial disease. As this story has evolved an increasingly wide range of medical disorders, many but not all of them neurological, have been identified relating to genetic problems in either the intra-mitochondrial or the nuclear genome where mitochondrial protein encoding genes are affected.

This work is very important as relatively rare conditions continue to be identified and in addition it has become increasingly evident that mitochondrial dysfunction could play a role in some very common conditions. In our own laboratory years ago we made significant observations about a decrease in mitochondrial respiratory chain efficiency in human ageing and with others were able to show mitochondrial dysfunction as a contribution, if not the main cause in some neurodegenerative conditions including Alzheimer’s disease. In these conditions it is likely that non-mitochondrial factors played a major role but mitochondrial inefficiency possibly related to ageing may also contribute.

Treatments for mitochondrial respiratory chain disorders are getting ever closer with research around the world continuing in these important areas. Australia has made a particular contribution in mitochondrial medicine because of the predominance of Australian research in early years in basic science related to mitochondria and the presence of a number of groups in Australia that have been working on mitochondrial dysfunction for many decades. This area, as much or more than any other, has advanced because of cooperation between clinicians and basic scientists and as that approach continues further advances will undoubtedly occur.

This report is a very important document portraying the very personal human impact of these debilitating and often life threatening conditions on children, adults and their families. It is one of the most important documents in this area to be produced in Australia and I commend the Australian Mitochondrial Disease Foundation for their work in developing this landmark initiative.

Yours sincerely,

Professor Ed Byrne AO
Dr Doug Lingard is chairman and a co-founding director of the Australian Mitochondrial Disease Foundation. He and his wife Margie are the parents of two children with mitochondrial disease. Doug is a radiologist and nuclear physician who has been active in public and private medicine in Australia for more than 30 years. He is a co-founder of the largest diagnostic imaging practice in Australia, Pittwater Radiology and Medical Imaging Australasia Ltd.

Like the vast majority of Australians, I hadn’t heard of mitochondrial disease when my adult daughter Rose first developed symptoms six short years ago. She was finally diagnosed after countless seizures, two induced comas, months in hospital and many tests. Her diagnosis spurred me to find out all I could about the disease but, in so doing, I was struck by the huge void. There was so little information available about mito, no support network, and almost total lack of awareness among GPs; the RACGP ‘bible’ Principles of General Practice contained no mention of mito at all. Consequently many people, particularly those with milder forms, were (and still are) undiagnosed, misdiagnosed, unknowingly at risk of passing the disease on to their children, labelled hypochondriacs or told it was psychosomatic, often for years.

We now know that mito is too common to be called a rare disease – at least one person in 200 is at risk – yet paradoxically it was, and still is, not sufficiently well known to attract the major funding, sponsorship or public support enjoyed by less prevalent conditions such as motor neurone disease or muscular dystrophy.

It was in this context that the Australian Mitochondrial Disease Foundation (AMDF) was formed in 2009. It aims to provide support and information, build awareness and raise funds for research to develop better diagnostic methods and targeted treatments, and to help discover a cure.

The AMDF has achieved much in just four years, including funding four PhD scholarships and laboratory equipment at major Australian research institutes. It has helped establish an Australia-wide mitochondrial patient database, and has funded priority access to a new Next-Generation DNA Sequencing Facility at Royal Perth Hospital that will enable faster, cheaper and more accurate diagnoses of mitochondrial disease.
However, there is much to be done and progress is at times frustratingly slow.

The human mitochondrial genome was first sequenced in the 1970s and the Cambridge Reference Sequence for human mtDNA was first published in 1981. This led on to the initiation of the human nuclear genome project which was completed in 2003 at a cost of around $3 billion. Since then the tangible benefits in the eyes of many have been surprisingly few. However, the cost and time of DNA sequencing have come down considerably, and genomic data have steadily accumulated along with our knowledge and understanding of how cells work and fail. Such an accumulation lays the foundation for advances in human health, leading a number of experts to express the feeling that major breakthroughs could be imminent. Unfortunately, there are as yet no targeted treatments for mito, although a few in the pipeline look promising.

In publishing this report, the AMDF aims to provide a credible resource and reference document, and to promote discussion and debate on issues related to mitochondrial disease, its research, treatment and prevention.

The Mito Report focuses attention on the vital role to be played by a wide range of stakeholders, including GPs, specialists, funding bodies, researchers and patients who are championing the “mito fight”.

The Australian Mitochondrial Disease Foundation is part of the solution, but we cannot do it alone. Concerted and collaborative action is urgently required.

- Governments and funding bodies must increase investment in mito and genetic research. It remains an urgent priority to improve diagnostic methods and identify gene mutations responsible for the many forms of mitochondrial disease, and to educate medical practitioners.
- Federal and state governments need to work together to facilitate and fund faster, less expensive diagnoses; this is an important first step along the path to better care and support for all Australians with mito, no matter where they live and what their financial circumstances.
- GPs must continue to become better informed and include mito in their curriculum to facilitate early diagnosis and appropriate healthcare. The AMDF urges the RACGP to update texts to ensure future medical practitioners are informed on the latest developments.
- The AMDF calls on the Australian government to reconsider its position against the human embryo research necessary to develop mitochondrial replacement techniques. In so doing it can assist women with maternally inheritable mitochondrial disease to have children and subsequent descendants free of this debilitating and potentially fatal condition.

The AMDF has made significant progress in the “mito fight” since 2009 but appreciates that the work is hardly over. There are funds to be raised for research, support provided to the mito community, education to be organised, and many more highs and lows to withstand before mito is considered treatable, or preferably joins that blessed list of curable diseases.

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Mitochondrial Disease - The Facts

What is mitochondrial disease and what causes it?
Mitochondrial disease (or mito) is a debilitating genetic disorder that robs the body’s cells of energy, causing multiple organ dysfunction or failure and potentially death. Most patients have a genetic mistake (mutation) in the mitochondrial or nuclear DNA. The condition can be inherited from the mother, the father or both parents, or can arise as a spontaneous genetic mistake at conception.

What are mitochondria?
Mitochondria are the energy source in almost every body cell. Often called the cells’ powerhouses or generators, mitochondria transform food to produce 90 per cent of the energy needed by the human body to function, sustain life and support growth. Mitochondria are most plentiful in tissues that require a lot of energy to function; the disease therefore causes most damage to the cells of the brain, muscles, heart, liver, inner ear and eye.

How are sufferers affected?
Depending on which parts of their bodies are affected and to what degree, people with mitochondrial disease can:
• lose their sight or hearing
• suffer muscle weakness and pain
• be unable to walk, eat, swallow or talk normally
• have strokes or seizures
• develop liver disease or diabetes
• suffer heart, respiratory or digestive problems
• experience developmental delays or intellectual disability.

Needing to stay in bed to rest and recharge is a common outward symptom of mitochondrial disease. Inside the body, it’s much more serious and complex: mitochondrial disease may literally cause any symptom in any organ at any age.

Who is affected by mitochondrial disease?
Mitochondrial disease can affect both children and adults; due to its genetic basis, the disease often affects multiple family members. Adult onset is becoming more commonly recognised. In many cases, the impaired mitochondrial load (cell injury and cell death) increases with age, until organ systems begin to fail and symptoms develop.

How common is mitochondrial disease?
Until the 1990s, mitochondrial disease was thought to be rare (1 in 20,000 people), but it is now recognised as the most common subgroup of inherited metabolic disorders. Recent research shows 1 in 200 people, or more than 100,000 Australians, may carry genetic mutations that put them at risk for developing mitochondrial disease or other related symptoms such as diabetes, deafness or seizures during their lifetimes. Many of these people are symptomatic but undiagnosed or misdiagnosed, some are not yet symptomatic, and others are unknowingly at risk of passing the disease on to their children.

Put another way, up to 20 children born in Australia each week are at risk for developing a mild form of mitochondrial disease, while one Australian child born each week – or 50 children every year – will develop a severe or
life-threatening form of mitochondrial disease (1 in 5000 people), making it the second most commonly diagnosed serious genetic disease after cystic fibrosis, which has an incidence of around 1 in 3500 people.

How is mitochondrial disease diagnosed and treated? Is there a cure?
Mitochondrial disease is a complex condition that is difficult to diagnose due to the widespread range, type and severity of symptoms and its varying onset and impact on patients' lives (from none to severe). Multiple tests may be required to confirm mitochondrial disease, including genetic tests, muscle biopsies or brain scans (depending on the type of disease suspected).

There are currently very few effective treatments and as yet no cure for mitochondrial disease. It impacts differently on every patient, so doctors can’t predict the progression of the disease or symptoms, or the outcome for patients.

Links with ageing and major diseases
Whereas people with mitochondrial disease have a genetic mutation that predisposes their mitochondria to fail early, mitochondrial dysfunction is thought to be one of the major factors contributing to ageing and the reason why humans have a finite lifespan. Over a lifetime, our mitochondria slowly suffer inevitable damage from environmental and lifestyle factors and become less effective at producing the energy our organs need to function properly.

Researchers increasingly believe mitochondrial dysfunction may be a significant factor in a wide range of major diseases – particularly chronic degenerative disorders and those associated with ageing – including:
- Parkinson disease
- Alzheimer disease
- Huntington disease
- motor neurone disease / amyotrophic lateral sclerosis (ALS)
- cardiovascular disease
- diabetes
- cancer, particularly solid tumours and tumour metastasis (spread to other organs).

Research into mitochondrial medicine therefore offers hope not only to people with primary mitochondrial disease (due to a genetic mutation), but also to the millions suffering from other major diseases commonly associated with ageing.

Improvements in mitochondrial medicine may eventually provide the key to better health and quality of life in old age for all.

Why haven’t we heard much about mitochondrial disease before?
Mitochondrial medicine is a newly established and rapidly evolving field thanks to major advances in our understanding of genetics. It was not until 1988 when mutations in mitochondrial DNA were discovered to cause disease, and not until 1995 when nuclear gene mutations were also found to cause mitochondrial disease. Since then, more than 100 clinical syndromes and disorders have been recognised as coming under the category of mitochondrial disease.

Mitochondrial disease is a debilitating genetic disorder that robs the body’s cells of energy, causing multiple organ dysfunction and potentially death.
Improving the diagnosis of mitochondrial disease patients: the role of the GP

Professor Carolyn Sue

The diagnosis of mitochondrial disease often involves a long, complex journey for both patient and clinician, but greater understanding by doctors and the development of new diagnostic tests provide hope for earlier diagnosis, prompt symptom management and better introduction of supportive treatments and preventative strategies.

A key issue to improve the early detection of mitochondrial disease requires a high index of suspicion and knowledge about this disorder. Clinicians need to consider mitochondrial disease when specific symptoms cluster within an affected individual. Unfortunately, many sufferers remain undiagnosed or misdiagnosed (particularly adults with milder symptoms), because of the vast range of symptoms that can present in affected patients. Because mitochondrial disease is often inherited and can follow an unpredictable disease course, undiagnosed individuals may unknowingly pass the disease on to their unborn children or are at risk of developing severe, life-threatening symptoms themselves.

There are several challenges that the GP faces in identifying mitochondrial disease.

Firstly, the field of mitochondrial medicine is a newly established, complex and evolving specialty that may not be at the top of the clinician’s mind when assessing affected patients. Originally thought to be a rare disorder affecting predominantly children, it is now recognised to be the most common inherited form of metabolic disease that may

Professor Carolyn Sue is a neurologist and research scientist with a major interest in understanding the disease processes involved in mitochondrial disorders and developing diagnostic and treatment options. Professor Sue runs Australia’s largest clinic specialising in the diagnosis, assessment and treatment of adults with mitochondrial disease and heads a research team at the Kolling Institute of Medical Research. She is a professor at the University of Sydney, director of the Department of Neurogenetics at Royal North Shore Hospital and the director of the National Centre for Adult Stem Cell Research (Sydney Node). Professor Sue is co-founding director of the AMDF and a member of its Scientific and Medical Advisory Panel.
manifest from early in the neonatal period to late in adulthood. Children often present with different clinical features when compared to adults and thus inheritance patterns may not be obvious on first review. Children typically present with failure to thrive, lactic acidosis, motor regression, encephalopathy or seizures. In contrast, adults frequently develop hearing loss, muscle weakness, diabetes, gastrointestinal dysmotility and fatigue. Specific clusters of symptoms may alert the clinician to the diagnosis. For example, an affected patient may have migraine-like headaches, early onset and mild asymmetrical hearing loss associated with diabetes and occasional abdominal bloating. Doctors or patients may overlook these mild symptoms, yet a combination of symptoms may point to a diagnosis of mitochondrial disease. Thus, it is important for the primary physician to take a detailed history to assess each symptom individually and be alerted to those signs that are indicators of mitochondrial disease (refer to table).

Although often inherited, mitochondrial disease may follow several different inheritance patterns and may also occur sporadically in the population. This complex pattern of inheritance is because the mitochondria use

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<th>Characteristics that might raise the suspicion of a mitochondrial disease</th>
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<td>Hearing loss</td>
<td>Early onset&lt;br&gt;Asymmetrical onset&lt;br&gt;Sudden onset&lt;br&gt;Worse after metabolic stress&lt;br&gt;Partial recovery after an auditory insult</td>
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<td>Stroke-like episodes</td>
<td>Early age of onset&lt;br&gt;Preceded by nausea, vomiting, constipation or drowsiness&lt;br&gt;Non-vascular territory on neuroimaging&lt;br&gt;Concomitant basal ganglia calcification&lt;br&gt;Neuroradiological features out of proportion to clinical deficit&lt;br&gt;Associated focal seizures or status epilepticus</td>
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<td>Seizures</td>
<td>Status epilepticus of cryptogenic origin&lt;br&gt;Seizures worsened by sodium valproate&lt;br&gt;Concomitant basal ganglia calcification&lt;br&gt;Additional features of hearing loss, diabetes or short stature</td>
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<td>Ptosis (droopy eyelids)</td>
<td>Accompanying external ophthalmoplegia or retinal pigmentary changes&lt;br&gt;Asymmetrical onset&lt;br&gt;Slowly progressive with little diurnal variation</td>
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<td>Retinal pigmentary changes</td>
<td>Perimacular distribution&lt;br&gt;Non-vision threatening&lt;br&gt;No associated drusen&lt;br&gt;Associated with hearing loss and diabetes</td>
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<tr>
<td>Diabetes</td>
<td>Associated with hearing loss and retinal pigmentary changes&lt;br&gt;No associated diabetic retinopathy/peripheral neuropathy with respect to the length of diabetes onset&lt;br&gt;Easily controlled with oral hypoglycaemic agents with respect to duration of diabetes</td>
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Table: Clinical features suggestive of a mitochondrial disorder
proteins that are encoded by two different genomes: mitochondrial and nuclear DNA. Because mitochondrial DNA is typically passed on from mother to all children, mitochondrial disease caused by mutations in this gene follow a maternal pattern of inheritance (all children of an affected mother are potentially at risk of the disease, but affected fathers do not pass on the disease). Given that other genetic mutations that cause mitochondrial disease are found in the nuclear DNA, other types of mitochondrial disease may be inherited via Mendelian traits such as autosomal recessive (both parents who are asymptomatic of the disease have to be carriers to have affected children) or autosomal dominant (only one parent has to be affected to have an affected child) modes of inheritance. Finally, mutations in mitochondrial DNA may arise sporadically and thus there may be no family history at all. Adding to this complexity, the range of impact that genetic mutations have on different individuals is highly variable, resulting in the fact that even family members with the same genetic mutation may have different disease courses and certain individuals may have gene mutations and remain asymptomatic until later in life. Unfortunately there is no single diagnostic test that is abnormal in each patient with mitochondrial disease. Thus, multiple investigations are often required. Diagnostic investigations include blood tests, urinary analysis, biochemical and genetic tests. Serum lactate and pyruvate levels, imaging studies, and neurophysiological studies may help to define the patient’s disease syndrome, but are only supportive of the diagnosis and are not definitive. Muscle biopsy is the historical gold standard to diagnose mitochondrial disease, but this is invasive and may present an additional risk to the affected patient if performed under general anaesthesia. Histological, biochemical and genetic analysis of the muscle tissue can be performed, but these may have poor sensitivity or have limited availability. Genetic testing is also not freely available throughout Australia and can be prohibitively expensive, given that the responsible genetic mutations may require analysis of both the mitochondrial and nuclear DNA.

![Diagram](image_url)  
*Figure: Comparison between current and proposed future diagnostic pathways for mitochondrial disease. Note the proposed future diagnostic pathway would avoid muscle biopsy and result in an increased number of patients receiving a genetically confirmed diagnosis of mitochondrial disease.*
New investigations to simplify and improve the diagnosis of mitochondrial disease are currently being evaluated. Two recent studies published by Suomalainen (Lancet Neurology) and Davis (Neurology) have reported the promising development of a new serum biomarker (serum FGF21 levels) that may preclude the need for a muscle biopsy in most cases and simplify the screening for mitochondrial disease to a simple blood test. Secondly, new advances in methods to sequence large amounts of DNA (known as massively parallel sequencing or “next-generation” sequencing or “NGS”), have been shown to be effective in detecting mutations in both mitochondrial and nuclear genes. These new sequencing techniques are able to determine the genetic code of millions of bases of DNA both rapidly and cheaply. As a consequence, NGS has increased the diagnostic yield and improved the ability of researchers to confirm a genetic cause in many cases of suspected mitochondrial disease. It is possible that using a combination of these new methods to diagnose mitochondrial disease may improve the diagnostic accuracy of mitochondrial disease. Further evaluation of relatively inexpensive new biomarkers (such as a simple blood test e.g. serum levels of FGF21) to screen for mitochondrial disease will encourage GPs to order it when mitochondrial disease is suspected, particularly for adults with milder symptoms or for asymptomatic family members.

GPs need to continue to become more informed about mitochondrial disease and consider this group of disorders as a diagnostic possibility. Increased awareness and improved understanding among frontline clinicians, combined with improved pathways to diagnosis may assist us to provide earlier and more accurate diagnosis, providing answers and better symptom management for more mitochondrial disease sufferers around the world.
A GP and mother’s plea: Doctors must face up to mito for patients’ sakes

Dr Karen Crawley

Dr Karen Crawley is a GP and the mother of three children affected by mitochondrial disease. She is dedicated to educating doctors about mito, raising awareness and empowering patients to manage their condition, and answers the AMDF’s Helpline: 1300 977 180.

People often feel powerless and isolated when dealing with a very sick child or their own illness. This is intensified when the disease is so complex and relatively unknown that rounds of doctors and tests can’t provide answers, multiple symptoms remain unexplained, or patients are labelled as unlucky, ‘difficult’, malingering or suffering a psychosomatic illness.

This is a common refrain from callers to the Australian Mitochondrial Disease Foundation (AMDF) Helpline, which I answer in a volunteer capacity. We not only provide information and support, but also help empower patients (and those who suspect they have mitochondrial disease) to take control of their diagnostic journey, understand their disease, and actively manage it in partnership with a compassionate medical practitioner who is determined to assist their patient. Knowledge is a powerful tool that can ease the path for patients and families, and for their healthcare providers.

However, it’s not as simple as it sounds. I am a GP myself and mitochondrial diseases were an unknown entity at university more than twenty years ago. We were told not to worry about what was then considered a ‘rare problem’ affecting only 1 in 20,000 people (recent research shows 1 in 200 people may develop mito in their lifetime).

Despite my medical training, I was unaware anything was seriously wrong when my elder daughter Kara started showing what I now know were signs of mitochondrial disease (mito) when she began school in 2005.

Her slowness in all tasks, poor balance and ever poorer sporting skills made Kara simply seem hard work. The excess body hair, early loss of teeth, repeated spontaneous vomits, plummeting percentile bands, awkward running style, stiff muscles, ‘stick legs’, stress incontinence, jerky eye movements, poor concentration and a failed occupational therapy assessment should have rung a few mitochondrial alarm bells if I had heard of it.
After Kara turned eight, I found her semiconscious and vomiting in bed. A bad gastro seemed the most obvious to me, not her first stroke-like episode. The diagnosis was quick: a progressive neurodegenerative form of mito called MELAS (Mitochondrial Encephalomyopathy, Lactic Acidosis and Stroke-like episodes syndrome).

My knowledge of mitochondrial disease was zero and my medical textbooks said nothing, so when an internet search revealed what she and us as a family were to face…you can imagine our devastation.

Even though there is as yet no cure and very few targeted treatments for mito, getting a diagnosis is a vital step.

Painful as the news was, we are thankful for Kara’s quick diagnosis because the insidious, variable, ill-defined and often slow development of mito unfortunately puts many patients – particularly with adult-onset mito – on a long and harrowing diagnostic odyssey that doctors can do much more to alleviate, judging by the calls for help I answer.

Frequent visits to the doctor before a mito diagnosis is even considered – sometimes at the behest of a patient who has turned in desperation to their own research – may lead to the patient or caring parent being pre-labelled, which hinders their whole medical care. Patients may be left waiting months or years for tests to be performed and results obtained. Some are told “it might be mito, but it’s too difficult to diagnose”.

Despite my medical training, I was unaware anything was seriously wrong when my daughter Kara started showing signs of mito.
Yet while confirming mito can be difficult and time consuming and may not change the eventual outcome, leaving a patient stranded without a diagnosis or a way forward is not why I became a doctor.

Even if the exact genetic mutation remains unknown, many patients and carers speak of the ‘dignity in diagnosis’, of the overwhelming comfort it is not in their head, they are not alone, and they or their child/ren have one disease rather than several. When both patient and GP understand mito well, it can lead to better quality of life, particularly for adults with debilitating yet non-life threatening disease. Patients may be able to manage their condition and prevent worsening health through exercise, diet and medications or surgery for symptom management in collaboration with their GP. Referrals to specialists like neurologists, cardiologists, gastroenterologists, endocrinologists and ophthalmologists also become easier (although specialists also need to be better informed about mito).

Diagnosis helps relatives who may have been experiencing mito symptoms and/or who are planning to have children, and can now approach their doctor with specific questions.

The overwhelming and unnecessary cost to Medicare also needs to be considered. If mitochondrial disease is not considered as a real possibility by a patient’s doctor, they and other affected relatives remain on the diagnostic government-funded merry-go-round, with thousands of dollars being spent on unnecessary investigations.

Given the pivotal role of GPs in diagnosing mito and managing patients’ health, the AMDF engages with GPs through General Practitioner Conference & Exhibition (GPCE) educational events and Mitochondrial
Information Days at hospitals and research institutes, and provides literature such as the AMDF’s Medical Information Booklet for General Practitioners. The AMDF Helpline is also for medical practitioners, not just for patients.

Slowly but surely, good progress is being made and significantly increased requests for testing are being reported by specialist mito centres at the Kolling Institute and the Murdoch Childrens Research Institute.

But moving ahead on mito requires action from the medical profession too. We know there are many thousands of people in the community with mito who are undiagnosed or misdiagnosed, and not receiving the care they deserve.

As a doctor and a ‘mito mum’ working to help other affected families, I urge all doctors to seek information and consider mito when patients have symptoms that don’t seem to add up; to be prepared to say “I don’t know much about mito, but I’ll find out”; to remember mito as the number one metabolic disorder in their list of differential diagnoses, preferably before they ring the psychiatrist or dismiss the patient as a malingering or ‘too much hard work’.

It’s also past time for an update of the GP’s ‘bible’, The Principles of General Practice, to reflect the latest understanding of mitochondrial disease and its devastating impact on Australians like our family, and ensure future doctors are better informed than I was.

In the meantime, we take each day as it comes with Kara. Early dementia has set in, she’s in a wheelchair, has a feeding tube and hearing aids and her eyesight is slowly disappearing. With a few years at most to live, our gorgeous girl is disappearing with each stroke. We are cautiously hopeful for the future of our younger son and daughter, who have also tested positive for the defective gene but are relatively healthy. At least we now know what we are dealing with.
Technology steps up to aid mito diagnosis

Professor David Thorburn

Even at rest, humans need to constantly generate about 100 Watts of energy, the same amount as a bright light globe. This allows our neurons to send messages, our heart to pump and our other organs to perform their roles. To do this, we must be able to efficiently use fuels such as fats, sugars and proteins to make a small molecule called ATP, which is our chemical energy store. Mitochondria are our cellular power plants, responsible for generating the majority of energy required for cellular function and survival. Each day we generate and consume about 65 kilograms of ATP, emphasising how much we rely on our mitochondrial power plants!

Mitochondrial disorders pose great challenges in diagnosis and treatment. Most patients suffer from mitochondrial disease because one of the 1500 or so genes needed to make healthy mitochondria is not working properly. Over 150 of these are already known to be “disease” genes with perhaps another 100 or more awaiting identification. Most of these genes are the regular nuclear genes that are present in two copies, one inherited from our mother and the other from our father. However, mitochondrial disorders are unique since they can also be caused by mutations (changes in the genetic coding sequence) in the mitochondrial DNA (mtDNA). The mtDNA is a small genome present in thousands of copies, with each mitochondrion (cellular powerhouse) containing about 1000 copies.
of copies in each cell and is inherited only from our mother. This makes the genetics of mitochondrial disorders particularly complicated. Recently, thanks to work by Professor Carolyn Sue in Sydney and two research groups in the UK, we have realised that at least 1 in 200 people carry a change in their mtDNA that can cause disease. Only about 1 in 50 of these carriers is currently being diagnosed with mtDNA disease. While some may be healthy, it is likely that a substantial number of these individuals have symptoms caused by their mtDNA mutation but they are not being investigated properly and not being diagnosed with mtDNA disease.

So how do we diagnose mitochondrial disorders? For over 10 years, genetic testing has been available in Australia for some DNA mutations. However, the complicated genetics and cost of DNA testing means that this has typically been limited to testing for a small number of mutations, mostly in the mtDNA. Hence only a minority of patients are diagnosed quickly and easily by DNA testing on a blood sample. The next step for most patients has been a muscle biopsy. The mitochondrial energy pathway consists of five major components or enzymes, known as Complexes I to V. Muscle biopsies are usually tested for how much of these complexes are present and how well they work. Finding a deficiency of one or more of these Complexes can be diagnostic. Sometimes the combination of the patient’s clinical and enzyme findings suggest one or two obvious genes that should be tested. Other combinations, such as Leigh Syndrome with Complex I deficiency may still leave more than 20 different genes and several different types of inheritance as
plausible. Genetic diagnosis is often a slow process and most centres can only afford to test a handful of genes so many patients and families remain on a diagnostic odyssey without a clear answer to the genetic cause of their symptoms.

The game changer in genetic diagnosis is the emergence of new technologies called “Next-Generation” or “Massively Parallel” DNA sequencing. Instead of having to decide on the most likely handful of genes that we can afford to sequence, we can now sequence panels of 100 genes, 1,000 genes or all 20,000 different genes at once. The latter is called “Whole Exome Sequencing” and cost a few million dollars in 2007 but can now be done for a few thousand dollars.

In theory this means we should be able to sequence blood first and sift through the sequencing data to pull out the genetic cause without needing to do a muscle biopsy. This approach is starting to be used more widely and should be the reality for most patients within a few years’ time. It has been made possible by the extraordinary fall in DNA sequencing costs in the last 6 years and advances in bioinformatic analyses of the data. However, it is still transitioning from being a research tool into routine diagnostic use. The AMDF has been active in supporting the use of this technology in Australia, by supporting a PhD student in my laboratory and by funding use of the technology in Perth.

The power of massively parallel sequencing is demonstrated by a study we published last year on 42 infants with deficient activity of one or more of the mitochondrial enzyme complexes. In conjunction with Prof. Vamsi Mootha’s group in Boston, we sequenced over 1000 genes encoding all the known mitochondrial proteins in each patient. We identified genetic diagnoses in 10 children in mtDNA or in 7 nuclear genes previously linked to mitochondrial disease. We also identified 15 novel “candidate” genes not previously linked to mitochondrial disease with mutations and showed that at least two of these 15 candidate genes were true novel disease genes. We have subsequently shown that at least another 5 of these candidates are true novel disease genes. This illustrates that we still have plenty to learn about which genes can cause mitochondrial disease and that these approaches will identify many more such genes in the next few years. The US National Institutes of Health kindly chose this study as one of 12 “Genome Advances of the Month” for 2012 (www.genome.gov/27547295).

What are the incentives to develop this technology when we don’t yet have effective treatments for most patients? Firstly, a diagnosis can end the diagnostic odyssey and provide dignity in diagnosis. In some cases an accurate diagnosis does guide treatment options and in others it can aid access to additional benefits and support. It can also enable patients to proactively manage health to manage disease progression and quality of life. It provides precise estimates to couples on their risk of having further affected children and allows them access to effective reproductive options. Finally it is important to
understanding the true incidence and impact of mitochondrial disorders, both of which are likely to be highly underestimated.

So what do we still need to do? A number of challenges remain in improving its sensitivity so that we can detect and interpret virtually all DNA changes that cause mitochondrial disorders. On average, each individual has about 20,000 DNA sequence differences in their 20,000 genes when compared to anyone else. Finding the needle in the haystack remains an issue. Hence we may still need to do muscle biopsies on most patients for a few years in order to help interpret all the DNA variants that we find. This technology requires additional investment in equipment, informatics, data storage, training of the scientific workforce and education of medical practitioners. At present there is no direct government funding for these techniques so costs are usually coming either from research budgets or families’ hip pockets. By working together, scientists, clinicians, the AMDF and professional bodies like HGSA and the RCPA will seek to develop a standardised approach for diagnosing mitochondrial disorders to ensure faster, less expensive diagnosis and better care and support for all Australians, no matter where they live and what their financial circumstances. AMDF members and professionals will also need to lobby governments to achieve this outcome.

Eleven year old Tom (pictured right) has had mitochondrial disease since birth although it took five years to clinically diagnose. Although he looks healthy he enteral feeds; has undergone over 30 procedures; had problems with his eyes, extreme pain and poor speech. His family helps raise funds to find a cure.
Surviving the diagnostic odyssey of mito

Despite the often poor outlook, receiving a diagnosis of mitochondrial disease can be a relief for patients and their families. Personal stories abound of years of tests, countless medical appointments, various diagnoses and significant financial costs, heartache and uncertainty.

Annaliese

Annaliese Hodge and her family endured a twelve-year diagnostic odyssey before she was diagnosed with mitochondrial disease at 17 thanks to advances in genetic testing. Her mother, Joanne Edwards, who now runs the Melbourne support group of the Australian Mitochondrial Disease Foundation, says while it was obvious something was wrong with Annaliese, doctors were unable to find the cause of her symptoms and the family had no answers on how to help their daughter.

“We noticed from a young age that Annaliese was intellectually delayed and had tremors in her hands. At age five, a paediatrician diagnosed her with ADHD and put her on Ritalin, but this didn’t help,” says Joanne.

“During primary school, academic testing showed Annaliese was functioning four to five years behind her age group. Her tremors got worse and it became harder for her to do day-to-day tasks such as eating, doing up buttons and shoe laces, writing and reading. It also affected her speech and her limbs, making her extremely clumsy. And because she was different, she had very few friends, which was upsetting.

“If you don’t know what’s wrong, you can’t be confident you’re doing the right thing for your child, so we persevered in trying to find answers. However, neurologists, movement specialists, a new paediatrician and geneticists still couldn’t help.

“As a parent I got to the point where I felt I was looking for something that didn’t exist and couldn’t understand how a child could be so impacted by this condition and the doctors not be able to find anything.”

The breakthrough came when, just before her 17th birthday, Annaliese was sent for further blood tests following yet another neurological review.

“Finally, after 12 years and thousands of dollars, we got a diagnosis of mitochondrial disease. I’m told we have advances in medical research and genetic testing to thank.

Although the diagnosis doesn’t open up doorways to targeted treatments or a cure, at least we know Annaliese’s form of mitochondrial disease is not life-threatening and that it’s maternally inherited.”

Tests showed Joanne’s older daughter and son also have the genetic mutation that puts them at risk of mitochondrial disease like their sister’s: MERRF syndrome (Myoclonic Epilepsy with Ragged Red Fibres, the latter being characteristic microscopic abnormalities seen on muscle biopsy in some mito patients).

“Thankfully my other children are not symptomatic, but we know the onset can occur in adulthood and symptoms can differ among family members. Because their mitochondrial disease is maternally inherited, my daughters are at risk of passing it on to their children, but now at least they can make informed decisions.”
If you don’t know what’s wrong, you can’t be confident you’re doing the right thing for your child… Finally, after 12 years and thousands of dollars, we got a diagnosis of mito.
Miranda Kirk with her aunt Lucy
Miranda

Brian Kirk and his wife endured a similar four-year ordeal of misdiagnoses, hospital stays, multiple tests and worry with their daughter, Miranda, before she was finally diagnosed with mitochondrial disease at the age of five.

“We suspected something was wrong when Miranda was a baby – she had developmental delays, was failing to thrive and didn’t seem to feel pain. She didn’t start walking and talking until she was four,” says Brian.

During what Brian describes as a ‘horrific’ diagnostic journey, doctors variously thought Miranda might have colon cancer or epilepsy or even that she was ‘just small’ and the Kirks were imagining her symptoms. Their distress at the lack of answers was compounded by the fact they lost their first child at the age of nine months to a diaphragmatic hernia.

After years of tests including invasive muscle, liver and skin biopsies, Miranda was eventually diagnosed with mitochondrial Complex I disease, which is damaging her intellect, muscles, eyes and ears. Conventional genetic testing has not identified the gene responsible for Miranda’s disease but this has not yet included next-generation DNA sequencing, which will be performed in the next year and will hopefully provide the answer.

“Although there are no targeted treatments for mitochondrial disease, it was some comfort to get a diagnosis because it enabled us to access support, specialist counselling and treatment.”

After years of struggling to put on weight, Miranda had a breakthrough after being fitted with a feeding pump that delivers her a high-fat concentrate solution for one-and-a-half hours each night.

“She got the feeding device in December 2012 and put on a stone over eight months, which has really improved her strength and stamina and possibly slowed her deterioration.

“It’s made such a difference that we do wonder whether an earlier diagnosis, and therefore getting this nutrition earlier, could have improved her physical development when she was younger and reduced the severity of her problems.”

Now aged seven, Miranda’s muscles don’t properly support her and she uses a walking frame as well as a wheelchair for longer activities and stability in the schoolyard. She has retinal dystrophy and is losing her sight, and wears hearing aids; cochlear implants may be necessary in a couple of years.

“Miranda’s future quality of life is uncertain, but for now she’s a happy little girl who’s making the most of life.”

“...we do wonder whether an earlier diagnosis... could have improved her physical development and reduced the severity of her problems.
Approaches to mitochondrial therapy: the next frontier

Professor John Christodoulou

Professor John Christodoulou is a senior geneticist based at The Children’s Hospital at Westmead, where he is Director of the Western Sydney Genetics Program, one of the few integrated clinical and laboratory diagnostic genetics services in Australia. He is also Professor, Disciplines of Paediatrics and Child Health and Genetic Medicine, in the Sydney Medical School at the University of Sydney, and a board member of the Australian Mitochondrial Disease Foundation and its Scientific and Medical Advisory Panel.

Mitochondrial disease – or, more specifically, mitochondrial respiratory chain disorders (MRCDs) – can encompass a wide array of health problems, and require a methodical and rigorous approach to testing to establish the diagnosis with certainty. Although our understanding of mitochondrial disease and its impact have increased over the past decade, this has not yet translated into treatments. Once diagnosed, there are no curative therapies and very few effective treatments for patients with mitochondrial disease; these represent the next frontier for researchers.

Research is underway around the world, including Australia. Most efforts are focused on improving patients’ quality of life and on early identification and management of secondary side effects. Some treatment approaches include the use of nutriceuticals (nutritional type products) in an attempt to improve the efficiency of cells’ energy production through metabolic manipulation, while others attempt to improve energy production by altering the balance between mutated and normal working versions of mitochondrial DNA (mtDNA), by enzyme replacement therapy, or by the use of specific gene activators.

Metabolic manipulation

Abnormalities in the mitochondrial energy production pathways in our cells can lead to an accumulation of free radicals, which potentially have damaging effects on a number of key cell processes, and are believed to contribute to disease progression in many cases. By modifying the nutritional composition of the patient’s diet through supplementation with vitamins and co-factors, it is hoped that such metabolic manipulation may reduce the accumulation of free radicals.
A number of such supplements, including vitamin C and vitamin E, have been trialled with some appearing to have beneficial effects in some mitochondrial disease patients. Coenzyme Q10 is also popular, based on suggestions that it is able to reduce free radicals, and so is creatine, based on its ability to function as an alternate energy source. However, there is a lack of objective studies demonstrating therapeutic benefit in using these supplements, either on their own or in combination.

On the other hand, there is a body of evidence supporting the use of the amino acid L-arginine in patients with MELAS (Mitochondrial Encephalopathy, Lactic Acidosis and Stroke-like episodes syndrome) by decreasing the frequency and the severity of acute stroke-like episodes, and more recently, it has been suggested that L-citrulline may be of better therapeutic value than L-arginine in MELAS.

In some cases, a ketogenic diet (high fat/low carbohydrate) has been shown to be of benefit for patients with difficult-to-control seizures, and this has been considered to be safe even for children with mitochondrial disorders.

**Altering the balance between mutated and normal mtDNA**

For those MRCDs that are due to a primary mtDNA mutation, altering the balance between mtDNA with and without the mutation could potentially improve mitochondrial function. New drug- or gene-based technologies are currently being developed with this aim in mind, but none have translated into clinical trials yet.

**Exercise as a therapy**

While drug and gene-based technologies are still under development, there is a growing body of research literature showing that carefully supervised exercise training can result in muscle regeneration with an improved ratio of normal to mutated mtDNA, and this leads to improved exercise tolerance and capacity.

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*Mitochondria are found in most cells of the body, with each cell having tens to hundreds of mitochondria. They are the powerhouses of the cell, using fat and sugars in our diet to convert them into energy (ATP), through a complex set of finely regulated biochemical reactions.*

*The final common pathway, generating most of the body’s ATP, is the mitochondrial respiratory chain, which is physically located within the inner membrane of the mitochondrion.*

*The ATP which is generated by oxidative phosphorylation can then be used by the cell for a multitude of essential purposes.*
Enzyme replacement
Mitochondrial-Neuro-Gastro-Intestinal Encephalopathy (MNGIE) is a form of mitochondrial disease caused by loss of activity of the enzyme thymidine phosphorylase (TPase). There is early evidence to suggest that restoration of TPase activity may be of benefit, using either a gene therapy approach (based on studies in a mouse model), or by bone marrow transplantation (this form of therapy has been applied to a number of human patients). However, the long term benefit of this approach remains to be demonstrated.

Regulation of specific gene activators
Some therapies are focused on increasing the number of healthy mitochondria. It is possible to increase the level of a transcriptional coactivator protein PGC-1α (peroxisome proliferator activated receptor gamma co-activator 1 alpha), which is a key player in promoting increases in the number of mitochondria. Exercise, especially endurance exercise, and specific drugs such as bezafibrate and resveratrol can increase PGC-1α levels.

"Once diagnosed, there are no curative therapies and very few effective treatments for patients... these represent the next frontier for researchers."

New therapies under evaluation
A chemically modified form of Coenzyme Q10 called idebenone is believed to penetrate key organs such as the brain more effectively, and there is some evidence to suggest it is of some benefit in mitochondrial diseases such as MELAS, Leber Hereditary Optic Neuropathy (characterised by sudden, profound loss of central vision) and Friedreich ataxia (progressive nervous system degeneration).

Another synthetic analogue of Coenzyme Q10, EP1-743, is believed to be even more potent and safer. Studies have shown EPI-743 to be of benefit in patients with a number of MRCDs. A Phase 2B randomised, placebo-controlled, double blind clinical trial is currently underway into EPI-743 as a treatment for children with Leigh Syndrome, the most commonly recognised mitochondrial disease of childhood (http://clinicaltrials.gov/ct2/show/NCT01642056).

Despite these advances, there are many people with mitochondrial disease for whom no effective treatment is currently available. For them it remains an incurable, debilitating and potentially life-threatening disorder. Prenatal diagnosis to prevent recurrences in future generations is an important option that should be explored. This can be an option for most families where a nuclear DNA mutation has been identified, but only for a minority of families with mtDNA mutations. Similarly, pre-implantation genetic diagnosis is also an option for families with nuclear DNA mutations. but only for some families with mtDNA mutations.

There is therefore an urgent need for further research focusing on improving our understanding of the cellular and molecular biology of these disorders. Such research, it is hoped, will give rise to new and powerful therapeutic agents that prove their value in extensive clinical trials and will eventually provide significant benefits for patients.
...it is hoped [further research] will give rise to new and powerful therapeutic agents that prove their value... and eventually provide significant benefits for patients.
Reading the blueprint of the mitochondrial genome

Professor Aleksandra Filipovska

Professor Aleksandra Filipovska is an ARC Future Fellow at the Western Australian Institute for Medical Research and the University of Western Australia in Perth, and established her research group in Mitochondrial Medicine and Biology in 2006. Her research focuses on studying mitochondrial gene regulation and function in health and mitochondrial disease. Professor Filipovska’s group has made advances in the development of methods for studying mitochondrial gene function and developing therapeutics for inherited mitochondrial diseases. She is a member of the AMDF’s Scientific and Medical Advisory Panel.

Mitochondria play a fundamental role in cell and energy metabolism and consequently mitochondrial dysfunction can lead to severe multi-system disorders with a wide range of clinical presentations that commonly include neurodegeneration, muscle defects and exercise intolerance. To understand these conditions better and identify therapeutic targets it is necessary to understand how gene expression is regulated within mitochondria, as some of the most significant gaps in our knowledge of mitochondrial function and disease are in the regulation of mitochondrial gene expression. In all living things genes provide the blueprints for cells and our bodies. When genes are turned on they make RNA, which acts as instructions to make the protein building blocks of the cells. These processes are well understood for most of the genes in the cell, however the small set of genes which reside in mitochondria follow different rules, which are only now beginning to be understood.

In a recent collaboration with Professor John Mattick’s team (Garvan Institute, Sydney) we performed the first comprehensive census of all the RNA instructions in human mitochondria. We discovered an unanticipated variety of different RNAs, many of which had never been observed before. These RNAs are very dynamic and vary dramatically depending on the energy demands of the cell. For example, we found that mitochondrial RNAs were far more abundant in tissues with high energy demands, such as the heart and brain, compared to those that require less energy, such as the skin.

Although little is known about how the levels of mitochondrial RNAs are controlled in cells, recent new and exciting findings emerging from groups around the world including our
team at the University of Western Australia indicate that RNA-binding proteins play a central role in the lifecycle of the mitochondrial genetic blueprint. The basic components and mechanisms of RNA regulation have recently been discovered; however, the fine-tuning of mitochondrial gene expression at the level of RNA remains a worthy pursuit for our future research endeavours and will provide new avenues for therapeutic interventions for mitochondrial diseases.

RNA is one of the essential macromolecules for life; it regulates how genes are turned on and made into proteins. RNA is present in all cells and in organelles within cells such as mitochondria. Defects in RNAs or the regulation of RNAs can cause or contribute to many important human diseases including mitochondrial diseases.
Professor Jus St John is Director of the Centre for Genetic Diseases at the Monash Institute of Medical Research in Melbourne. The overall aim of Professor St John’s research is to understand how maternally inherited mitochondrial DNA is transmitted, segregated and replicated. His current research focuses on defining key mitochondrial DNA replication events and how they influence the transmission of mutant mitochondrial DNA from one generation to the next. Professor St John is currently developing models of mitochondrial DNA diseases and testing the safety and efficacy of maternal spindle transfer. He has advised the UK government, parliament and the Royal College of Obstetricians and Gynaecologists on policy related to stem cells and reproduction, and also advised the Human Fertilisation and Embryology Authority on stem cells and embryo policy.

The challenge for a woman who is a carrier of mitochondrial DNA disease and wants to have children is that each of her eggs will have different amounts of damaged or mutated mitochondrial DNA. As one egg is ovulated at each menstrual cycle, she will not know if that egg has high or low levels of mutated mtDNA. Furthermore, current genetic tests do not allow us to predict whether the egg has high or low levels of mutated mitochondrial DNA and then allow the woman to proceed with her egg to make a child. However, recent developments using in vitro fertilisation (IVF) technologies are now opening up new research avenues that could prevent mitochondrial DNA disease from being transmitted from the mother to her children.

Two approaches have been proposed that would prevent children from inheriting these severe forms of mitochondrial DNA disease. One of the techniques, known as Maternal Spindle Transfer (M-ST), enables the mother’s chromosomes to be transferred from one of her eggs into an egg from a donor. In this case, the donor egg retains its healthy mitochondrial DNA but has had its chromosomes removed. The eggs are fertilised with her partner’s sperm, as is normal during IVF treatment, and then allowed to develop into an embryo in the laboratory. After a few days, the developing embryo is transferred to the mother. Normally, it would then implant into her womb and a pregnancy is established.

The other technique is known as Pronuclear Transfer (PNT). It is similar to M-ST except that the partner’s sperm fertilises the egg.
before the parents’ chromosomes, which are contained within each of the pronuclei, are transferred into a healthy donor egg. These fertilised oocytes can then develop into embryos and be transferred to the mother to establish a pregnancy.

**Debate on ethics of such treatments**
Admittedly, there is controversy associated with these techniques. Some people regard these techniques as cloning. Although they use the technology that produced ‘Dolly the Sheep’, they do not produce an identical individual as s/he is produced from the mother’s and father’s chromosomes. Dolly was produced from a single adult cell introduced into an egg. However, through M-ST and PNT, the baby will have three genetic parents. S/he will inherit his or her chromosomes from the mother and father, as is normal following natural fertilisation and IVF. However, the ‘third parent’ is the mitochondrial DNA mother who donated the egg.

Whilst some groups regard these procedures as unacceptable, others believe that the significant benefits outweigh the unacceptability. This is because there is the potential to eradicate these terrible diseases. Indeed, there have been two important reports

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that support these procedures. However, they insisted that some important reservations were implemented before the techniques are used to produce children. Firstly, the UK’s Nuffield Council on Bioethics stated: “If further research shows these techniques to be sufficiently safe and effective, we think it would be ethical for families to use them if they wished to, provided they receive an appropriate level of information and support.”

The other key statement came from the UK’s regulator of all fertility procedures, the Human Fertilisation and Embryology Authority (HFEA), which was commissioned by the Secretary of State for Health to seek public opinion. This year, it reported that: “... there is general support for permitting mitochondria replacement in the UK, so long as it is safe enough to offer in a treatment setting and is done so within a regulatory framework.”
These reservations are very important. If met, they will ensure that no mutant mitochondrial DNA is transferred with the chromosomes into the donor egg. This will ensure that there is no risk of even low levels of mutant mtDNA being preferentially replicated and the baby suffering from mitochondrial disease. Furthermore, they will ensure that the baby would not suffer from any harmful side effects of the technology.

Scientists are not proposing to conduct any of these approaches without the appropriate regulations in place. Firstly, scientists are proposing to do the work in animal models to demonstrate that they are absolutely safe.

“Recent developments using IVF technologies are now opening up new research avenues that could prevent mitochondrial DNA disease from being transmitted from the mother to her children.”

They will not demonstrate this in just one animal model but several and in those that have the most relevance to humans. Just as important, they are working with governments to ensure that any procedures performed in the human are under the control of the respective regulators. For example, in the UK, scientists have been working with Government to legalise these procedures. This will ensure that they are regulated by the HFEA. Equally so, scientists in the USA would operate under the jurisdiction of the FDA, which regulates all medical and drug procedures.

Looking ahead for Australian families
If mitochondrial DNA disease is to be eradicated in Australia then Australian law will need to embrace these technologies. The Australian Mitochondrial Disease Foundation’s recently released position statement welcoming further research adds to the weight of support from many scientists as well as people affected by mitochondrial disease, who advocate for the opportunity to develop safe and effective methods to prevent transmission of the disease.

Nevertheless, significant progress will need to be made in order to determine the safety and effectiveness of these technologies and they require extensive validation. Once validated, they could prevent mitochondrial disease from being passed from the mother to her children and to subsequent generations. The female children will not have to undergo the dilemma that current carriers do.
Seeking every parent’s wish: a healthy baby

The first Rhonda Murray’s family knew of mitochondrial disease was when her brother Peter became progressively ill from his mid-thirties with fatigue, vagueness, hearing loss and eye problems. Following his diagnosis, tests showed Rhonda, her two sisters, her brother and their mother also had the genetic defect that causes a debilitating and potentially fatal form of maternally inheritable mitochondrial disease called MELAS (Mitochondrial Encephalomyopathy, Lactic Acidosis and Stroke-like episodes syndrome).

“I was pregnant with our eldest daughter Annie, who’s now 14, not long after Peter had his first stroke-like episode. We had Annie’s cord blood tested and hoped for the best, but our baby had the defective gene too,” says Rhonda.

“Later, my husband and I agonised about having a second child. Mito affects every individual differently, from no health problems to severe illness, so we finally decided to go ahead and had our daughter Cassie, who’s now 12. We’ll discuss testing with her when she’s older.”

The Murray family diagnoses were an eye-opener that seemed to explain a family history of ill health, early deaths, miscarriages and still births. Rhonda’s mother died in 2011, aged 70, after having a serious stroke-like episode; she started going deaf in her forties and became tired very easily. Now 47, Rhonda is also losing her hearing and has fatigue, which raises the spectre of whether her health will decline like her mother and Peter, who died in 2009.

“Both our daughters are currently well, but there’s an added level of anxiety when they seem tired or get sick… I think, is this the first sign of mitochondrial disease?”

“Being able to bear a healthy child is something most Australian women take for granted, so I hope for my daughters’ sakes that IVF technology becomes available that enables them to have children free of mitochondrial disease.

“Mitochondrial disease is a dark cloud hanging over our family, so preventing mito being passed to future generations is a prospect we welcome with open arms. We hope the Australian government follows the lead of the UK and moves to support and legalise research to make this technology a safe and effective reality for families like ours.”
Currently, for women at risk of maternally inheritable mitochondrial disease, the only reproductive options to have a healthy child are donor egg donation or, in some cases where the exact gene mutation is known, prenatal diagnosis or IVF using pre-implantation genetic diagnosis (PGD). However, these options are not available to many women at risk because most of their eggs may be carrying substantial amounts of a mitochondrial DNA mutation. Also, PGD can only reduce and does not eliminate the risk of mitochondrial disease in the resulting child, or of her passing the disease to her children.

A further issue is that mitochondrial disease is so often undiagnosed or misdiagnosed that many people unknowingly pass the disease on to their children.

“Dion wasn’t growing or putting on weight, was lethargic, often had vomiting and diarrhoea and eventually just couldn’t eat at all without medical intervention,” says Tracy.

“We cried rivers of tears when we finally got the diagnosis after an MRI showed lesions in Dion’s brain, and then 12 weeks of waiting on muscle, liver and skin biopsies to confirm he had a fatal form of mitochondrial disease.”

Tracy and Warren eventually decided to try for another baby, a brother or sister for their daughter Erin, thankfully unaffected by mito. Tracy became pregnant naturally in 2010, but genetic tests showed the foetus had the genetic defect and they made the difficult decision to terminate the pregnancy.

“Having IVF using pre-implantation genetic diagnosis was our only alternative to taking another chance with a normal conception.

“Thanks to advances in mitochondrial disease research – and the legacy of Dion’s diagnosis and biopsy samples – scientists could identify the specific gene mutation responsible.

Tracy and Warren Taprell’s son Dion died in June 2009 just after his third birthday, having been diagnosed at 20 months with a severe form of mitochondrial disease called Leigh syndrome, which affects the central nervous system.
Specialists were then able to test the embryos for a defective copy of the gene.”

This accurate identification was vital because mitochondrial disease can be caused by multiple genetic defects (at least 26 are known for Leigh syndrome), may involve mutations in the nuclear and/or mitochondrial DNA, and can have different inheritance patterns.

On 18 November 2011, Tracy gave birth to a healthy little boy named Levi. And, on 9 August 2013, the family welcomed a new baby, Bree, also conceived using PGD.

“I know PGD isn’t an option for many women who carry mitochondrial disease. We are just so grateful to have been able to have two more children who are healthy and have their whole lives ahead of them.”
Glossary of terms

ACIDOSIS:
Elevated amounts of organic acids in the blood, which accumulate when food is not properly metabolised.

ADP:
Adenosine diphosphate; the low energy product produced when ATP releases energy to the cell.

APHASIA:
Impaired or absent language function, usually referring to speech; which results from an injury to brain structures usually in the dominant hemisphere (the side of the brain that controls language function is usually the side opposite to the handedness of the person and is referred to as the dominant hemisphere by definition).

ATAXIA:
Un-coordination; inability to coordinate the muscles in voluntary movement.

ATP:
Adenosine triphosphate; cellular energy is stored in the third phosphate bond. ATP is formed from ADP and phosphate in the process known as oxidative phosphorylation.

BETA-OXIDATION:
A series of metabolic reactions necessary for burning fatty acids (fats)

CARNITINE:
Responsible for the transport of long chain fatty acids into mitochondria.

CLONUS:
An abnormal movement characterised by rapid contraction and relaxation of muscles.

COENZYME Q:
(Ubiquinone): A vitamin cofactor responsible for transferring electrons in the oxidative phosphorylation system.

COMPLEX I:
The first component of oxidative phosphorylation, which transfers electrons from NADH to Coenzyme Q.

COMPLEX II:
(Succinate dehydrogenase). The third component of oxidative phosphorylation, which transfers electrons from succinate to Coenzyme Q.

COMPLEX III:
The third component of oxidative phosphorylation, which transfers electrons from Coenzyme Q to cytochrome c.

COMPLEX IV:
(Cytochrome c oxidase, COX). The fourth component of oxidative phosphorylation, which transfers electrons from cytochrome c to oxygen.

COMPLEX V:
(ATP synthase). The final component of oxidative phosphorylation, which uses the proton gradient generated by Complexes I, III and IV to convert ADP to ATP.

COX:
Cytochrome c oxidase (Complex IV).

CPEO:
Chronic Progressive External Ophthalmoplegia Syndrome; the combination of ptosis and restricted eye movements is referred to as ophthamoplegia.
**DEMENTIA:**
Loss of cognition and mental functions due to a disease or disease process.

**DNA:**
Deoxyribonucleic acid; a two-stranded molecule that contain the genes that provide the blueprint for all of the structures and functions of a living being. Most human DNA is nDNA, which is a huge molecule that is folded tightly and stored in the nucleus of the cell. MtDNA is a much smaller molecule stored in the mitochondria.

**mtDNA:**
Mitochondrial DNA contain the genes that code for some of the enzymes and some of the necessary molecules needed to make those enzymes of the respiratory chain. Mitochondria are the only part of the body cell with their own separate and unique DNA. Regardless, most of the mitochondria and the respiratory chain are coded by nDNA. MtDNA is inherited only from the mother.

**nDNA:**
Nuclear DNA; located in the nucleus of the cell, this DNA contains the blueprints for cells which make up the body.

**RNA:**
Ribonucleic acid (RNA) is one of the essential macromolecules for life; it regulates how genes are turned on and made into proteins. RNA is present in all cells and organelles within cells such as mitochondria. Defects in RNAs or the regulation of RNAs can cause or contribute to many important human diseases including mitochondrial diseases.

**DRUSEN:**
Tiny yellow or white accumulations of extracellular material that build up in the eye.

**DYSPHASIA:**
Lack of coordination in speech, and failure to arrange words in an understandable way; due to brain lesion. Aphasia is the complete or near complete absence of speech, and is used to describe a more severe situation than dysphasia.

**ELECTRON TRANSPORT CHAIN:**
See respiratory chain.

**ENCEPHALOPATHY:**
Any disease of the brain.

**ENZYME:**
A protein that speeds up a chemical reaction or causes a chemical change in another substance. Enzymes do their work without being changed or used up in the process.

**GENE:**
The fundamental unit of heredity. Genes are located on strands of DNA found in the cells and mitochondria.

**HYPOTONIA:**
Poor muscle tone, such as seen in “floppy babies”.

**LACTATE or LACTIC ACID:**
A chemical that is formed when sugars are broken down for energy without the presence of adequate oxygen. Lactic acid cannot be used by the body and will accumulate in blood and urine. Lactic acid causes the muscle pain when one runs too fast for too long. In people with mitochondrial disorders, lactic acid forms when the oxidative capacity (ability to burn foods using oxidative phosphorylation) of the person is impaired.

**LEIGH DISEASE OR SYNDROME:**
Subacute Necrotizing Encephalomyelopathy; a form of mitochondrial disease associated with neurodegeneration, usually with onset in infancy.
**LHON:**
Leber Hereditary Optic Neuropathy; a form of mitochondrial disease associated with blindness, usually with onset in adulthood.

**MELAS:**
Mitochondrial Encephalomyopathy Lactic Acidosis and Stroke-like episodes; a form of mitochondrial disease.

**MERRF:**
Myoclonic Epilepsy and Ragged-Red Fibre disease; a form of mitochondrial disease.

**METABOLISM:**
The process of cells burning food to produce energy. This is similar to a car’s engine (the cell’s mitochondria) burning petrol (the food we eat) to produce the energy or torque that turns the drive train that spins the car’s wheels (the energy we need to move and think).

**MITCHONDRIA:**
A part of the cell (organelle) that is responsible for energy production. The organelle consists of two sets of membranes: a smooth continuous outer coat and an inner membrane arranged in tubules or in folds that form plate-like double membranes (cristae). The principal energy source of the cell, containing the cytochrome enzymes of terminal electron transport and the enzymes of the citric acid cycle, fatty acid oxidation, and oxidative phosphorylation. Responsible for converting nutrients into energy as well as many other specialised tasks.

**MITCHONDRIAL ENCEPHALOPATHY:**
Disease process characterised by mitochondrial dysfunction in the brain.

**MNGIE:**
Mitochondrial Neuro-Gastro-Intestinal disorder and Encephalopathy; a form of mitochondrial disease.

**MUTATION:**
A change in the DNA sequence, which can disrupt the normal function of a gene.

**MYOCLONUS:**
A single spasm or twitching of a muscle. Myoclonus can be a single event (twitch) or repeated events. Myoclonus can be a normal event (the jerks that occur when we fall asleep) or an abnormal event (those that occur while awake, or those associated with seizures or mitochondrial diseases). Clonus is the repeated spasms of muscles, due to a seizure or increased muscle tone.

**MYOPATHY:**
Any abnormal conditions or disease of the muscle tissues, which include the muscles over our bones (skeletal muscle) and the heart (cardiac muscle).

**NARP:**
Neuropathy, Ataxia and Retinitis Pigmentosa; a form of mitochondrial disease

**NYSTAGMUS:**
Involuntary, erratic eye movements

**OPHTHALMOPEGIA:**
A paralysis or weakness of one or more of the muscles that control eye movement.

**OXIDATIVE PHOSPHORYLATION:**
The mitochondrial enzymes comprising complexes I, II, III and IV, which generate the mitochondrial electron and proton “gradient”, plus complex V, which utilises this gradient to drive conversion of ADP to ATP.

**PERIPHERAL NEUROPATHY:**
Damage to the nerves of the peripheral nervous system (i.e. outside of the brain and spinal cord). Symptoms depend on the type of nerves affected and where they are located in the body. May be associated with varying combinations of muscle weakness, loss of balance, cramps and spasms (motor
nerves); tingling, numbness, pain, usually in the hands or feet (sensory nerves); abnormal blood pressure and heart rate, reduced ability to perspire, constipation, bladder dysfunction (autonomic nerves).

**POINT MUTATION:**
The substitution of one nucleotide for another nucleotide in a gene. This can result in changing the amino acid sequence of the protein encoded by a gene or affect the expression of the gene.

**PTOSIS:**
Droopy eyelids.

**RESPIRATORY CHAIN:**
Also known as Electron Transport Chain: The mitochondrial enzymes (also known as complexes I, II, III and IV) that are needed to generate the electron and proton "gradient" across the mitochondrial inner membrane.

**RETINOPATHY:**
Persistent or acute damage to the blood vessels inside the retina, causing vision loss and blindness.

**SEIZURES:**
Disturbances of brain function, manifested as episodic impairment or loss of consciousness, abnormal movement, or sensory disturbances. Caused by paroxysmal disturbances in the electrical activity of the brain.

- **Myoclonic:** Seizures characterised by jerking a body extremity or generalised tonic-clonic seizures within an hour or two of waking from sleep.
- **Partial:** (Formerly known as focal seizures.) The seizure is limited to one area in the brain. During this type of seizure, the patient may experience a range of strange or unusual sensations including sudden, jerky movements of one body part, distortions in hearing or seeing, stomach discomfort, or a sudden sense of fear. Partial seizures are classified as either simple or complex. In simple partial seizures, there is no loss of consciousness. In partial complex seizures, consciousness is impaired.

- **Petit-mal:** Now called generalised absence seizures. These are characterised by 5 to 15 second lapses in consciousness. During an absence seizure, the child appears to be staring into space and the eyes may roll upwards. Absence seizures typically occur in childhood and resolve in adolescence. Absence seizures are rare in adults.

**SODIUM VALPROATE:**
Anticonvulsant drug.

**STATUS EPILEPTICUS:**
A life-threatening condition in which the brain is in a state of persistent seizure for longer than five minutes.

**UBIQUINONE:**
Coenzyme Q.
Further reading


**Websites**

Australian Mitochondrial Disease Foundation: www.amdf.org.au

United Mitochondrial Disease Foundation: www.umdf.org

Association of Genetic Support of Australasia: www.agsa-geneticsupport.org.au

ClinicalTrials.gov (worldwide registry and results database): www.clinicaltrials.gov


Human Genome Organisation: www.hugo-international.org

Mitochondria Research Society: www.mitoresearch.org

MITOMAP human mitochondrial genome database: www.mitomap.org

OMIM (Online Mendelian Inheritance in Man - catalog of human genes and genetic disorders): www.omim.org

Rare Mitochondrial Disease Service for Adults and Children: www.mitochondrialncg.nhs.uk
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