Symposium: Mitochondria and human conception

New approaches to the treatment of mitochondrial disorders

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Abstract

Mitochondrial disorders are among the most common inherited metabolic diseases and the issue of treatment arises on a regular basis. There is no established treatment for mitochondrial disorders and current management is largely supportive, but recent advances in our understanding of the pathophysiology provide hope for novel treatments. Patients with mitochondrial myopathy due to mutations of mitochondrial DNA (mtDNA) may benefit from treatments that move normal mitochondrial genomes from the muscle satellite cells into skeletal muscle, but there are concerns about the long-term effects of this approach. A greater understanding of the pathophysiology of a number of nuclear genetic mitochondrial disorders suggests new avenues for treatment (such as copper-histidine in children with *SCO2* gene mutations, and strategies modifying intra-mitochondrial nucleoside pools in the various disorders of mtDNA maintenance). A number of different strategies are also being explored at the molecular level, including the use of antigenomic molecules to mutated mtDNA and the allotropic expression of mutated mtDNA genes within the cell nucleus. Nuclear transfer techniques also provide hope for women at risk of transmitting pathogenic mtDNA mutations.

Keywords: *gene therapy, mitochondrial disease, mitochondrial encephalomyopathy, mtDNA*

Introduction

Recent epidemiological studies have established that mitochondrial disorders are among the most common inherited metabolic diseases, affecting at least 1 in 8000 of the general population (Chinnery *et al*., 2000; Darin *et al*., 2001). They therefore cause substantial morbidity and premature mortality, and the issue of treatment arises on a weekly basis in most large clinical centres.

Mitochondria are essential intracellular organelles that are the main source of ATP. ATP is a high energy phosphate molecule that is required for all active cellular processes. ATP is produced by the mitochondrial respiratory chain which is situated on the inner mitochondrial membrane. The respiratory chain is composed of over 70 interacting polypeptide units that form five complexes. Thirteen polypeptides are encoded by mitochondrial DNA (mtDNA, **Figure 1**), but the remainder are synthesized within the cytosol from nuclear gene transcripts (**Figure 2**). The abnormal mtDNA transmission during assisted reproduction, degradation of mRNA after fertilization, and relationships between spindles, mitochondria and redox potenetial are discussed elsewhere in this symposium by St John *et al*. (2003), Sutovsky *et al*. (2003) and Eichenlaub-Ritter *et al*. (2003).

In general terms, mitochondrial disorders can be divided into two clinical and genetic groups. Clinically, some patients present with well-defined clinical syndromes (**Table 1**), but others present with unique phenotypes. These usually involve tissues and organs that are highly dependent upon oxidative phosphorylation such as skeletal muscle, the brain, the eye, myocardium and endocrine organs. Adults who present with mitochondrial disease usually harbour a primary pathogenic defect of mtDNA. These patients are usually heteroplasmic (i.e. individuals harbour a mixture of mutant and wild-type mtDNA). Heteroplasmic mtDNA mutations only cause a biochemical defect when the percentage level of mutant mtDNA exceeds a critical threshold level (the threshold effect) (Schon *et al*., 1997) (**Figure 3**). Some adults presenting with

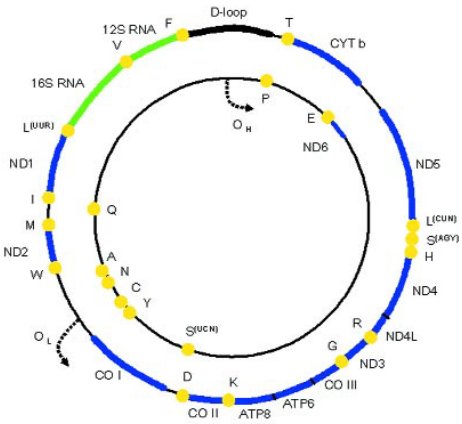

Figure 1. The human mitochondrial genome. The human mitochondrial genome (mtDNA) is a small 16,569 kb molecule of double stranded DNA. mtDNA encodes for 13 essential components of the respiratory chain. *ND1–ND6*, and *ND4L* encode seven subunits of complex I (NADH-ubiquinone oxidoreductase). *Cyt b* is the only mtDNA encoded complex III subunit (ubiquinol-cytochrome *c* oxidase reductase). *COXI* to *COXIII* encode for three of the complex IV (cytochrome *c* oxidase, or COX) subunits, and the *ATP6* and *ATP8* genes encode for two subunits of complex V (ATP synthase). Two ribosomal RNA genes (12S and 16S rRNA), and 22 transfer RNA genes are interspaced between the protein-encoding genes. These provide the necessary RNA components for intra-mitochondrial protein synthesis. D-loop = the 1.1 kb non-coding region which is involved in the regulation of transcription and replication of the molecule, and is the only region not directly involved in the synthesis of respiratory chain polypeptides. O_H and O_L are the origins of heavy and light strand mtDNA replication.

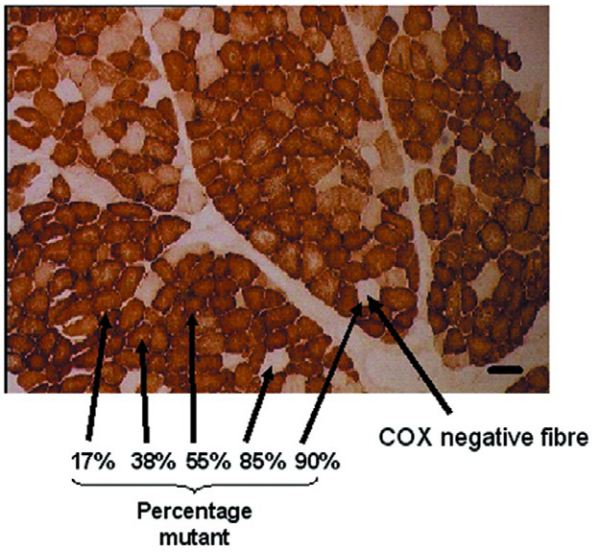

Figure 3. Muscle histochemistry from a patient with a pathogenic mitochondrial DNA defect. Transverse section through vastus lateralis showing cytochrome c oxidase activity within individual muscle fibres. Normal COX activity = brown, COX-deficient = white. Arrows show the percentage of mutated mtDNA within individual skeletal muscle fibres, illustrating the 'threshold effect' – a high percentage level of mutant mtDNA is required (>85% for this mutation) before the cell expresses a COX defect. These appearances are characteristic of patients with a primary pathogenic mtDNA mutation. Scale bar 50 μm.

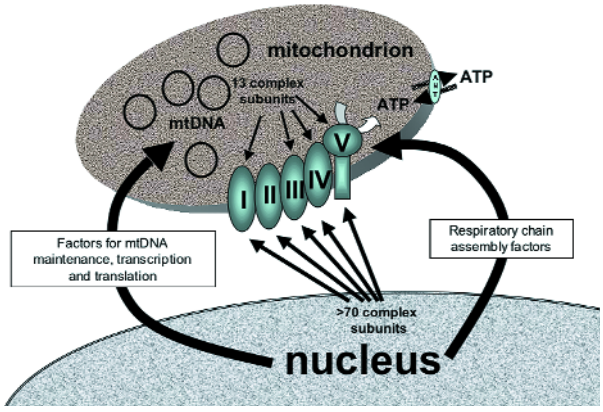

Figure 2. Nuclear–mitochondrial interactions and the respiratory chain. The mitochondrial respiratory chain has a dual genetic basis. Thirteen essential polypeptide subunits are synthesized within the mitochondrial matrix from mitochondrial DNA (mtDNA) transcripts, and ~1000 different mitochondrial proteins are synthesized within the cytosol from nuclear gene transcripts. These proteins include over 70 nuclear-encoded respiratory chain polypeptides. Nuclear genes may also influence mitochondrial function through an effect on mtDNA replication, transcription and translation, and the assembly of an intact respiratory chain is dependent upon nuclear-encoded assembly factors. Nuclear gene-encoded mitochondrial proteins are also involved in many other processes including intermediary metabolism, cellular calcium homeostasis, and the induction of apoptotosis through the release of cytochrome c (cyt c). It is likely that many other perhaps unknown nuclear genes will be important in maintaining mitochondrial homeostasis, either directly through an effect on the respiratory chain, or indirectly through a more general effect on mitochondrial function. (ANT = adenine nucleotide transferase).

primary mitochondrial disease are found to have a nuclear genetic disorder associated with the formation of secondary mtDNA mutations that are important in the pathogenesis (for example, autosomal dominant chronic progressive external ophthalmoplegia due to mutations in the genes *POLG1*, *C10orf2*, or *ANT1* (Kaukonen *et al*., 2000; Spelbrink *et al*., 2001; Van Goethem *et al*., 2001)). By contrast, children who present with mitochondrial disease usually have a nuclear genetic disorder, which often involves a respiratory complex assembly protein (particularly affecting cytochrome *c* oxidase, for example *SURF1*, *COX10* and *SCO2* (Papadopoulou *et al*., 1999)). These mechanisms are illustrated in **Figure 2**, and the different genes involved are summarized in **Table 2**. Different strategies are being employed to develop new treatments for these two different clinical and genetic groups (DiMauro *et al*., 2000).

Current management of mitochondrial disease

The current clinical management of mitochondrial disease is largely supportive. After making a firm diagnosis at the biochemical or molecular level, the next step is to provide prognostic guidance and genetic counselling for the index case and their family. This is a complex area (Thorburn and Dahl,

Table 1. Clinical syndromes associated with mitochondrial disease.

Syndromes	*Primary features*	*Additional features*
Chronic progressive external ophthalmoplegia (CPEO)	External ophthalmoplegia and bilateral ptosis	Mild proximal myopathy
Infantile myopathy and lactic acidosis (fatal and non-fatal forms)	Hypotonia in the first year of life; feeding and respiratory difficulties	Fatal form may be associated with a cardiomyopathy and/or the Toni–Fanconi–Debre syndrome
Kearns–Sayre syndrome (KSS)	PEO onset before age 20 with pigmentary retinopathy, plus one of the following: CSF protein greater than 1 g/l, cerebellar ataxia, heart block	Bilateral deafness; myopathy; dysphagia; diabetes mellitus and hypoparathyroidism; dementia
Leber hereditary optic neuropathy(LHON)	Subacute painless bilateral visual failure; males:females approx. 4:1; median age of onset 24 years	Dystonia; cardiac pre-excitation syndromes
Leigh syndrome (LS)	Subacute relapsing encephalopathy with cerebellar and brain-stem signs presenting during infancy	Basal ganglia lucencies
Mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes (MELAS)	Stroke-like episodes before age 40 years; seizures and/or dementia; ragged-red fibres and/or lactic acidosis	Diabetes mellitus; cardiomyopathy (hypertrophic leading to dilated); bilateral deafness; pigmentary retinopathy; cerebellar ataxia
Myoclonic epilepsy with ragged-red fibres (MERRF)	Myoclonus; seizures; cerebellar ataxia; myopathy	Dementia, optic atrophy; bilateral deafness; peripheral neuropathy; spasticity; multiple lipomata
Neurogenic weakness with ataxia and retinitis pigmentosa (NARP)	Late childhood or adult onset peripheral neuropathy with associated ataxia and pigmentary retinopathy	Basal ganglia lucencies; abnormal electroretinogram; sensori-motor neuropathy
Pearson syndome	Sideroblastic anaemia of childhood; pancytopenia; exocrine pancreatic failure	Renal tubular defects

2001; Chinnery, 2002), and along with prenatal diagnosis, forms another productive area of research. The subsequent clinical approach involves regular surveillance, looking for known complications of mitochondrial disease (particularly diabetes, deafness, cataracts, ptosis and cardiac involvement), and their management (for example, ptosis surgery and cardiac pacing) (Chinnery and Turnbull, 1997; DiMauro *et al*., 2000). A number of different pharmacological agents and vitamin supplements have been used, or are currently being used in patients with mitochondrial disease (DiMauro *et al*., 2000). There is no robust evidence base for these treatments, although anecdotal reports have shown promise in individual cases (reviewed extensively in Chinnery and Turnbull, 2001). These issues were the focus of a recent multinational workshop on the treatment of mitochondrial disease which led to the publication of a consensus document on the current best practice for the treatment of mitochondrial disease (Chinnery and Bindoff, 2003).

Novel treatment strategies – exercise therapy

Aerobic training has been shown to be of benefit in patients with a prominent muscle phenotype of mitochondrial myopathy (Taivassalo *et al*., 1997), improving symptoms, muscle strength and in-vivo measures of mitochondrial function (particularly phosphorus magnetic resonance spectroscopy), but in one serial study the percentage level of mutant mtDNA increased in the skeletal muscle in some patients (Taivassalo *et al*., 2001). This raises concerns about the long-term consequences of this treatment, and particularly that a short-term benefit may compromise muscle function later in life. A more rigorous trial of the effects of aerobic exercise is currently under way to establish whether this treatment has a sustained response and whether it will be detrimental in the longer term.

Table 2. Gene mutations causing human mitochondrial diseases.

Mitochondrial genetic disorders

Rearrangements (large-scale partial deletions and duplications)[a]

Chronic progressive external ophthalmoplegia (CPEO)
Kearns–Sayre syndrome
Diabetes and deafness
Pearson marrow-pancreas syndrome
Sporadic tubulopathy

Point mutations

Protein-encoding genes
LHON (G11778A, T14484C, G3460A)
NARP/Leigh syndrome (T8993G/C)
tRNA genes
MELAS (A3243G, T3271C, A3251G)
MERRF (A8344G, T8356C)
CPEO (A3243G, T4274C)
Myopathy (T14709C, A12320G)
Cardiomyopathy (A3243G, A4269G, A4300G)
Diabetes and deafness (A3243G, C12258A)
Encephalomyopathy (G1606A, T10010C)
rRNA genes
Non-syndromic sensorineural deafness (A7445G)
Aminoglycoside induced non-syndromic deafness (A1555G)

Nuclear genetic disorders

Disorders of mtDNA maintenance

Autosomal dominant progressive external ophthalmoplegia (with secondary multiple mtDNA deletions)
Mutations in adenine nucleotide translocator *(ANT1)*
Mutations in DNA polymerase γ *(POLG)*
Mutations in Twinkle helicase (*C10orf2*)
Mitochondrial neuro-gastrointestinal encephalomyopathy (with secondary multiple mtDNA deletions)
Mutations in thymidine phosphorylase *(TP)*
Myopathy with mtDNA depletion
Mutations in thymidine kinase *(TK2)*
Encephalopathy with liver failure
Mutations in deoxyguanosine kinase *(DGK)*

Primary disorders of the respiratory chain

Leigh syndrome
Complex I deficiency – mutations in complex I subunits (*NDUFS2,4,7,8* and *NDUFV1)*
Complex II deficiency – mutations in complex II flavoprotein subunit *(SDHA)*
Leukodystrophy and myoclonic epilepsy
Complex I deficiency – mutations in complex I subunit (*NDUFV1*)
Cardioencephalomyopathy
Complex I deficiency – mutations in complex I subunit (*NDUFS2)*
Optic atrophy and ataxia
Complex II deficiency – mutations in complex II flavoprotein subunit *(SDHA)*

Disorders of mitochondrial protein import

Dystonia-deafness
Mutations in deafness-dystonia protein DDP1 *(TIMM8A)*

Disorders of assembly of the respiratory chain

Leigh syndrome
Complex IV deficiency – mutations in COX assembly protein (*SURFI*)
Complex IV deficiency – mutations in COX assembly protein *(COX10)*
Cardioencephalomyopathy
Complex IV deficiency – mutations in COX assembly protein *(SCO2)*
Hepatic failure and encephalopathy
Complex IV deficiency – mutations in COX assembly protein *(SCO1)*
Complex IV deficiency – mutations in protein affecting COX mRNA stability (*LRPPRC)*
Tubulopathy, encephalopathy and liver failure
Complex III deficiency – mutations in complex III assembly *(BCS1L)*

[a]Mitochondrial DNA (mtDNA) nucleotide positions refer to the L-chain, and are taken from the 'standard Cambridge' sequence.
AD = autosomal dominant, AR = autosomal recessive, M = maternal, S = sporadic, XLR = X-linked recessive.

Patients with a pure mitochondrial myopathy may benefit from 'gene shifting' procedures. This is possible because some patients with mitochondrial myopathies due to mtDNA defects have low levels of mutated mtDNA in skeletal muscle satellite cells. Satellite cells are small muscle precursor cells that are situated adjacent to the normal skeletal muscle fibre. The proliferation of satellite cells can be induced by myotoxins (for example, bupivacaine (Clark *et al*., 1997)), surgery (Fu *et al*., 1996), or eccentric exercise (lengthening contractions (Taivassalo *et al*., 1999)). It is thought that the satellite cells fuse with the mature muscle fibres and deliver wild-type mtDNA to the affected fibres. This approach has been shown to reduce the overall proportion of mutant mtDNA within single cells to subthreshold levels, thereby correcting the biochemical phenotype (Clark *et al*., 1997; Fu *et al*., 1996). This provides us with hope of 'physiological' gene shifting through exercise therapy, which may correct the biochemical defect within muscle cells in the short term, but again there are concerns that this may be detrimental over longer periods of time because of satellite cell exhaustion (as is seen in the muscular dystrophies), or because the level of mutant mtDNA may increase over time. While promising, this approach is unlikely to help patients with severe central neurological features, which are the main cause of morbidity and mortality in mitochondrial patients as a whole.

Treatments at the laboratory experimental stage

A number of different approaches are currently being explored in cell culture systems or in animal models. Part of the difficulty in developing new treatments for mitochondrial disease is the lack of good animal models. This has recently been addressed by a number of groups, and there are now a number of good animal models of mitochondrial disease available for study (summarized in **Table 3**).

'Gene therapy' for mtDNA disorders

Many mtDNA mutations are heteroplasmic, and there have been various attempts to reduce the percentage level of mutant mtDNA within cells. Early strategies aimed at delivering wild-type mtDNA into mitochondria, (in the form of a self-replicating plasmid), provided initial promise (Seibel *et al*., 1995), but have subsequently been fraught with difficulty. An alternative approach has been to use antigenomic molecules targeted to mutant mtDNA within mitochondria by a peptide presequence (Chinnery *et al*., 1999) or lipophilic cations (Muratovska *et al*., 2001). Unlike nuclear DNA, mtDNA replicates continuously, even in non-dividing cells. The aim of this approach is to block the replication of mutant mtDNA, while leaving wild-type mtDNA replication intact, and thereby allowing the percentage of wild-type to increase over time. This approach works *in vitro* (Taylor *et al*., 1997), but despite a sustained effort it has not been possible to manipulate the level of heteroplasmy in primary cell lines from patients with mtDNA disorders (Taylor *et al*., 2000). An alternative method of removing mutant mtDNA is to deliver restriction enzymes that specifically recognize and destroy pathogenic genomes (Tanaka *et al*., 2002). There have been more positive results using this technique on cultured cells, but this strategy has limitations because of difficulties designing and expressing restriction endonucleases that are specific for the pathogenic mtDNA mutations found in humans.

Alternative strategies for mtDNA disorders

Rather than tackle the problem at the level of the mitochondrial genome, others have used a different approach to tackle the biochemical defects caused by the pathogenic mtDNA mutations. One approach has been to express normal mitochondrial protein within the cytoplasm by nuclear-genetic engineering (so-called allotopic expression) (Sutherland *et al*., 1994). This was recently attempted for the mtDNA *ATPase 6* gene which is mutated in neurogenic weakness with ataxia and retinitis pigmentosa (NARP) (Manfredi *et al*., 2002; Ojaimi *et al*., 2002), and the *ND4* gene which is mutated in Leber hereditary optic neuropathy (Guy *et al*., 2002) (see **Table 1** for a description of these disorders). An alternative has been to try and increase the amount of normal tRNA within the mitochondrial matrix for disorders that involve mtDNA and tRNA gene mutations (Kolesnikova *et al*., 2000).

A major obstacle that stands in the way of effective treatment for mtDNA disorders is how to deliver an effective agent into mitochondria throughout the body of a living organism. It is difficult to see how this problem will be overcome, particularly in the light of the problems facing conventional nuclear gene therapy strategies.

Arguably the 'best' strategy for treating mtDNA disorders is therefore to give a healthy mitochondrial 'transplant' at an early stage in development, correcting the underlying genetic defect and the biochemical defect in one fell swoop. In principle this could be achieved by the transfer of the nucleus from a fertilized oocyte to a healthy donor cytoplast (nuclear transfer). It is important to stress that this approach is not cloning, because it would not involve nuclear gene manipulation and the offspring would remain genetically distinct from their parents. This strategy is currently being studied in mice, but is at a very preliminary stage.

Nuclear genetic mitochondrial disorders

Recent advances in our understanding of two groups of disorders provides some hope for treatment. In a proportion of cases, childhood onset cardioencephalomyopathy due to cytochrome *c* oxidase deficiency is due to mutations in the *SCO2* gene (Papadopoulou *et al*., 1999). *SCO2* is involved in the delivery of copper to cytochrome *c* oxidase, and the addition of copper-histidine to cultured cells from patients with *SCO2* mutations improved the biochemical defect (Jaksch *et al*., 2001; Salviati *et al*., 2002). Copper-histidine has been used in the treatment of Menke's disease with some success (Christodoulou *et al*., 1998), and the same treatment may also help children with *SCO2* mutations.

Recent studies in patients with autosomally inherited mitochondrial disorders have identified a number of diseases that appear to be due to a disturbance of nucleoside metabolism within mitochondria. Autosomal recessive mitochondrial neurogatrointestinal encephalomyopathy (MNGIE) is due to mutations in the thymidine phosphorylase gene (Nishino *et al*., 1999), and most families with autosomal dominant chronic progressive external ophthalmoplegia (AD-PEO) transmit mutations in one of three genes: *POLG1, ANT1 and Twinkle* (Kaukonen *et al*., 2000; Spelbrink *et al*., 2001; Van Goethem *et*

Table 3. Animal models of mitochondrial disease (adapted from Larsson and Rustin, 2001).

Type	*Mouse model*	*Gene(s)*	*Biochemical abnormality*	*Phenotype*
Nuclear gene knockouts	Adenosine nucleotide translocase	*ANT1* (Graham *et al*., 1997)	Defect of coupled respiration	Myopathy and cardiomyopathy
	Mitochondrial superoxide dismutase	*SOD2* (Li *et al*., 1995)	Mitochondrial superoxide deficiency	Myopathy and cardiomyopathy
	Mitochondrial transcription factor A			
	Germ line	*TFAM*, germ line (Larsson *et al*., 1998)	Respiratory chain defect	Embryonic lethal. Abnormal development with absence of heart and optic disc
	Heart-specific	*TFAM*, tissue-specific (Wang *et al*., 1999)	Respiratory chain defect	Dilated cardiomyopathy and cardiac conduction block
	Pancreatic β-cell-specific	*TFAM*, tissue-specific (Silva *et al*., 2000)	Respiratory chain defect	Diabetes
	COX assembly protein SURF1	*SURF1* (Agostino *et al*., 2003)	Respiratory chain defect	Embryonic lethality
	Thymidine phosphorylase	*TP* (Haraguchi *et al*., 2002)	Reduction in liver TP activity	None
Mitochondrial DNA	BALB/NZB heteroplasmic	NZB/BALB mtDNA (Jenuth *et al*., 1996)	None	Random drift during transmission. Tissue specific selection of different genotypes.
	Chloramphenicol resistance	CAP-R T2443C mtDNA (Sligh *et al*., 2000)	None	Myopathy, cardiomyopathy, perinatal death
	ΔmtDNA	4.7 kb mtDNA deletion (Inoue *et al*., 2000)	Respiratory chain defect	Growth delay, nephropathy, myopathy
Spontaneous mutants	Defect of nuclear–mitochondrial communication	Not known (Johnson *et al*., 2001)	None	Deafness

al., 2001). These two groups of disorders are associated with the formation of multiple secondary mtDNA deletions within post-mitotic tissues. More recently, mutations in the thymidine kinase gene *TK2* and deoyguanosine kinase gene *dGK* have been identified in children with mtDNA depletion syndromes (Mandel *et al*., 2001; Saada *et al*., 2001). All of these disorders appear to be due to a disturbance of mtDNA maintenance, and it is possible that manipulating the levels of free nucleotides within the mitochondrial matrix will be a useful treatment (for example, lowering thymidine levels in patients with MNGIE). Vitamin B12 may also prove useful because of its effect on nucleoside metabolism (Chinnery and Bindoff, 2003).

Conclusions

Throughout the 1990s we saw major advances in our understanding of the molecular pathology of mtDNA disease, and more recently in our understanding of nuclear genetic mitochondrial disorders. There is no doubt that this has been of great benefit to patients and their families, providing an accurate diagnosis, forming the basis for genetic counselling, and alerting clinicians to the possible complications of mitochondrial disease that can be managed appropriately. Although there are currently no universally accepted disease-modifying treatments for mitochondrial disease, there is now real hope for effective treatments in the near future. It is therefore fundamentally important to form multinational cohorts of patients and to collect natural history data on the various mitochondrial diseases, ready for new treatment trials as and when the new agents become available.

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