A neurological perspective on mitochondrial disease

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Disruption of the most fundamental cellular energy process, the mitochondrial respiratory chain, results in a diverse and variable group of multisystem disorders known collectively as mitochondrial disease. The frequent involvement of the brain, nerves, and muscles, often in the same patient, places neurologists at the forefront of the interesting and challenging process of diagnosing and caring for these patients. Mitochondrial diseases are among the most frequently inherited neurological disorders, and can be caused by mutations in mitochondrial or nuclear DNA. Substantial progress has been made over the past decade in understanding the genetic basis of these disorders, with important implications for the general neurologist in terms of the diagnosis, investigation, and multidisciplinary management of these patients.

Introduction

Over 20 years ago, Holt and colleagues reported the first association between a defect in mitochondrial DNA and human disease, and this was quickly followed later the same year by a second report from Wallace and colleagues. Since then, the number of disease-associated mitochondrial DNA mutations has expanded rapidly and mutations have been identified that cause classic mitochondrial syndromes such as mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS), myoclonic epilepsy and ragged red fibres (MERRF), Kearns-Sayre syndrome, and maternally inherited Leigh syndrome. The importance of nuclear genetic mutations in causing mitochondrial dysfunction and human disease has become increasingly clear over the past decade. Indeed, the putative role of mitochondrial respiratory chain deficiency in the pathogenesis of a wide range of neurological disorders has been the subject of intense scientific scrutiny. In this Review, we discuss mitochondrial respiratory chain disease in the context of clinical neurology, providing an overview of not only the neurological aspects but also the multisystem effects of mitochondrial disease that neurologists must consider in both children and adults with this disorder. The genetic aetiology of mitochondrial disease has a substantial effect on the investigations requested by treating physicians and the type of counselling that is provided to families. We have therefore discussed the underlying genetics in depth, along with diagnostic and management strategies.

Mitochondria and mitochondrial genetics

Mitochondria undertake many vital metabolic functions, probably the most important of which is oxidative phosphorylation, the principal method for generating ATP. This process is dependent on five intramembrane complexes and two mobile electron carriers (coenzyme Q, and cytochrome c), which transport electrons between them. Supercomplexes (ie, respirasomes) are combinations of two or more respiratory chain complexes that can further enhance electron transfer. Although their role in the in-vivo action of the human mitochondrial respiratory chain remains contentious, evidence in favour of a multimeric organisation is accumulating.

An interesting legacy of the primeval origins of mitochondria is the persistence of a 16-6 kb, double-stranded circle of DNA (mitochondrial DNA). This semi-autonomous genome encodes 13 structural subunit polypeptides and the machinery (22 transfer RNA molecules and 2 ribosomal RNA molecules) necessary for intramitochondrial protein synthesis. Mitochondrial DNA is present in multiple copies, and in any single cell a small number of these genomes might contain mutations; however, the proportion of mutated DNA is usually so small that for practical purposes the tissue can be regarded as homoplasmic (genetically uniform). By contrast, for several mitochondrial DNA mutations, tissue variation in the level of heteroplasmy (the existence of two or more distinct mitochondrial genomes at high concentrations within the same tissue) has a direct effect on the resultant phenotype and even small decreases in the concentrations of wild-type mitochondrial DNA might be sufficient to cause disease. However, the variable or single organ phenotypes that occur with homoplasmic mutations and the apparent dominant nature of some mitochondrial transfer RNA (mitochondrial tRNA) mutations suggest that other, as yet undefined, factors are also important in determining the phenotype. Although nuclear genetic mutations causing mitochondrial dysfunction have been associated with several so-called new clinical phenotypes, some nuclear gene mutations can result in clinical phenotypes that are similar to those in primary mitochondrial DNA disease and the distinction between the two is not always clinically obvious.

Prevalence of mitochondrial disease

Recent estimates of prevalence suggest that mitochondrial disease is more common than previously thought. Both the mitochondrial tRNA mutations MTTL1, m.3243A>G and MTRNR1, m.1555A>G (aminoglycoside-induced deafness) have frequencies of up to 1 in 400 in the general population, but many patients with these mutations remain asymptomatic. Clinical prevalence studies report that mitochondrial disease caused by mutations in mitochondrial DNA affects 9.2 in 100 000 adults aged less than 65 years, and up to 16.5 in 100 000 people aged less than 65 years who have a
maternal relative with mitochondrial disease.\textsuperscript{9} These studies did not include nuclear genetic mutations and are therefore probably rather low estimates of the true prevalence of mitochondrial respiratory chain disease.

**Recent developments in mitochondrial genetics**

**Point mutations of mitochondrial DNA**

Novel point mutations in mitochondrial DNA are still being identified by use of high-throughput sequencing technology, some 22 years after the first mutation was identified.\textsuperscript{2} Most mitochondrial DNA mutations occur in a few families worldwide, but some, such as those that cause Leber’s hereditary optic neuropathy (LHON), and the m.3243A>G mutation (in the \textit{MTTL1} gene), account for a large proportion of cases of mitochondrial disease. A disproportionately large number of these point mutations occur in mitochondrial tRNA genes according to the human mitochondrial genome database MITOMAP. Determining the pathogenicity of novel mutations in these mitochondrial tRNA genes can be difficult,\textsuperscript{2} particularly if the mutations are associated with unusual characteristics such as dominance,\textsuperscript{15} skewed segregation within a tissue, or homoplasmy.\textsuperscript{15} Other areas of the mitochondrial genome, such as the \textit{MTND} genes, which encode structural subunits of complex I, are also mutation hotspots.\textsuperscript{23,24} Although some mitochondrial DNA mutations are readily transmitted through the germline (eg, primary LHON mutations), many others are sporadic and recurrent.\textsuperscript{23,24} Some of these sporadic mutations are present at high rates of tissue heteroplasmy, but cause severe or fatal early-onset disease, such as Leigh syndrome (subacute necrotising encephalopathy), which limits their transmission. The phenotypes of many mitochondrial DNA mutations, although sometimes severe, usually occur in late childhood or early adolescence and females survive to reproductive age; thus all offspring are at risk of inheriting the mitochondrial DNA mutation. The proportion of cells, particularly oocytes, that inherit the mutation depends on the mitochondrial genetic bottleneck: a sharp developmental reduction in the amount of mitochondrial DNA present in primordial germ cells, which is a variable filter that influences the transmission of mitochondrial DNA mutations.\textsuperscript{25-27}

**Nuclear basis for mitochondrial disease**

Nuclear genes encode most information necessary for manufacturing the individual structural subunits of mitochondrial respiratory chain complexes. Mutations have been identified in several of the so-called structural genes for complex I,\textsuperscript{28-34} complex II,\textsuperscript{35-37} complex III,\textsuperscript{38} and complex IV.\textsuperscript{39} Furthermore, mutations in genes encoding assembly factors and chaperone proteins, which are essential for the synchronised formation of the complexes, have been identified for complex I,\textsuperscript{40-44} complex II,\textsuperscript{45} complex III,\textsuperscript{46-48} complex IV,\textsuperscript{49-53} and complex V.\textsuperscript{54} Recently, a mutation in the apoptosis-related gene \textit{FASTKD2} has been associated with complex IV deficiency,\textsuperscript{55} which further extends the range of nuclear–mitochondrial interactions. Although some of the resulting clinical problems, such as cardiomyopathy, encephalopathy, and Leigh syndrome, are indistinguishable from those caused by mitochondrial DNA mutations, other clinical phenotypes are unique to nuclear gene mutations (table): three such examples are growth retardation, aminoaciduria, cholestasis, iron overload, lactic acidosis, and early death (GRACLE syndrome), which is caused by mutations in the \textit{BCS1L} gene;\textsuperscript{56} leukoencephalopathy with brainstem and spinal cord involvement and high brain lactate (LBSL), which is associated with mutations in the mitochondrial aspartyl-tRNA synthetase gene \textit{DARS2};\textsuperscript{57} and the deafness-dystonia syndrome (Mohr-Tranebjaerg syndrome), which results from mutations in the \textit{TIMM8A} gene.\textsuperscript{57} Understanding of nuclear–mitochondrial interactions is limited, but over the past decade the major pathological consequences of disruption or malfunction of this interaction have become apparent. The mitochondrial genome has no capacity for independent replication and relies on the nuclear-encoded DNA polymerase gamma, Twinkle helicase, and several other proteins, including \textit{ANT1} and thymidine phosphorylase, which are involved in the transport and metabolism of substrates necessary for mitochondrial DNA synthesis. Defects in this replication and repair machinery result in qualitative defects (multiple deletions of mitochondrial DNA), quantitative defects (mitochondrial DNA depletion), or both, depending on the mutated gene (figure 1). For some genes (eg, \textit{POLG} and \textit{PEO1}) the site of the mutation determines the defect in mitochondrial DNA.\textsuperscript{58}

**Qualitative and quantitative deficiencies of mitochondrial DNA**

Mutations in nuclear genes encoding parts of the mitochondrial DNA replication and repair machinery can lead to multiple deletions of mitochondrial DNA molecules of variable sizes or to a decrease in the total amount of mitochondrial DNA within a tissue (DNA depletion; figure 1). Both of these changes result in unpredictable, but often multiple, respiratory chain deficiencies and are associated with several different genetic mutations and clinical phenotypes, a number of which are specific to children.\textsuperscript{59} Depletion syndromes often seem to be tissue-specific, with both hepatocerebral and encephalomyopathic forms.\textsuperscript{59} In addition to the genes involved in mitochondrial DNA replication and repair, there is an emerging group of disorders that indirectly lead to multiple mitochondrial DNA deletions, mitochondrial DNA depletion, or both, in which the primary defect is one of mitochondrial dynamics.\textsuperscript{60} Mutations in the \textit{OPA1} gene, a cause of dominant optic atrophy,\textsuperscript{61} have also been associated with ophthalmoplegia, deafness, dysphagia, proximal myopathy, ataxia, and peripheral (predominantly sensory) neuropathy.\textsuperscript{62} At the cellular level, mutations in \textit{OPA1} impair mitochondrial...
and this is also true for mutations in MFN2, the commonest cause of Charcot-Marie-Tooth disease type 2A.64

**Diagnosis of mitochondrial disease**

**Clinical evidence**

The clinical presentation of mitochondrial disease is varied and can occur at almost any stage of life, often with involvement of an unusual combination of organs.1 Multisystem involvement in adult patients commonly occurs in the so-called classic mitochondrial syndromes, but, with the exception of Alpers-Huttenlocher syndrome (AHS) and Leigh syndrome, these classic syndromes are less common in young children with mitochondrial disease.65 A detailed family history can be informative, although factors such as mitochondrial DNA heteroplasmy and the genetic bottleneck mean that maternal inheritance might not always be evident from the family tree. Blood tests including lactate measurements, urinalysis, examination of CSF, neuroimaging, neurophysiology, audiometry, cardiology, and ophthalmology are important in defining the phenotype and, together with repeated systematic assessment, can serve as a baseline against which to judge disease progression.66,67

**Laboratory evidence**

In addition to detailing the phenotype, clinical investigation of suspected mitochondrial disease can be used to direct genetic investigations, particularly in cases in which the clinical features are somewhat unusual, for example the mild methylmalonic aciduria in patients with *SUCLA2* mutations.68 However, the laboratory investigation of a skeletal muscle biopsy (figure 2), from which histochemical, biochemical, and genetic information can be obtained, is often crucial for the diagnosis of mitochondrial disease.69

**Histochemical studies**

Histochemical analysis of skeletal muscle can reveal important clues to a mitochondrial cause. A mosaic, or global, pattern of cytochrome c oxidase (COX)-deficient fibres or presence of ragged red fibres (evidence of mitochondrial proliferation) are key histochemical features of mitochondrial disease. However, reliable histochemical assays are not available to assess activity of complexes I, II, or V, and COX defects might only be apparent in affected organs, such as the liver. Also, a small number of COX-deficient fibres (1–2%) is not unusual in patients over 65 years of age, owing to age-related accumulation of somatic mitochondrial DNA mutations, and should not be taken as definitive evidence of mitochondrial disease.

**Biochemical analysis of mitochondrial respiratory chain complexes**

A biochemical diagnosis of mitochondrial respiratory chain deficiency is invaluable in the selection of further molecular genetic testing strategies, particularly in the paediatric population, in whom abnormalities of a single respiratory chain complex are more common (figure 2). Direct spectrophotometric assays of enzyme activities are useful in the initial assessment of respiratory chain function. These measurements can be used to direct genetic investigations, particularly in cases in which respiratory chain enzyme deficiencies are not evident from the family tree. Blood tests including lactate measurements, urinalysis, examination of CSF, neuroimaging, neurophysiology, audiometry, cardiology, and ophthalmology are important in defining the phenotype and, together with repeated systematic assessment, can serve as a baseline against which to judge disease progression.

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<thead>
<tr>
<th>Mitochondrial respiratory chain deficiency</th>
<th>Examples of nuclear genes involved</th>
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<td>Encephalomyopathy</td>
<td>Complex I</td>
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<td>Tubulopathy and hepatopathy (GRACILE syndrome)</td>
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<td>Cardiomyopathy and tubulopathy</td>
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<td>Hepatopathy and seizures (Alpers-Huttenlocher syndrome)</td>
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<td>Enteropathy, neuropathy, and progressive external ophthalmoplegia (mitochondrial neurogastrointestinal encephalopathy, MNGIE)</td>
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<td>Myoglobinuria and seizures (coenzyme Q10 deficiency)</td>
<td>Complex I-III and II-III</td>
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<td>Cardiomyopathy, nephrotic syndrome, deafness, optic atrophy, and ataxia (coenzyme Q10 deficiency)</td>
<td>Complex I-III and II-III</td>
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**Subacute necrotising encephalopathy (Leigh syndrome)**

|                                    | Complex II                        |
|                                    | Complex IV                        |
|                                    |                                  |
| Leukodystrophy                     |                                  |
|                                    |                                  |
| Cardiomyopathy                     |                                  |
|                                    |                                  |
| French-Canadian ethnicity          |                                  |
|                                    |                                  |
| Growth retardation and dysmorphic features | Normal or secondary |
|                                    |                                  |
| Growth retardation, ataxia, and deafness (coenzyme Q10 deficiency) | Complex I-III and II-III |

**Progressive external ophthalmoplegia (progressive external ophthalmoplegia, PEO)**

|                                    | Multiple or variable |
|                                    | POLG, POLG2, PEO1, ANT1, RRMB2 |
| Spinocerebellar ataxia with epilepsy | Multiple or variable |
|                                    | POLG |
| Neuropathy and dysarthria (sensory ataxic neuropathy, dysarthria, and ophthalmoplegia, SANDO) | Multiple or variable |
|                                    | POLG |
| Optic atrophy, ataxia, and deafness (coenzyme Q10 deficiency) | Multiple or variable |

**Isolated myopathy**

|                                    | Complex I-III and II-III |
|                                    | Multiple or variable |
| Ataxia, cerebellar atrophy, and seizures (coenzyme Q10 deficiency) | Complex I-III and II-III |
| Deafness and dystonia (Mohn-Trainbjaerg syndrome) | Normal activities |

**Hereditary spastic paraparesis**

|                                    | Often normal activities |
|                                    | SPG7? |

Clinical features are grouped in relation to a major neurological feature (eg, encephalomyopathy) or clinical presentation (eg, subacute necrotising encephalopathy), and additional features are then grouped according to known biochemical deficiencies and mutations in nuclear genes that might be responsible for the clinical and biochemical phenotype. For example, encephalomyopathy with hepatic failure in combination with complex IV deficiency might suggest a mutation in *SUCLA2*, but with multiple respiratory chain deficiency then mutations in *DGUOK*, *FRM1*, or *MPV17* might be more likely. An additional clinical feature of seizures would raise the possibility of Alpers-Huttenlocher syndrome and POLG sequencing should be considered. *TIMM8A* encodes a translocase situated in the inner mitochondrial membrane and is responsible for transport of proteins into the mitochondrial matrix. *TIMM8A* is an example of a nuclear gene with a clear phenotype (deafness and dystonia) but no mitochondrial respiratory chain deficiency evident in muscle. *15PG7* encodes paraplegin, a metalloprotease complex located on the inner mitochondrial membrane that degrades misfolded proteins and regulates ribosome assembly. Mutations in *15PG7* have a distinct phenotype—hereditary spastic paraparesis—and mitochondrial respiratory chain activity is often normal.

Table: Biochemical and nuclear genetic causes of common neurological manifestations of mitochondrial disease
activities in tissue homogenates are widely used and are readily adaptable for the study of frozen tissue, which enables samples to be transported to specialist centres for analysis. Blue (or clear) native polyacrylamide gel electrophoresis permits assessment of intact complexes in-gel and is particularly useful for investigating complex V activity and for assessing whether the assembly of specific respiratory chain complexes is affected.

Measurement of coenzyme Q₁₀

Deficiency of coenzyme Q₁₀ can result in various clinical phenotypes including isolated myopathy, encephalomyopathy, cerebellar ataxia, and Leigh syndrome. Biochemical analysis of skeletal muscle using linked assays often reveals deficiencies of complexes I–III and complexes II–III. Replacement therapy with high-dose coenzyme Q₁₀ (20 mg/kg per day) as soon as possible might be of clinical benefit. Thus, prompt diagnosis, by measuring coenzyme Q₁₀ levels in skeletal muscle or blood leucocytes, is important. There is a strong correlation between the genotype and observed phenotype for several different genes involved in coenzyme Q₁₀ metabolism, including COQ2, ABC1/ADCK3, COQ9, PDSS1, PDSS2, ETFDH, and APTX. Thus, clinical characterisation of the phenotype is important in deciding which genetic investigations to undertake.

Genetics

The precise genetic tests undertaken and the timing of these tests in the diagnostic process are greatly influenced by the clinical phenotype and histochemical and

Figure 1: Relation between genetic and biochemical findings in mitochondrial disease

(A) Maternally inherited mtDNA (green circles, semicircles, and fragments) can have large-scale single deletions, rearrangements, or point mutations (yellow star). If a threshold level of heteroplasmy is exceeded, activity of complexes I, III, IV, or V will be variably affected depending on the site of the mutation or size of the deletion. Complex II activity remains unaffected.

(B) Autosomal dominant inheritance of a nuclear DNA mutation (red star) in genes responsible for mtDNA replication machinery (polymerase gamma and Twinkle helicase) results in multiple deletions of mtDNA, with variable effects on the activity of complexes I, III, IV, and V. Complex II activity remains unaffected because the effect of the mutation is exerted through proteins synthesised by mtDNA. Some autosomal dominant mutations, in genes such as OPA1 and MFn2, affect mitochondrial integrity, and can cause either mtDNA depletion or multiple deletions.

(C) Autosomal recessive mutations (homozygous or compound heterozygous) in nuclear DNA genes that encode polymerase gamma and Twinkle helicase, might lead to either depletion or multiple deletions of mtDNA. Again, because these autosomal recessive mutations affect mtDNA, the resulting biochemical abnormalities might affect complexes I, III, IV, and V alone, or in various combinations, but complex II activity is unaffected. (D) Autosomal recessive mutations in nuclear DNA genes encoding protein subunits of respiratory chain complexes, proteins governing the assembly of complexes, or the electron carrier coenzyme Q₁₀, can cause isolated biochemical deficiencies of the corresponding complex or, in the case of coenzyme Q₁₀, a biochemical abnormality evident on a linked assay of complexes II and III. mtDNA copy number remains normal and there is no evidence of multiple deletions. mtDNA=mitochondrial DNA.
biochemical findings (table). Except for patients in whom a classic mitochondrial syndrome is suspected, making a genetic diagnosis in the index case directly from blood or urine specimens without a muscle biopsy is difficult and is not recommended. Once such exception is mitochondrial neurogastrointestinal encephalomyopathy (MNGIE), a syndrome with clinical features of ophthalmoplegia, ptosis, severe gastrointestinal dysmotility, peripheral neuropathy, and leukoencephalopathy. MNGIE usually manifests in the second decade of life and is not caused by defects in the replication machinery but by mutations in TYMP, the gene encoding thymidine phosphorylase. Disruption of the salvage pathway for thymidine leads to an imbalance of the deoxynucleotide pool that is necessary for mitochondrial DNA synthesis, which results in multiple deletions, point mutations, and depletion of mitochondrial DNA. The diagnosis of MNGIE is most easily confirmed by detection of high levels of thymidine (or deoxyuridine) in plasma and urine or by identification of decreased thymidine phosphorylase activity in buffy coat preparations of blood samples, followed by sequence analysis of the TYMP gene.

**Multiple mitochondrial DNA deletion syndromes**

Mutations in the POLG gene are the most common cause of multiple mitochondrial DNA deletions and are associated with various clinical phenotypes, including sensory ataxic neuropathy, dysarthria, and ophthalmoplegasia (SANDO), spinocerebellar ataxia with epilepsy (SCAE), autosomal dominant and recessive forms of progressive external ophthalmoplegia (PEO), and MELAS. Mutations also occur in genes encoding ancillary elements of the mitochondrial DNA replication machinery, such as Twinkle helicase (encoded by the PEO1 gene) and the adenine nucleotide transporter (ANTI gene). Although ptosis and ophthalmoplegia associated with mutations in ANTI and PEO1 are clinically indistinguishable from those caused by...

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**Figure 2: Algorithm for investigation of mitochondrial disease**

Whenever possible, mitochondrial disease should be assessed in an affected tissue such as the liver, heart, or skeletal muscle. In-vivo access to some of these tissues is often not possible and muscle biopsy serves as an alternative even though clinically there might be no evidence of myopathy. Skeletal muscle biopsy is important in the diagnosis of mitochondrial disease. In the context of common features of adult-onset mitochondrial disease, the histochemical analysis of skeletal muscle is particularly important in directing genetic investigation, and biochemistry is often unnecessary for establishing the diagnosis. In some circumstances, however, biochemistry might be necessary (eg, insufficient histochemical sample) or it might be possible (eg, patient has a specific phenotype) to move directly to biochemistry or molecular genetics (dashed arrows).
mutations in POLG, additional features such as epilepsy (POLG) and neuropathy (POLG and PEO1) might help to decide the order in which these genes are sequenced.

Mitochondrial DNA depletion syndromes
AHS is a form of hepatocerebral mitochondrial DNA depletion that occurs in infants and young children. AHS is associated with a sudden onset of focal or generalised seizures, developmental delay or regression, and hepatic dysfunction. Hepatic dysfunction is often terminal, but can predate the seizures, and progression of the dysfunction can be increased by the use of sodium valproate therapy. Neuroimaging reveals thalamic and parieto-occipital cortical atrophy, which result from the spongiform degeneration of grey matter. Many mutations in POLG have been associated with AHS, often with involvement of the linker region of this gene. Three mutations—p.A467T, p.W748S, and p.G848S—occur with sufficient frequency that some diagnostic centres screen for these mutations before sequencing the entire gene (figure 2). Although seizures are a less prominent feature of other forms of hepatocerebral deficiency can also cause myopathy and these mutations in mitochondrial DNA and coenzyme Q10 often occur with involvement of the linker region of this gene. Three mutations—p.A467T, p.W748S, and p.G848S—are present. Cardiac arrhythmias are not uncommon in infants and young children. AHS is a form of hepatocerebral mitochondrial DNA depletion that occurs in infants and young children.

Genetic testing of nuclear DNA
New so-called deep sequencing technologies are enabling increasingly sophisticated analysis of the nuclear genome and, with appropriate bioinformatic support, such sequencing might become a mainstream component in the investigation of mitochondrial disease. Careful clinical characterisation of large families with autosomal dominant disease or smaller families with autosomal recessive disease is crucial for successful identification of disease-related genes.

Clinical assessment and surveillance
Baseline assessment
In view of the progressive, often multisystem, nature of mitochondrial disease, the health needs of all patients should be assessed in detail at the time of diagnosis and at regular intervals thereafter. We have developed and validated paediatric and adult clinical disease-rating scales specifically for this purpose. Basic data relating to weight, height, and body-mass index should be recorded at each clinic visit, and in children this data should be plotted on appropriate growth charts.

Cardiac assessment
An experienced cardiologist who understands the progressive nature of mitochondrial disease should assess patients on a regular basis if signs of cardiac involvement are present. Cardiac arrhythmias are not uncommon in mitochondrial disease and electrocardiography should be routinely done. Echocardiography or cardiac MRI should also be done at diagnosis in all patients with mitochondrial disease, irrespective of their symptoms, because hypertrophic cardiomyopathy can remain asymptomatic until it reaches an advanced stage. Some groups of patients with particular genotypes, such as m.3243A>G, seem to be at greater risk of cardiac progression and warrant closer surveillance (unpublished).

Swallowing assessment
Many patients with mitochondrial disease will experience some degree of dysphagia. In a proportion of patients this will progress to an extent at which the risk of aspiration is substantial and nasogastric tube or gastrostomy feeding is necessary. Assessment of patients with dysphagia by a
speech and language therapist, often including a videofluoroscopy, is important to avoid aspiration pneumonia, which can be fatal.99 Swallowing assessment is usually accompanied by dietetic advice regarding the moisture content and texture of suitable foods.

**Ophthalmology assessment**

Various eye signs accompany mitochondrial disease including ptosis, ophthalmoplegia, optic atrophy, retinal hyperpigmentation, strabismus, visual field defects, and nystagmus. Although ptosis can be asymmetrical at onset, eventually both eyes are affected, which results in progressive obscuring of vision. More detailed eye examination is necessary in forms of mitochondrial disease in which the optic nerve is directly involved, such as LHON and autosomal dominant optic atrophy-plus syndrome. Optical coherence tomography is a useful technique in measuring the subtle progression of these diseases.97,98

**Physiotherapy assessment**

Abnormalities of tone, posture, power, and balance complicate mitochondrial disease in patients of all ages. Specially adapted seats, for infants who are unable to sit unsupported, place the child in an upright posture, help them to develop axial tone, and enable them to have a more comprehensive visual engagement with their environment. Management of spasticity and dystonia with splinting, botulinum toxin, and pharmacotherapy is also important and should involve regular physiotherapy assessment. In adult patients, ataxia and proximal weakness are more prominent features and the risk of serious falls is high. The treating physiotherapist should advise the patient on appropriate exercise and walking aids, and assess whether the patient might benefit from a wheelchair. Functional assessment of the patient in their home often reveals areas of difficulty (eg, tasks they are unable to do), which is helpful in planning health care support such as mobility and hygiene aids. The physiotherapist also has an important role in the management of respiratory problems that arise from muscle weakness and aspiration pneumonia.

**Treatment and prevention of mitochondrial disease**

**Specific treatment of mitochondrial disease**

No specific pharmaceutical drugs have been clearly shown in large-scale clinical trials to treat mitochondrial disease effectively.99 However, there are anecdotal reports of improvement in fatigue and relief of myalgia associated with use of coenzyme Q₁₀ and its analogue idebenone.100 In patients with coenzyme Q₁₀ deficiency, coenzyme Q₁₀ replacement therapy can also be beneficial but requires much higher doses.7 Riboflavin is also effective in some patients with complex I deficiency.101 Some patients with MNGIE have had bone marrow transplantation to replace thymidine phosphorylase in bone marrow tissue.102 However, bone marrow transplantation is not suitable for most mitochondrial diseases because the deficiencies that occur are caused not by toxicity but by inherent malfunction of specific proteins located within the mitochondrial matrix. Prescription of arginine for both acute treatment of stroke-like episodes and as stroke prophylaxis is increasing because of reports of success in Japan.103 However, both the number of stroke-like episodes and the interval between episodes is variable in these patients, and the results of these initial studies have yet to be replicated in placebo-controlled, double-blind trials.

Exercise has been tested as a treatment for mitochondrial disease and has shown some success both in mouse models (COX10 knockouts)104 and in patients.105 Endurance training of patients with mitochondrial disease has shown many benefits, including improved oxidative phosphorylation and tissue perfusion, but unfortunately these advantages are lost with cessation of exercise and subsequent deconditioning.106,107 Long-term exercise studies are in progress to assess the potential of this therapy. An alternative so-called gene shifting approach is based on stimulation of muscle regeneration.108 Patients undergo resistance training, involving repeated concentric and eccentric muscle contractions, which is harmful to muscle fibres; the damage incurred is repaired by fusion of muscle fibres with undifferentiated myogenic precursors (satellite cells), which proliferate in response to injury. Satellite cells often have much lower levels of mutated mitochondrial DNA than mature skeletal muscle fibres and fusion of the two cells can be sufficient to lower heteroplasmy to subthreshold levels. Early results from resistance training studies in patients with large-scale mitochondrial DNA deletions suggest that satellite cell proliferation is associated with improvements in muscular strength and is accompanied by a decrease in the proportion of COX-deficient fibres.108

**Epilepsy**

Seizures are a frequent complication of mitochondrial disease in both adults and children. Various seizure types can occur, but myoclonic and focal epilepsy are the most common. Anticonvulsants, such as levetiracetam, lamotrigine, carbamazepine, and clonazepam, alone or

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in combination, are, in our experience, successful in attaining good seizure control in most patients. We use carbamazepine cautiously (with a low threshold for checking serum sodium concentrations if the patient has an increased seizure frequency, is encephalopathic, or is vomiting) in patients with the m.3243A>G genotype because we find that hyponatraemia can occur. Sodium valproate has been associated with liver disease in patients with POLG mutations and is probably best avoided in all forms of mitochondrial disease.41

Cardiomyopathy and arrhythmias
In patients with cardiomyopathy, early intervention with β blockers and an angiotensin converting enzyme inhibitor (or angiotensin II receptor antagonist) is thought to delay progression of cardiomyopathy, as it does in other causes of heart failure.111,112 but large-scale clinical trials of these drugs in patients with mitochondrial disease have not been done. Wolff-Parkinson-White syndrome and conduction block are common rhythm disturbances identified on the electrocardiogram in patients with mitochondrial disease.113,114 Wolff-Parkinson-White syndrome can lead to re-entrant tachyarrhythmia and patients who are repeatedly symptomatic require radiofrequency ablation. Right bundle branch block can progress to trifascicular block115 and ultimately to complete atioventricular block, which necessitates an implanted pacing device.116 Occasionally, cardiac deterioration is rapid and cardiac transplantation occurs before a diagnosis of mitochondrial disease is considered. This is often the case when cardiac involvement is the only, or earliest, manifestation of mitochondrial disease. After cardiac transplantation, survival depends on the degree of multi-organ involvement and the extent of immune-mediated rejection of the new organ.

Respiratory failure
In the terminal stages of mitochondrial disease, respiratory insufficiency can dominate the clinical picture. This can be either as a consequence of central hypopnoea, as occurs in Leigh syndrome, or from weakness of the diaphragm. In the latter, non-invasive positive pressure ventilation can help patients with their breathing and can also help during the additional physical stresses of pregnancy.117

Renal and adrenal dysfunction
Renal involvement leading to electrolyte abnormalities, including hyponatraemia and hypokalaemia, is not uncommon in children with mitochondrial disease, and both proximal and distal renal tubular acidosis have been identified.119,120 In patients with Kearns-Sayre syndrome, electrolyte disturbances should prompt investigation of adrenal function, because replacement therapy might be necessary to avoid life-threatening adrenal crises. In the absence of cerebral or major cardiac involvement and if quality of life of the patient is regarded as good, the diagnosis of mitochondrial disease should not necessarily prevent selection to receive a kidney transplant.

Liver disease
Hepatic failure is rarely, if ever, an isolated manifestation of mitochondrial disease, but might precede the often associated cerebral dysfunction and refractory epilepsy. A few patients with AHS or other forms of hepatocerebral depletion have unwittingly received liver transplants but the survival rates have been extremely poor.119,121

Ocular disease
Optic neuropathy in patients with LHON is often the only clinically and functionally relevant feature of mitochondrial disease. In other patients, optic neuropathy increases the complications associated with multisystem disease. In either case, optic neuropathy is very difficult to treat. The treating ophthalmologist should maximise any residual visual acuity by the use of visual aids and should help the patient to gain access to financial and other social support by formally registering the patient as blind. Corrective ptosis surgery can dramatically improve both vision and appearance, but surgery should be carefully considered and done only by suitably trained professionals because complications of over correction can occur. Surgically adjustable frontalis slings or less invasive ptosis props might be preferable alternatives when an ophthalmologist experienced in ptosis correction is not available. Strabismus with consequent diplopia is a common complication of ophthalmoplegia and can be treated with surgery.122 However, given the progressive nature of the muscle disease, a detailed preoperative assessment of eye movements is essential.

Malnutrition
Bulbar or pseudobulbar complications are common in children and adults with mitochondrial disease. As well as greatly increasing the risk of aspiration pneumonia, dysphagia can result in chronic malnutrition in adults or growth faltering in children. Nasogastric tube feeding is a temporary solution before a gastrostomy can be done. Involvement of speech and language therapists, dietitians, and gastrostomy support nurses is crucial if gastrostomy feeding is to be successful.

Abnormalities of muscle tone
Patients with dystonia and spasticity require specialist physiotherapy as well as pharmacotherapy. Drugs such as tetrabenazine, baclofen, and diazepam can be used, but management with botulinum toxin should be offered only when the extent of the dystonia and spasticity is severe and when patients are also treated with physiotherapy.123 Deep brain stimulation can be done in some patients with severe generalised dystonia that is resistant to drug treatment, but experience of this technique in patients with mitochondrial disease is limited.124 Hypotonia in infants is also difficult to treat and requires intensive physiotherapy that is aimed at developing core motor skills related to posture and balance.
Diabetes mellitus

Diabetes mellitus is a frequent complication of mitochondrial disease and occurs particularly in patients with mitochondrial DNA mutations. Dietary advice, graded exercise, weight loss, and drug treatment are all used to manage the disorder in patients with mitochondrial disease in much the same way that type 2 diabetes is managed in the general population. There is a risk of precipitating or exacerbating lactic acidosis by use of metformin, and this biguanide should be avoided in patients with mitochondrial disease known to have hyperlactataemia. Some patients will require insulin and the local diabetes management team should be involved in the decision-making process, follow-up, and appropriate health surveillance.

Prevention of transmission

For an inheritable disorder for which effective disease-modifying treatment is not available, the possibility of preventing transmission to future generations is one that patients and parents are keen to explore. An accurate presentation of the risk for each pregnancy based on a genetic diagnosis is ideal, but in paediatric mitochondrial disease the genetic diagnosis is often not known. In such circumstances, sequencing of the mitochondrial genome to exclude maternal inheritance is justified. When the genetic defect (mitochondrial DNA or nuclear DNA) is known, antenatal diagnosis, on cells from a chorionic villus biopsy, can be offered. Preimplantation genetic diagnosis is an in-vitro fertilisation-based technique that enables genetic analysis of an individual cell from an eight-cell stage embryo (blastomere) and, for mitochondrial DNA mutations, enables assessment of the level of mitochondrial DNA heteroplasmy. Preimplantation genetic diagnosis is not widely available for mitochondrial DNA disease, but in a situation where all eggs are likely to carry at least some mutations, it does offer the prospect of returning embryos with the lowest amount of heteroplasmy to the womb. An alternative strategy to prevent disease transmission is egg or sperm donation, the latter only being possible when mitochondrial DNA mutations have been excluded. For some prospective parents, preserving their genetic link with the child is of the utmost importance and egg or sperm donation is less acceptable. Pronuclear transfer is a technique that overcomes these difficulties by allowing the transfer of maternal and paternal chromosomes from an oocyte containing a mitochondrial DNA mutation to an enucleated normal donor oocyte; biparental nuclear genetic inheritance is preserved, but mitochondrial DNA is of donor origin. A recent study of the use of human eggs with an abnormal number of pronuclei has shown that after transfer of two pronuclei, less than 2% of mitochondrial DNA is carried over and that this technique has the potential to prevent the transmission of mitochondrial DNA disease in humans. A variation of this technique, using metaphase 2 spindle transfer, has been done successfully in macaque monkeys.

Search strategy and selection criteria

We searched PubMed (1966–November, 2009) using the term “mitochondria” in combination with “disease”, “neurology”, “myopathy”, “epilepsy”, “Leigh disease”, “encephalopathy”, “respiratory chain”, “genetics”, and “lactic acidosis”. Articles were also identified through searches of the authors’ own files. Only papers published in English were reviewed.

Conclusions

In a previous review, we concluded that nuclear–mitochondrial interaction must be important in the expression of mitochondrial disease and suggested that we were edging towards effective therapy. Although research now supports the first of these conjectures, we remain somewhat short of the mark on the latter. Nevertheless, with the development of national cohorts and registries, the potential for doing systematic large-scale studies of possible treatments is slowly becoming a reality. In addition, the fundamental pathogenetic mechanisms underlying mitochondrial diseases can now be explored in mouse and Drosophila disease models, with the real prospect that preliminary trials of novel therapeutic drugs can be done in these models before clinical trials in humans. Developing an effective treatment for mitochondrial disease is an enormous challenge, requiring a cohesive clinical research strategy that is underpinned by a comprehensive understanding of the molecular genetic mechanisms that are involved. With progress being made on so many fronts, it remains an exciting and stimulating time to be practising mitochondrial medicine.

Contributors

All authors jointly decided the concept of the Review. RM wrote 80% of the Review and designed figure 1. RWT wrote 10% of the Review and designed figure 2. DMT wrote 10% of the Review. Both RWT and DMT contributed to regular review of the paper during the writing process by RM.

Conflicts of interest

We have no conflicts of interest.

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