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## **Diagnosis and Therapy in Neuromuscular Disorders: Diagnosis and New Treatments in Mitochondrial Diseases**

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### **Key words**

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**Mitochondrial disease enters the differential diagnosis of a wide range of CNS and PNS presentations. Respiratory chain ATP production is under bigenomic genetic control. Adult mitochondrial diseases are mainly caused by mutations in mitochondrial DNA, and nuclear gene defects usually present with more severe childhood phenotypes. Recently, mutations in certain nuclear genes, for example *POLG*, *MFN2* and *OPA1*, have been associated with an increasing number of adult onset phenotypes. Achieving an accurate diagnosis can be complex and requires the coordinated interplay of clinical assessment, muscle histochemistry, muscle respiratory chain enzymology and genetics. Factors influencing the transmission and expression of mtDNA defects are not fully defined, presenting difficulties in calculating accurate recurrence risks for patients. Curative therapy exists for primary coenzyme Q<sub>10</sub> deficiency. For certain mtDNA mutations new therapeutic strategies, including resistance training, have the potential to reduce mutant mtDNA load. Allogeneic stem cell transplant may produce benefit in the nuclear recessive mitochondrial disorder MNGIE and should be considered in this nuclear driven multiple mtDNA deletion disorder. Supportive therapies in a multidisciplinary team environment are essential to reduce morbidity and mortality.**

## **Introduction**

The mitochondrial respiratory chain is critical for aerobic ATP production and it is perhaps unsurprising that its dysfunction can affect virtually any organ system in the body. Skeletal muscle and brain have an especially high dependence on ATP which partly accounts for the significant frequency of neurological and neuromuscular clinical presentations. The neurologist often has to consider the possibility of a mitochondrial disease in the differential diagnosis of neuromuscular weakness, exercise intolerance or complex central nervous system presentations.<sup>1</sup>

Mitochondrial respiratory chain disease may be more common than previously considered. One recent study indicated possibly 9.2 per 100000 adults in the UK population have mitochondrial DNA (mtDNA) related disease.<sup>2</sup> Although most adult patients with mitochondrial disease harbour mutations in mtDNA, mutations in nuclear encoded genes can cause respiratory chain dysfunction resulting in neurological illness. The majority of the nuclear genes identified present with a neonatal or early childhood onset.<sup>1</sup> Childhood presentations of mitochondrial respiratory chain disease tend to be more severe with more frequent involvement of many organ systems in addition to neurological involvement (e.g. cardiac, renal, hepatic, endocrine and haemopoetic system). Recently mutations in the nuclear gene encoding the catalytic subunit of the mitochondrial DNA polymerase gamma (*POLG*) have been found in adults presenting with one of a number of phenotypes that the adult neurologist is more likely to encounter.<sup>3</sup>

In this review we briefly summarize important aspects of mitochondrial genetics and the range of possible clinical presentations. We then outline an approach to the diagnosis of mitochondrial disease which incorporates the increasing knowledge of the genetic basis of respiratory chain dysfunction. Finally, we discuss issues in relation to clinical management and potential treatments. This review focuses mainly on adolescent and adult onset presentations of mitochondrial disease.

## **The respiratory chain: biochemistry and genetics**

ATP generation through oxidative phosphorylation under aerobic conditions is a key function of mitochondria. Mitochondria contain their own DNA (mtDNA) in the form of a circular double stranded molecule about 16.6 Kb long. Thirteen of the approximately 90 proteins that make up the respiratory chain are encoded in mtDNA and the remainder are nuclear encoded. In addition to genes encoding the 13 proteins involved in the respiratory chain, mtDNA encodes two rRNA subunits and 22 tRNA molecules necessary for intramitochondrial protein synthesis. An elaborate mitochondrial importation process allows the cytosolically

synthesized nuclear encoded proteins to be co-assembled with mtDNA encoded counterparts in the inner mitochondrial membrane. The first genetic defects associated with human neurological disease were in mtDNA but the last few years have seen a dramatic increase in nuclear gene mutations.<sup>1,4</sup>

The mitochondrion is exclusively maternally inherited. Therefore, with the exception of most large scale deletions in mtDNA, defects in mtDNA are maternally inherited. Nuclear gene defects causing mitochondrial respiratory chain diseases follow Mendelian rules of inheritance and may be autosomal dominant, recessive, or X-linked. MtDNA exists in many copies in each cell, in some cases thousands, and is constantly replicating, even in terminally differentiated cells. Normally all copies of mtDNA are identical, a state termed homoplasmy. In contrast, mutated mtDNA commonly coexists with normal [wild-type] mtDNA - a state known as heteroplasmy. With heteroplasmy, a 'threshold level' of mutant load may need to be reached before cellular and tissue function is impaired sufficiently to cause clinical disease. This threshold may vary between cell types. Mutations in mtDNA may take the form of deletions, rearrangements, or point substitutions.

The majority of adult mitochondrial phenotypes associate with primary mutations in mtDNA (Table1). Multiple deletions with autosomal inheritance are suggestive of a defect in nuclear DNA, causing a secondary abnormality in mtDNA. Recently, mutations in the nuclear gene *POLG* have been shown to result in such deletions. A new genetic classification has now emerged (Table 1 provides examples of phenotypes associated with mtDNA or nuclear gene mutations).<sup>1,5</sup>

**Table 1 Genetic classification of the mitochondrial respiratory chain diseases**

<u>DEFECTS IN MTDNA</u>	
<u>Mutations in protein coding genes</u>	Phenotype
Mitochondrial complex I (ND) genes	LHON, MELAS, Leigh syndrome
Mitochondrial complex V (ATPase) genes	NARP, Leigh syndrome
<u>Mutations in protein synthesis genes</u>	
tRNA genes	PEO, myopathy MELAS, MERRF
rRNA genes	Deafness
Rearrangements	Myopathy, PEO, KSS

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## DEFECTS IN NUCLEAR GENES

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### Defects in mtDNA replication

*POLG*

*PEO1*

*SLC25A4*

*TYMP*

### Phenotypes

AD PEO, AR PEO, MERRF, SANDO

AD PEO

AD PEO

MNGIE

### Defects in respiratory chain subunit genes

Complex I, II, III or IV

Encephalomyopathies

### Defects in respiratory chain assembly

Complex I e.g. *NDUFAF1*

Complex III *BCS1L*

Complex IV e.g. *SURF1*

Encephalomyopathy and cardiomyopathy

Bjornstad syndrome (SNHL and pili torti)

Leigh syndrome

### Defects in mitochondrial dynamics

*MFN2*

*OPA1*

CMT2

AD optic atrophy, auditory neuropathy,  
peripheral neuropathy

### Defects in coenzyme Q10 biosynthesis

e.g. *CABC1*

Cerebellar ataxia, exercise intolerance, seizures

### Defects in mitochondrial translation

Aminoacyl tRNA synthetases  
e.g. *DARS2*

LBSL

tRNA modification  
e.g. *PUS1*

Myopathy, sideroblastic anaemia, lactic acidosis

### Defects in mitochondrial import

*TIMM8A*

*SLC25A3*

Deafness dystonia syndrome

Cardiomyopathy, hypotonia, growth retardation

### Defects in mitochondrial membrane lipids

Tafazzin protein

Barth syndrome (cardiomyopathy)

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Abbreviations: LBSL leukoencephalopathy with bilateral striatal involvement and lactate accumulation. See main text for other abbreviations.

## **Neurological clinical presentations of mitochondrial respiratory chain disease**

The clinical presentations of mitochondrial respiratory chain disease are extremely variable. Patients may present with a fatal encephalopathic or acidotic illness in the first few weeks of life, or at the other extreme, with a late onset indolent mild myopathy. Although patients with mitochondrial disease may present to a range of different clinical specialties, because of the potential for a variety of different organ systems to be involved, neuromuscular and neurological presentations are the commonest.<sup>6</sup>

### **Isolated mitochondrial myopathy**

Myopathy is a common finding in patients with mitochondrial disease. It may be isolated or part of a more complex syndrome such as Kearns-Sayre (KSS).<sup>7,8</sup> Myopathy may accompany CNS disease and indeed the coexistence of myopathy with CNS symptoms such as seizures, strokes, myoclonus, ataxia or encephalopathy should prompt consideration of mitochondrial disease. The myopathy is often isolated and may vary widely in severity. Many patients with isolated myopathy present with relatively nonspecific symptoms such as exercise intolerance and fatigue.<sup>9</sup> Examination in such cases may not show clear static weakness at the bedside but often a more “give-way” weakness which may lead the neurologist to suspect chronic fatigue. Other patients have a more typical proximal myopathy with clear fixed weakness on examination. Distal weakness is uncommon but may occur. Facial and bulbar muscles may be affected and this is especially the case in KSS. Some patients begin with an isolated myopathy but subsequently develop associated features due to additional organ involvement e.g. diabetes, deafness, cardiomyopathy or other CNS features listed above.<sup>10</sup>

### **Isolated progressive external ophthalmoplegia**

Progressive external ophthalmoplegia (PEO) in isolation is a common manifestation of mitochondrial disease. Onset may be before 20 or after 50 in most cases. There is a slow evolution of symmetrical extraocular muscle weakness and diplopia is uncommon. Ptosis progresses over time, and measures to raise the eyelids may be required such as eyelid props or sometimes eyelid surgery. A single clonal deletion of mtDNA is the commonest genetic defect in patients with PEO, although point mutations in tRNA genes and multiple deletions of mtDNA may be the cause.<sup>4,7,8</sup>

### **Autosomal dominant or recessive PEO with multiple deletions**

Autosomal dominant or recessive PEO is an adult onset condition caused by nuclear genes which affect mtDNA maintenance, resulting in multiple deletions of mtDNA. In addition to PEO there may be a variety of clinical features including peripheral neuropathy, ataxia, tremor, Parkinsonism, mental depression, cataracts, pigmentary retinopathy, deafness, rhabdomyolysis and hypogonadism. There is also a multiple deletion PEO variant in which there is dramatic sensory ataxia and neuropathy termed SANDO - **S**ensory **A**taxic **N**europathy **D**ysarthria

and Ophthalmoplegia. A number of different nuclear genes have been implicated in these different multiple deletions disorders and one of the most common is *POLG*.<sup>11-14</sup>

### **Kearns-Sayre Syndrome**

KSS is defined by the triad of PEO, pigmentary retinopathy and onset before the age of 20 years, with at least one of the following: high CSF protein greater than 100mg/dL, cerebellar ataxia or cardiac conduction block. It is most commonly caused by single large deletions in mtDNA but has a far more serious prognosis compared to isolated PEO. Patients often have a progressive limb myopathy and frequently require a pacemaker for AV block.<sup>7</sup>

### **Mitochondrial peripheral neuropathy**

A mild axonal sensorimotor neuropathy is a common finding in patients with complex mtDNA-associated mitochondrial phenotypes involving the CNS such as MERRF (**M**yoclonic **E**pilepsy with **R**agged **R**ed **F**ibres) or MELAS (**M**itochondrial **E**ncephalomyopathy, **L**actic **A**cidosis and **S**troke-like episodes). However, neuropathy can be the dominant clinical feature associated with two nuclear mitochondrial genes. Mutations in the *MFN2* gene encoding a protein which influences mitochondrial dynamics have been shown to be a major cause of Charcot Marie Tooth disease (CMT).<sup>15</sup> Mutations in *POLG* can cause a prominent large fibre sensory neuropathy with significant proprioceptive loss in the SANDO syndrome.<sup>3</sup> Axonal sensorimotor polyneuropathy may also be a feature of dominant optic atrophy caused by mutations in the *OPA1* gene encoding a mitochondrial dynamin.<sup>16</sup>

### **Mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes**

MELAS is a serious stroke-like syndrome that most commonly associates with point mutations in mtDNA transfer RNA genes. The commonest is the m.3243A>G mutation in *MT-TL1*, the tRNA gene for Leucine [UUR], accounting for around 80% of MELAS cases. The stroke-like episodes are often dramatic and are different from typical ischaemic strokes in that the patients are encephalopathic. Typically episodes begin with severe “migraine-like” headache with nausea and vomiting. There may be focal or generalized seizures in an episode followed by hemiparesis, hemianopia or cortical blindness. The strokes are often parieto-occipital and do not conform to vascular territories. Patients often experience multiple stroke-like episodes. Dementia, ataxia, deafness, muscle weakness, cardiomyopathy and diabetes are frequent accompaniments. Treatment of acute episodes is supportive, and L-arginine may play a role in prevention, as discussed below.<sup>17-26</sup>

### **Myoclonus epilepsy with ragged red fibres**

MERRF patients experience myoclonus, epileptic seizures, ataxia and muscle weakness. Onset is usually in childhood but adult onset is described. Myoclonus is stimulus sensitive and the seizures may be tonic-clonic and often there is

photosensitivity. There is a common mtDNA mutation m.8344A>G in the *MT-TK2* gene for tRNA lysine associated with MERRF.<sup>27</sup> In addition to myopathy, approximately one third of MERRF cases have significant cardiomyopathy.<sup>28, 29</sup>

### **Mitochondrial neurogastrointestinal encephalopathy syndrome**

MNGIE patients experience a combination of features including PEO, leukoencephalopathy, myopathy, peripheral neuropathy, and gastrointestinal motility problems. The gastrointestinal problems may dominate the clinical picture with recurrent episodes of pseudo-obstruction and often a history of “exploratory” laparotomies. MNGIE is often an aggressive disorder with significant malnutrition developing. It is autosomal recessive and has been linked to mutations in the *TYMP* gene encoding thymidine phosphorylase.<sup>30</sup>

### **Mitochondrial DNA depletion syndrome**

MDDS is an autosomal recessive disorder caused by a quantitative reduction in the amount of mtDNA. A myopathic and a hepatocerebral form are recognized. Both are fatal in childhood, although patients with Navajo neurohepatopathy may survive into their late teens. MDDS may be caused by recessive defects of the mtDNA replication machinery (polymerase gamma or twinkle helicase) or from defects of maintaining the deoxyribonucleoside triphosphate pool necessary for mtDNA replication.<sup>31-36</sup>

### **Leber’s hereditary optic neuropathy**

Patients with LHON develop subacute, usually painless, loss of central vision. It is an important cause of blindness in young men. It is usually bilateral with an inter-eye delay of around two months. The majority of cases have one of three complex I gene point mutations in mtDNA: m.11778G>A, m.3460G>A and m.14484T>C. Recovery of vision is poor but seems to be related to genotype. Patients with m.14484T>C have a better chance of some visual recovery. LHON is usually a clinically isolated visual loss syndrome. However, occasionally it can be associated with extraocular symptoms, including cardiac pre-excitation, a multiple sclerosis like illness or dystonia.<sup>37-42</sup>

### **Leigh syndrome**

Leigh syndrome is a neurodegenerative condition that most commonly presents in infancy or childhood but adult onset presentations are recognized. The pathological process particularly affects the brainstem and basal ganglia with characteristic symmetrical necrotic lesions. Post mortem examination reveals cystic cavitation, demyelination, vascular proliferation and gliosis.<sup>43</sup> The clinical presentation is usually developmental delay and regression. In both childhood and adult onset forms there are combinations of brain stem/basal ganglia signs including nystagmus, ataxia, dystonia, and respiratory abnormalities. The clinical course is progressive and intercurrent infectious illness typically causes a step-wise decline. MRI/CT reveals symmetrical abnormalities (High signal on T2 MRI and hypodensity on CT) in the brain stem and basal ganglia.<sup>44</sup> Leigh syndrome is considered to be caused by a severe failure of respiratory chain function and



ATP production and appears to be the final common pathway of a variety of primary genetic defects. It can result from mtDNA mutations (e.g. high levels of the m.8993T>G mutation) and a range of nuclear defects (e.g. *SURF1* mutations).<sup>44-48</sup>

### **Neuropathy ataxia retinitis pigmentosa**

NARP is usually caused by the m.8993T>G mutation in the gene encoding subunit 6 of complex V of the respiratory chain [*MT-ATP6*]. The original family exhibited retinitis pigmentosa, dementia, seizures, sensory neuropathy, cerebellar ataxia, developmental delay and neurogenic muscular weakness. Subsequent families have been reported to have cardiomyopathy. High levels of this mutation may cause Leigh syndrome, and within families some members may have NARP and others Leigh syndrome.<sup>44,49</sup>

### **Sensorineural deafness**

Sensorineural hearing loss (SNHL) is a common associated feature in many of the mitochondrial encephalomyopathy phenotypes and may occur in MELAS, maternally inherited diabetes and deafness (MIDD),<sup>50</sup> MERRF or KSS. The presence of deafness in a patient with a complex CNS phenotype should be regarded as a clue to a possible mitochondrial disease. Deafness may also occur in isolation. Recently it has been shown that the m.1555A>G mitochondrial mutation (which confers extreme sensitivity to aminoglycoside-induced deafness and which may also cause non-syndromic deafness) is present in 1 in 500 of the general population.<sup>51,52</sup> Mutations in the tRNA gene for serine (UCN) are also described to cause isolated deafness.<sup>53</sup> SNHL is also a feature of some nuclear encoded mitochondrial disorders for example some patients with dominant optic atrophy associated with OPA1 mutations.<sup>16</sup>

## **Diagnostic strategy in suspected mitochondrial disease**

### **Clinical assessment**

The clinical manifestations of mitochondrial disease can vary widely. Careful clinical evaluation is essential. A multidisciplinary approach to investigation and diagnosis is often required (Figure 1). The typical mitochondrial syndromes described can usually be recognized when presenting in their fully developed form and this will trigger appropriate confirmative investigations. In practice, mitochondrial disease needs to be considered when the patient may only have elements of a number of different syndromes. Useful pointers to consider in patients with CNS syndromes that might suggest a mitochondrial aetiology include the coexistence of varying combinations of myopathy, neuropathy, diabetes, cardiomyopathy or deafness. Many of the symptoms that can occur in mitochondrial disease such as strokes, migraine, seizures, dementia, diabetes and deafness are common neurological presentations in isolation but it is the combinations of these symptoms in an individual that point to the possibility of a mitochondrial disease. Family history may indicate other members with full blown

mitochondrial syndromes or suspicious combinations. Maternal inheritance suggests a mtDNA defect but Mendelian inheritance can occur, as can sporadic cases. Routine blood tests are not usually helpful. There may or may not be an elevated lactate and lactate/pyruvate ratio. Creatine kinase is nonspecific and may be modestly elevated in the presence of myopathy but not usually above 1000. EMG may show mild myopathic changes or may be normal. Nerve conduction studies may confirm a mild sensorimotor axonal neuropathy or show more significant changes in patients with CMT2 caused by *MFN2* mutations or SANDO caused by *POLG* mutations. EEG may show epileptiform discharges or may be normal. Brain imaging is frequently abnormal in patients with complex CNS phenotypes. The abnormalities may be nonspecific e.g. cerebral or cerebellar atrophy. Alternatively there may be specific imaging findings of a MELAS stroke in the parieto-occipital region not conforming to a single vascular territory, or symmetrical lesions in the basal ganglia and brainstem in Leigh syndrome.<sup>54</sup>

### **Genetics and DNA-based diagnosis**

In practice if a mitochondrial respiratory chain disease is suspected it is sensible to select the available mtDNA point mutations to be analyzed in a blood sample (Figure 1). Genetic tests for mutations at positions 3243, 8344 and 8993 are now widely available in diagnostic laboratories. It should be noted that the level of m.3243A>G in blood declines with age and may become undetectable over the age of 30.<sup>55</sup> In contrast the levels of m.3243A>G remain constant in urine, which should be tested in addition to blood.<sup>56</sup> Increasingly, diagnostic labs offer other mtDNA point mutations that can be analyzed from blood samples. In a patient under the age of 20 years blood mtDNA deletion analysis can be requested. The hit rate from this limited analysis of blood for mtDNA point mutations and deletions is likely to be low. However, if positive, a secure genetic diagnosis of mitochondrial disease is achieved without the need for further invasive investigations. The majority of LHON cases will have one of the three common point mutations at positions 11778, 14484 or 3460. Table 2 lists common mtDNA mutations associated with adult mitochondrial phenotypes. Although most adult patients with mitochondrial disease harbour mutations in mtDNA, it is increasingly recognized that mutations in nuclear encoded genes can cause respiratory chain dysfunction resulting in neurological disease. To date most of the nuclear genes identified cause severe disease with a neonatal or early childhood onset. However, the discovery that mutations in the *POLG* gene can cause adult mitochondrial disease is important. Patients with *POLG* gene mutations may present with one of a number of phenotypes with late childhood or adult onset phenotypes (see Table 3). *POLG* gene analysis can be undertaken on DNA from blood and should be considered early in the diagnostic evaluation. In patients in whom the muscle biopsy shows a mosaic pattern of histoenzymatic changes and/or the respiratory chain biochemistry is abnormal, full mtDNA sequencing on mtDNA extracted from muscle should be considered, as discussed below.<sup>6</sup>

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**Table 2 Common mitochondrial phenotypes in adults**

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<b>Phenotype</b>	<b>Genetics</b>	<b>Inheritance</b>
PEO	Deletion in mtDNA Multiple deletions ( <i>POLG</i> ) MtDNA point mutations	Sporadic AR/AD Maternal
Myopathy	Deletion in mtDNA MtDNA point mutations	Sporadic Sporadic or maternal
KSS	Deletion in mtDNA	Sporadic
MELAS	MtDNA point mutation (m.3243A>G)	Maternal
MERRF	MtDNA point mutation (m.8344A>G)	Maternal
LHON	MtDNA point mutation (m.11778)	Maternal
Adult LS	mtDNA point mutation (m.8344A>G)	Maternal

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AD, autosomal dominant; AR, autosomal recessive; KSS, Kearns-Sayre syndrome; LHON, Leber's hereditary optic neuropathy; LS, Leigh syndrome; MELAS, mitochondrial encephalomyopathy with lactic acidosis and stroke like episodes; MERRF, myoclonus epilepsy with ragged red fibres; PEO, progressive external ophthalmoplegia. Further details about mtDNA mutations can be found in the MitoMap database [www.mitomap.org](http://www.mitomap.org)

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**Table 3 Phenotypes associated with *POLG* mutations**

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AD PEO with or without parkinsonism  
AR PEO with or without parkinsonism  
Sporadic PEO  
Cataracts  
Alpers syndrome  
SANDO - sensory ataxia, neuropathy, dysarthria, ophthalmoplegia  
MIRAS – mitochondrial recessive ataxia syndrome  
SCAE – spinocerebellar ataxia with epilepsy  
Premature menopause  
Male subfertility  
MERRF  
MELAS

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A full list of genotype-phenotype correlations can be found in the online Human DNA Polymerase Gamma Mutation Database <http://tools.niehs.nih.gov/polg/>.

### **Muscle biopsy – histochemistry, enzymology and genetic analysis**

Unless a specific genetic diagnosis has been achieved by evaluation of DNA extracted from blood, a muscle biopsy is important in the diagnostic evaluation of suspected mitochondrial disease (Figure 1). Histochemical techniques which detect abnormal focal accumulations of mitochondria include the Gomori trichrome stain; the red staining of focal subsarcolemmal mitochondria gives rise to the ragged red fibre (RRF). Evaluation with specific histoenzymatic reactions for succinate dehydrogenase (SDH) and cytochrome c oxidase (COX) are also important. The presence of RRF (which can also be detected by the SDH reaction) or of COX-negative fibres is highly suggestive of a defect in mtDNA. RRF may not be present in young children with mitochondrial disease and may be a normal finding in the muscle of healthy older individuals (>50 years) so expert interpretation, taking into account the age and clinical context, is essential. Most heteroplasmic defects in mtDNA will reveal a mosaic of staining abnormalities for SDH and COX. Combined staining with COX and SDH together may be helpful in highlighting COX-negative fibres containing proliferations of mitochondria. Muscle is a much better tissue than blood in which to search for pathogenic mtDNA mutations. In particular, mtDNA deletions are much more reliably detectable in muscle compared to blood. Even if the muscle histochemistry is normal, it is important to perform specific enzymatic assays of the individual respiratory chain enzymes, particularly in children with suspected mitochondrial disease. This can be undertaken on a small muscle homogenate (fresh or frozen). Multiple respiratory chain enzyme deficiencies most commonly associate with defects in mtDNA which affect intra-mitochondrial protein synthesis (e.g. tRNA mutations or large scale rearrangements) or MDDS. Isolated defects of individual respiratory chain complexes suggest either a nuclear gene defect or a mtDNA mutation in a mtDNA protein encoding gene.<sup>6,52</sup> Combined defect of complexes II+III when assayed together, with normal activities of the individual complexes, raises suspicion of coenzyme Q<sub>10</sub> deficiency. This is because the combined complex II+III assay requires endogenous coenzyme Q<sub>10</sub>, whereas the individual complex II and complex III assays are independent of coenzyme Q<sub>10</sub>.<sup>57,58</sup> Coenzyme Q<sub>10</sub> levels can be determined by HPLC in muscle and peripheral blood mononuclear cells; the latter may be used to monitor treatment with coenzyme Q<sub>10</sub>.<sup>59</sup>

### **Management and Treatment of Mitochondrial Disease**

Patients and families with confirmed mitochondrial disease require management and support in a multidisciplinary clinical team setting. Often this is coordinated by a neurologist with close links to a range of different disciplines as required such as rehabilitation medicine, physiotherapy, occupational therapy, cardiology, endocrinology/diabetes, ophthalmology, audiology and speech therapy.

## **Symptomatic therapy**

Symptomatic therapy is extremely important for patients with mitochondrial disease and can significantly improve quality of life and reduce morbidity. Early recognition of treatable complications is achieved through coordinated clinical surveillance.

### **Hearing**

Monitoring audiological function is important in patients with mitochondrial disease, who may benefit from aids or (in the case of profound hearing loss) from cochlear implantation.<sup>60-62</sup> Maximising audiological function is particularly important in patients who have coexistent visual impairment, for example those with optic atrophy or pigmentary retinopathy.<sup>63</sup>

### **Vision**

Ptosis may be alleviated by prosthetic inserts placed inside spectacles, but surgery may be needed in severe cases. Expert ophthalmic surgical correction for ptosis not only improves vision, but may also enhance psychological wellbeing and ability to function in society. Visual impairment may also result from cataracts, optic atrophy and retinopathy. Occipital lobe infarction in MELAS may result in field defects which may be compounded by cognitive difficulties.<sup>19-21</sup>

### **Seizures and movement disorders**

Epileptic seizures occur commonly in patients with CNS mitochondrial disease e.g. MELAS, MERRF and Leigh syndrome. Most available anticonvulsant drugs can be used with the exception of sodium valproate. Sodium valproate has been shown to inhibit mitochondrial oxidative phosphorylation and may cause clinical worsening, including precipitation of fatal hepatic failure in some cases of Alpers syndrome caused by *POLG* mutations.<sup>64</sup> Intractable myoclonus can be a particular problem in MERRF, where piracetam, leviracetam or clonazepam may be helpful.

Movement disorders may be especially prominent in Leigh syndrome, the commonest being dystonia. Antidystonia oral medications such as anticholinergics or neuroleptics may be helpful. Alternatively EMG-guided botox can be used although it may sometimes result in pronounced weakness.<sup>65</sup>

### **Diabetes**

Screening for mitochondrial diabetes is important. It is usually caused by pancreatic beta cell energetic failure rather than insulin resistance. For this reason, mitochondrial diabetes usually responds to oral hypoglycaemics and/or comparatively low doses of insulin. Metformin should be avoided because of the risk of lactic acidosis.<sup>66</sup> One study suggested that long-term coenzyme Q<sub>10</sub> administration prevented progressive insulin secretory defect, exercise intolerance and hearing loss in MIDD patients.<sup>67</sup>

### **Respiratory**

Respiratory muscle weakness may occur in advanced mitochondrial myopathy and may be compounded by bulbar weakness and aspiration. Patients with KSS and advanced PEO are at particular risk of respiratory muscle weakness resulting in respiratory failure.<sup>68</sup> Diaphragmatic and axial muscle weakness may also occur. FVC monitoring in patients with significant myopathy and KSS is important. Sometimes significant respiratory muscle weakness only comes to light when slow recovery of respiratory function is identified after a general anaesthetic. Bulbar muscle weakness and reduced oropharyngeal tone may predispose to obstructive sleep apnoea. In CNS mitochondrial disease, especially Leigh syndrome, central hypoventilation may develop. In addition to FVC, nocturnal oximetry should be undertaken if nocturnal hypoventilation is suspected. Formal respiratory support may be instituted if significant respiratory dysfunction is identified.

### ***Gastrointestinal***

Gastrointestinal symptoms are not uncommon in patients with mitochondrial disease. These include swallowing difficulties, failure to thrive, weight loss/cachexia, constipation, pseudo-obstruction, nausea and vomiting. Weakness of the bulbar and facial muscles may result in significant swallowing difficulties which may be compounded by cerebellar incoordination. Such dysphagia can be a particular problem in patients with KSS, some PEO patients and in Leigh syndrome. Aspiration can be a significant risk and detailed speech and language assessments are very important, supplemented by videofluoroscopy swallow assessments of swallowing. Percutaneous endoscopic gastrostomy (PEG) can be useful. Nonspecific anorexia and mild or more severe weight loss in the absence of dysphagia may occur. Weight loss can be dramatic, especially in the full blown MNGIE syndrome, when it may be accompanied by recurrent pseudo-obstruction. For severe cachexia, PEG feeding or parenteral nutrition should be considered. Constipation and/or diarrhoea may be a problem, especially in patients with the m.3243A>G mutation. Nausea and vomiting may accompany lactic acidosis.<sup>69</sup>

### ***Heart***

Cardiac screening is important in patients with mitochondrial disease. Pacing or insertion of an implantable cardiac defibrillator may be lifesaving in patients with identified cardiac conduction defects, such as those with KSS and some cases of PEO. Pre-excitation syndromes such as Wolff-Parkinson-White may cause supraventricular tachycardias in patients with m.3243A>G cardiomyopathy and in some patients with LHON. Progressive left ventricular hypertrophy (LVH) may be a particular feature in patients with the m.3243A>G and m.8344A>G mutations and may progress to left ventricular failure. Once identified, LVH should be monitored with ECG and ECHO and treated in conjunction with the cardiologist with agents such as ACE inhibitors.<sup>70</sup>

### ***Genetic counselling, prenatal diagnosis and reproductive options***

If a nuclear gene mutation is identified, genetic counselling according to the Mendelian rules for genetic diseases is appropriate. Prenatal diagnosis for mitochondrial nuclear gene defects is available, provided the causative nuclear gene mutation has been identified.

If an mtDNA mutation is identified in a woman with mitochondrial disease it is more difficult to provide accurate genetic counselling advice. Most large scale deletions of mtDNA are sporadic and the risk of transmission is low, but there is a small risk.<sup>71</sup> Certain point mutations also seem to be sporadic. For heteroplasmic mtDNA point mutations, the factors which determine the amount of a particular point mutation that will be transmitted are poorly understood. Although a heteroplasmic point mutation will be transmitted in the maternal line, because of the genetic bottleneck for mtDNA (where only a small number of mtDNA molecules in the mother are passed on to the next generation), large shifts in the proportion of mutant from mother to offspring may occur. It is therefore not possible to offer women who harbour heteroplasmic disease-causing point mutations accurate recurrence risks. The situation for homoplasmic point mutations (e.g. mutations associated with LHON) is also difficult. Although all the offspring of a mother with a homoplasmic mutation will be homoplasmic for the mutation, they may not all develop the disease. Other, presumably non mtDNA, factors are important in determining disease expression but at present cannot be reliably predicted.

It is our view that until current research efforts reliably identify the factors which influence the recurrence risks for point mutations, the only definite way to avoid maternal transmission is to consider ovum donation. However some preliminary studies of preimplantation genetic diagnosis for the NARP mutation have shown encouraging results.<sup>72</sup> One research approach is focussing on oocyte manipulation techniques to replace maternal mutant mtDNA with donor mtDNA, for example by transferring the pronuclei from an oocyte carrying a mtDNA mutation into an enucleated donor egg, but these studies are still at a very preliminary stage.<sup>73</sup>

Individuals at risk of inheriting a mtDNA mutation may present requesting genetic testing. If they have any symptoms suggestive of mitochondrial phenotype diagnostic genetic testing is appropriate. On the other hand, if they are symptom free the value of offering mtDNA testing for the mutation known to be in the family needs to be carefully considered. Since the correlation between the proportion of a given point mutation in blood and the chances of developing a clinical phenotype in the future are poorly understood it is hard to provide reliable predictive advice. Careful discussion with each patient requesting such predictive testing is required.<sup>71-79</sup>

## **Pharmacological approaches**

### ***Coenzyme Q<sub>10</sub> deficiency***

Coenzyme Q<sub>10</sub> is a lipophilic mobile electron carrier and antioxidant located in the inner mitochondrial membrane. Disorders of coenzyme Q<sub>10</sub> biosynthesis are clinically heterogeneous: presentations include recurrent rhabdomyolysis with

seizures;<sup>57,80</sup> multisystem disorder of infancy with prominent nephropathy;<sup>58,81</sup> ataxia with or without seizures;<sup>82</sup> Leigh syndrome;<sup>83</sup> and pure myopathy.<sup>84</sup> These disorders respond remarkably well to coenzyme Q<sub>10</sub> if treatment is started early but very large doses may be necessary, because of poor uptake into the mitochondrion.

Efficacy of coenzyme Q<sub>10</sub> in other respiratory chain disorders is variable and the results of randomised controlled trials have yielded conflicting results.<sup>85</sup> However, it is our practice to offer coenzyme Q<sub>10</sub> to all patients with a proven diagnosis of mitochondrial disease, initially at a dose of 100 mg three times daily in adults. MitoQ, a synthetic analogue of coenzyme Q<sub>10</sub> tagged with a lipophilic cation for easier uptake into the mitochondrion, does not function as an electron carrier in the respiratory chain, but may be an effective antioxidant.<sup>86</sup>

### ***Other pharmacological approaches***

In contrast to coenzyme Q<sub>10</sub> replacement in primary defects of coenzyme Q10 biosynthesis, no other pharmacological agent is of proven efficacy. A number of pharmacological agents have been tried in mitochondrial disease but with limited benefit, and a recent Cochrane systematic review concluded that there is insufficient evidence to recommend any standard treatment.<sup>85</sup> Although there are anecdotal reports of benefit of various agents (including riboflavin, succinate, L-carnitine, alpha-lipoic acid and vitamins C, E and K), the clinical heterogeneity and unpredictable natural history of mitochondrial disease, with a frequently relapsing and remitting course, means that interpretation of effectiveness of an agent in a single individual is very difficult. The few randomised double blind clinical trials which have been performed yielded inconclusive or conflicting results.<sup>85</sup> Recently, some novel pharmacological approaches have emerged, aimed at stimulating mitochondrial biogenesis via the transcriptional coactivator PGC1 $\alpha$ . Drugs which may stimulate this pathway include bezafibrate and resveratrol.<sup>87-89</sup> Limited data is available about these agents, and controlled clinical trials are planned.

### **Removal or neutralisation of toxic metabolites**

The pathomechanism of MNGIE syndrome (thymidine phosphorylase deficiency) is considered to result from an imbalance of intramitochondrial nucleosides, leading to stalling of the mtDNA replication apparatus. A rationale for treatment is thus to restore intramitochondrial nucleoside balance by removing accumulated nucleosides. In one study renal dialysis was used to remove accumulated plasma thymidine and deoxyuridine in patients with MNGIE. However these metabolites re-accumulated within 24 hours of the dialysis procedure.<sup>90</sup> Diuretics have also been used to increase renal excretion of thymidine and deoxyuridine, but without success.

Bicarbonate may be used to correct acute or chronic lactic acidosis.

Dichloroacetate (DCA) is an inhibitor of pyruvate dehydrogenase (PDH) kinase and thus maintains PDH in its active (phosphorylated) state, resulting in reduced lactate production. DCA can be effective in lowering lactate levels in acute acidotic states. Recently a double blind placebo controlled trial aimed to



investigate efficacy of DCA in the MELAS syndrome. However this trial had to be terminated prematurely because of peripheral nerve toxicity.<sup>91</sup> Peripheral neuropathy developed in all 15 cases, and was reversed on cessation of treatment.

## **Enzyme and metabolite replacement**

### ***Thymidine phosphorylase replacement therapy***

A number of strategies have been used to replace thymidine phosphorylase activity in patients with MNGIE. Replacement of thymidine phosphorylase activity by repeated platelet transfusions produced transient benefit only,<sup>92</sup> as might be expected from the short half life of platelets in the peripheral circulation. Administration of thymidine phosphorylase in encapsulated red cells also resulted in only transient reductions in plasma thymidine levels.<sup>93</sup> So far allogeneic stem cell transplantation has proved to be the most successful method of restoring thymidine phosphorylase activity in MNGIE patients.<sup>94</sup> Small numbers of patients have been treated with this procedure to date. However, a high mortality has been reported, possibly because these transplants have been performed late in the disease course, when the patients are in an advanced cachectic state. Further work and long term follow up are necessary to establish the efficacy of stem cell transplantation in MNGIE. Liver transplantation has been used to attempt to cure other disorders of nucleoside metabolism, such as deoxyguanosine kinase deficiency, with very limited benefit.<sup>95</sup>

### ***L-Arginine therapy in MELAS***

The precise mechanisms leading to stroke-like episodes in MELAS have not been determined but one possible factor is alteration in arterial vascular stability. Effective treatment of acute stroke-like episodes or their prevention has not yet been established. There has been recent interest in the possible role of L-arginine therapy. Based on the possibility that the strokes are caused by impaired vasodilation, the effects of administering L-arginine, a nitric oxide precursor, were assessed in patients with acute MELAS stroke-like episodes. L-arginine was given intravenously in the acute stroke phase or orally at the interictal phase. The authors concluded that L-arginine infusions significantly improved stroke-like symptoms. The authors suggested that oral administration within 30 minutes of a stroke significantly decreased frequency and severity of stroke like episodes.<sup>96</sup> In a further study the same group evaluated endothelial function in patients with MELAS using flow-mediated vasodilation and found a significant decrease in MELAS patients compared to controls. Two years of supplementation with oral L-arginine improved endothelial function to control levels and normalised plasma levels of L-arginine in patients.<sup>97</sup> It was suggested that L-arginine therapy improved endothelial dysfunction and may have potential in the prevention and treatment of stroke-like episodes in MELAS.<sup>98</sup> These studies are of interest but further evaluation and larger studies are required.

### ***Folate deficiency***

Low CSF folate levels were first reported in KSS 25 years ago.<sup>99,100</sup> More recently rapid clinical response to folinic acid was reported in an 8 year old boy with a mtDNA deletion associated with cerebral folate deficiency and leukoencephalopathy.<sup>101</sup> It seems likely that the folate deficiency is secondary in KSS and at present the prevalence of CSF folate deficiency in patients with mitochondrial disorders is not known. If central folate deficiency is suspected CSF must be analysed, because blood folate levels do not accurately reflect CNS folate status. Treatment needs to be with folinic acid rather than folate, since the latter does not cross the blood brain barrier.

### ***Other metabolic approaches***

Other experimental approaches aimed to restore copper in patients with SCO2 mutations, an infantile onset hypertrophic cardiomyopathy syndrome in which copper assembly into COX is impaired. Although in vitro studies yielded promising results, an attempt to treat a patient was unsuccessful.<sup>102,103</sup> Similarly, whilst replacing nucleosides into cells from patients with MDDS looks an attractive possibility the reality is likely to be much more complex.<sup>104</sup>

### ***Gene therapy***

It is possible that conventional gene therapy methods may be used to correct nuclear gene defects of mitochondrial function in the future. However, since potential techniques for nuclear gene therapy are relatively well established, they will not be discussed here. Instead we will focus on gene therapy strategies aimed at eliminating or reducing the quantity of mtDNA mutations.

### ***Resistance exercise training to shift mtDNA genotype***

The proportion of mutant mtDNA in muscle is correlated with the degree of reduction in oxidative capacity. Recently, there has been increasing interest in the role of exercise therapy to improve muscle respiratory chain oxidative capacity by potentially reducing mutant mtDNA load - so called 'gene shifting'. Certain mtDNA mutations such as deletions and some tRNA point mutations are present in high levels in mature skeletal muscle but, for reasons which remain unclear, are absent from the muscle satellite cell population, which harbours only wild-type mtDNA. Previous experimental work has demonstrated that activation of satellite cells has the potential to introduce wild-type mtDNA into the mature skeletal muscle, thereby lowering the proportion of mutant mtDNA and reversing the respiratory chain defect.<sup>105</sup> Certain types of exercise protocols have the potential to induce satellite cell activation and promote entry of wild type mtDNA into mature muscle. Initial studies employed endurance training and demonstrated improved aerobic capacity.<sup>106</sup> Recent studies using 12 week progressive overload leg resistance exercise training protocols demonstrated increased muscle strength and improved muscle oxidative capacity.<sup>107</sup> Although a measurable reduction in deleted mtDNA was not observed in either of these studies the results are encouraging. Further, larger exercise trials are underway.

### ***Other gene therapy approaches for mtDNA mutations***

A number of *in vitro* experimental approaches have recently been reported. An allotropic expression strategy used a mitochondrial targeting sequence added to an ATP6 gene recoded using the nuclear rather than the mitochondrial genetic code, to rescue the NARP phenotype in a cell culture model.<sup>108</sup> Cell growth was restored and ATP synthesis improved. More recently a similar approach was used in a rat model of LHON with an ND4 mutation.<sup>109</sup> Other approaches have attempted to introduce cytosolic tRNAs into the mitochondrion;<sup>110,111</sup> eliminate mutant mtDNAs using restriction enzymes targeted to the mitochondrion;<sup>112</sup> reduce deleted mtDNA molecules in cultured cells by growing in ketogenic conditions;<sup>113</sup> and to shift heteroplasmy with zinc finger nucleases which bind to mutant mtDNA molecules, leading to their selective degradation.<sup>114</sup> All of these approaches have yielded encouraging results but remain at a preclinical stage.

### **Conclusions**

Mitochondrial respiratory chain diseases frequently present to the neurologist with CNS and or neuromuscular symptoms. Accurate diagnosis and management rely on a team approach including multidisciplinary clinical assessment, muscle histochemistry, mitochondrial respiratory chain enzymology and genetics. Most adult onset phenotypes associate with mtDNA mutations and whole genome mtDNA sequencing is increasingly routinely available. Recently, nuclear genes, especially *POLG* and *MFN2* have been shown to be important in adult presentations. Curative therapy is available for only a small proportion of cases but it is especially important not to miss primary coenzyme Q<sub>10</sub> deficiency. Supportive therapy in a multidisciplinary environment is essential for all patients and families with genetic mitochondrial respiratory chain diseases.

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## Figure legend

### Figure 1 Queen Square Mitochondrial Disease Investigation Pathway

<sup>1</sup>mtDNA deletion screen can be performed in blood if patient <20 years old.

<sup>2</sup>Perform respiratory chain enzyme assays even if histochemistry normal if strong clinical suspicion.

<sup>3</sup>Sequence mtDNA even if respiratory chain enzyme assays normal if strong clinical suspicion.

CI complex I; CII complex II; CIII complex III; HCM hypertrophic cardiomyopathy; LS Leigh syndrome.

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# Figure 1 Queen Square Mitochondrial Disease Investigation Pathway

