

Mitochondrial Disease: Old and New

The mitochondrion is an intracellular organelle with the primary function of generating ATP, the energy currency of the cell, by oxidative phosphorylation. It also hosts beta oxidation, the Krebs cycle and other pathways. Mitochondrial dysfunction is particularly prominent in the energy dependent tissues brain and muscle. In some diseases, mitochondrial dysfunction is part of the pathophysiology (e.g. Parkinson's disease); in others a mitochondrial enzyme deficiency is the primary cause of the disease.

The complexity of mitochondrial disease is increased by the presence of copies of mitochondrial DNA (mtDNA) within the organelle. Human mtDNA is a 16.6 kb circular double stranded DNA molecule. It contains 13 genes for protein subunits of the mitochondrial respiratory chain. A further 2 genes encode ribosomal RNA, and there is a complete set of 22 tRNAs. The mtDNA encoded proteins are subunits of respiratory chain complexes I, III, IV and V, while the subunits of complex II are entirely nuclear encoded. Because there are multiple copies of mtDNA within each mitochondrion, cell and tissue, the concept of heteroplasmy arises, in which there is a mix of different mtDNAs. In mitochondrial disease heteroplasmy is common, with normal mtDNA coexisting with abnormal mtDNA.

This article is necessarily selective and describes:

- a scheme of investigation for classical mitochondrial respiratory chain disease (often easy to diagnose)
- a discussion of more recently described mitochondrial disorders and those more difficult to diagnose

Classical mitochondrial encephalomyopathies

It is often stated that mitochondrial disease is clinically and genetically heterogeneous, and is protean in its manifestations. It would therefore be expected that the diagnosis of mitochondrial disease is often overlooked and misdiagnosed. Whereas this may happen, most experienced neurologists meeting a patient with mitochondrial disease will consider the diagnosis in timely fashion, although genetic classification may take somewhat longer. The reason for the diagnostic success lies in the fact that although presentations are highly variable, mitochondrial disease is usually suggested by the cardinal features of classical mitochondrial encephalomyopathies (Table 1).

Mitochondrial disease is not rare, having a prevalence of approximately 10/100000,¹ which ensures that patients appear in general neurology clinics fairly regularly. Although the presenting complaint may be ataxia, ptosis, seizures or one of the other manifestations, these rarely

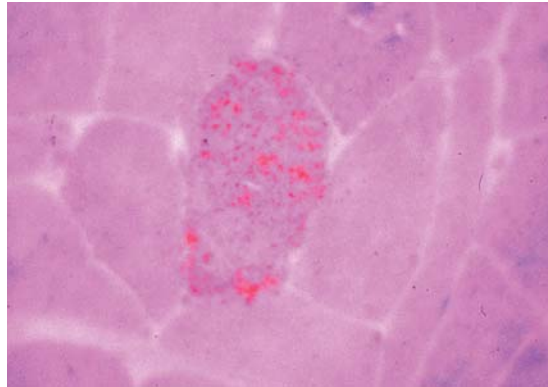


Figure 1: A muscle biopsy of a patient with a heteroplasmic mtDNA deletion. In situ hybridization using a probe detecting deleted mtDNA species showing them to be concentrated within ragged red fibres (RRF).

occur in isolation. For example, a patient may present with ataxia. If additional deafness or ptosis is noted, mitochondrial disease rises to the top of the differential diagnosis. This should lead to a comprehensive review to look for further features in the patient and their family. When the diagnosis is suspected, it may usually be confirmed by the appropriate use of genetic analysis of blood or muscle, and histochemical examination of muscle. Table 2 details the usual scheme of investigation. A genetic diagnosis from blood may be possible, but if not muscle biopsy usually shows focal histochemical abnormalities, with proliferation of mitochondria seen as ragged red fibres on the modified Gomori trichrome reaction. Research techniques such as in situ hybridisation show these abnormal fibres to be populated by mutant DNA (Figure 1). Such fibres react heavily with succinic hydroxylase, which is not mtDNA encoded, but are usually deficient in cytochrome oxidase (COX), which is.

Mitochondrial encephalopathies

Mitochondrial encephalopathies can present at any age. Presentations in childhood have a higher incidence of multisystem disease, with short stature, bone marrow, cardiac and renal involvement often seen. Mitochondrial encephalopathies sometimes conform to the acronyms MERRF (myoclonic epilepsy with ragged red fibres) or MELAS (mitochondrial encephalopathy with lactic acidosis and stroke-like episodes), but more often do not. The value of the acronyms is to provide a mnemonic for some of the more common manifestations of mitochondrial encephalopathy, and the clinical features have an approximate correlation with the two commonest mtDNA point mutations (mtDNA 8344 tRNA^{Leu} and 3243 tRNA^{Leu}(UUR) respectively). However, dementia, ataxia and deafness are also common CNS features and even within families the clinical features vary qualitatively and



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Table 1: Common features of classical mitochondrial disease

Eyes	Ptosis and ophthalmoplegia Retinopathy
Somatic	Diabetes Deafness Short stature
	Cardiac, renal involvement Sideroblastic anaemia
CNS	Dementia Ataxia Myoclonus Seizures Stroke-like episodes

Table 2: Investigation of mtDNA disorders

Presentation	First investigation	Further investigation
PEO, KSS or myopathy	Muscle biopsy	Muscle mtDNA analysis including deletions
Encephalopathies	mtDNA analysis (blood) for common point mutations	Muscle biopsy
Leber's hereditary optic neuropathy	mtDNA analysis (blood)	
NARP/MILS	mtDNA analysis (blood)	

KSS = Kearns Sayre syndrome, NARP = Neurogenic weakness, ataxia, retinitis pigmentosa, MILS = maternally inherited Leigh's syndrome.

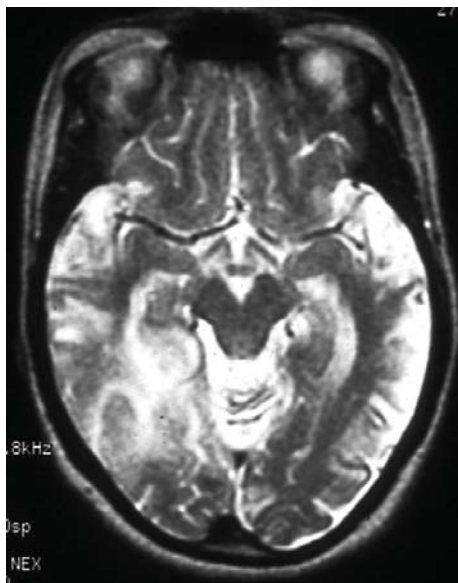


Figure 2: A T2 weighted magnetic resonance brain scan image showing atrophy and infarction in a young adult with MELAS.



Figure 3: A patient with Progressive external ophthalmoplegia (PEO), showing asymmetrical ptosis and squint.

quantitatively. Although screening for the common mutations in blood is suggested as a first investigation, if these are negative a muscle biopsy is required.

In modern practice diagnostic suspicion of mitochondrial encephalopathy may be generated by brain imaging. CT may show basal ganglia calcification. Stroke-like episodes appear on MRI as high signal change on T2 weighted images, particularly posteriorly, not necessarily following arterial territories (Figure 2). Atrophy of the cerebellum and/or cerebrum is common.

Mitochondrial myopathies

Mitochondrial disease may have its major manifestation in muscle. The commonest muscles affected are those of the eyes, with a progressive external ophthalmoplegia (PEO), often consisting of an asymmetrical ptosis and weakness of the extraocular muscles (Figure 3).

PEO is often combined with other clinical features, and can range from late onset pure PEO to more severe variants such as the Kearns-Sayre syndrome. Although some degree of proximal limb weakness is common, a pure limb girdle syndrome without clues such as PEO, deafness, or somatic features is relatively rare. Petty et al² pointed out that if the patient has no CNS manifestations of disease within 5 years of onset, then these are unlikely and the prognosis correspondingly much better than the mitochondrial encephalomyopathies. Muscle involvement rarely causes severe weakness, and loss of mobility secondary to muscle weakness alone is rare.

PEO can be sporadic with very low recurrence risk (single large heteroplasmic mtDNA deletion), or with maternal (heteroplasmic mtDNA mutation), or autosomal inheritance (see below). Thus genetic investigation is important to define recurrence risks.

The commonest abnormality causing mitochondrial PEO is a single large mtDNA deletion. This is present in much larger proportion in muscle, and a muscle biopsy remains the definitive investigation for histochemical studies and genetic analysis.

Therapeutics in mitochondrial disease

A Cochrane review concluded that there was no clear evidence supporting any therapeutic intervention in mitochondrial disorders.³ Exercise training appears to help patients and is probably safe.⁴ Some patients report subjective improvement in muscle symptoms on Coenzyme Q10 or creatine supplements. The author has observed abrupt improvement in treating stroke-like episodes acutely with dexamethasone, but this is unlikely to be trialled. Supportive treatment for the many other manifestations of these disorders remains valuable.

New and more difficult mitochondrial disease

The characteristics of classical mitochondrial disease establish useful guidelines to the clinician and scientist. Maternal inheritance, multi-system disease, focal histochemical abnormalities on muscle biopsy, and heteroplasmic mtDNA are all typical of these diseases. Clinical and molecular diagnosis becomes more challenging when these features are not present. Examples of such patients not conforming to the classical model are increasingly described, and are summarised here

Absence of typical histochemistry

Mitochondrial encephalomyopathies are historically defined by the presence of ragged red fibres (RRF) in muscle, an indicator of pathological focal mitochondrial proliferation.

Diseases without this hallmark or without any histochemical markers of mitochondrial dysfunction are more difficult to identify. The characteristic phenotype of Leber's hereditary optic neuropathy is not associated with mitochondrial histochemical defects, and similarly RRF are not found in the biopsies of NARP patients (which usually show evidence of denervation). More difficult are the few patients with classical mitochondrial phenotypes such as MELAS and others. Such phenotypes are usually associated with mtDNA tRNA mutations, but less commonly can be associated with mutations in protein coding genes. Mutations in protein coding genes such as mtDNA ND5, often do not cause histochemical abnormalities on muscle biopsy.⁵ When abnormalities are found, they are subtle, with increased staining of the succinic dehydrogenase reaction in cytochrome oxidase positive fibres.

Absence of family history or multisystem disease

Some patients with muscle symptoms such as fatigue and exercise intolerance (sometimes with episodic myoglobinuria) also have mutations in mtDNA protein coding genes. Such patients are difficult to identify because there may be no maternal family history and no manifestations outside muscle.⁶ This group of patients do have abnormalities on muscle biopsy which often acts as a spur to more directed mtDNA analysis. Unless the mtDNA mutation involves COX genes, the RRF are COX positive, which is an additional clue.

Absence of mtDNA heteroplasmy

LHON is more often due to mutations in the homoplasmic rather than heteroplasmic state. Homoplasmic mtDNA mutations causing other mitochondrial disease are rare, but has now been described in several families, although the pathogenicity of the mutation is always harder to prove.⁷

No mtDNA defect

MtDNA encodes only 13 of the 90 (approximately) proteins of the respiratory chain, leaving much scope for respiratory chain defects secondary to nuclear gene defects, as well as many other mitochondrial proteins. These disorders may have similar clinical and biochemical features to mtDNA disease and may be difficult to define in molecular terms. Many of these mutations cause severe or fatal early onset disorders, such as SURF1 mutations which are the commonest cause of typical Leigh's disease. These numerous disorders are beyond the scope of this review.

Intergenicomic disease

Whereas the initial thrust of research and understanding of mitochondrial disease was largely confined to the primary disorders of mitochondrial DNA, a more recent research priority has been the elucidation of disorders arising from defects in the interaction of the two genomes. These disorders are caused by nuclear gene defects involved in mtDNA maintenance; consequently Mendelian inheritance is observed. These defects give rise to direct or

Table 3: MtDNA disorders without RRF or histochemical deficit.	
Phenotype	Genetic abnormality
LHON	3 mtDNA mutations account for ~95% of disease
NARP/MILS	mtDNA ATPase mutations
Some patients with MELAS and other encephalopathies	mtDNA mutations in ND5 and other protein coding genes

LHON = Leber's hereditary optic neuropathy.

Table 4

Gene	Protein	MtDNA defect	Clinical syndrome
POLG1	polymerase γ -alpha subunit catalytic subunit p140	Multiple deletions and depletion	See text ad/arPEO, ad/ar multisystemic syndromes Alpers syndrome
POLG2	polymerase γ -alpha subunit accessory subunit p55	Multiple deletions and depletion	adPEO
C10orf2	mitochondrial helicase PEO1 (Twinkle)	Multiple deletions	adPEO, arIOSCA, ar hepatocerebral syndrome
ANT1	adenine nucleotide translocator 1	Multiple deletions	ad/arPEO
TP	Thymidine phosphorylase	multiple deletions and depletion	MNGIE
dGK	deoxyguanosine kinase	Depletion	Hepato-cerebral syndrome
SUCLA2	beta subunit of the ADP-forming succinyl-CoA synthetase ligase	Depletion	Encephalomyopathy and anaemia
MPV17	MPV17 inner mitochondrial membrane protein	Depletion	Hepato-cerebral syndrome
TK2	thymidine kinase 2	Depletion	Myopathic syndrome
RRM2B	P53 controlled ribonucleotide reductase	Depletion	Fatal infantile multisystem disease

ad/ar = autosomal dominant/autosomal recessive, IOSCA = infantile onset spinocerebellar ataxia.

Table 5: Clinical features suggestive of POLG mutations

• Onset at any age but neurological features commonly in teens
• Epilepsy is the most frequent presenting symptom (often with headache and sometimes resulting in status epilepticus)
• Axonal neuropathy is present in most patients
• Myopathy, ataxia, PEO are also common
• PEO is not invariably present and usually appears after age 20
• Alper's disease is caused by POLG mutations, and valproate may precipitate hepatic failure in neurological patients carrying POLG mutations

indirect injury to mtDNA through different pathways, either quantitative or qualitative. However, as might be predicted, the biochemical, pathological and clinical consequences are often suggestive of mitochondrial disease.

MtDNA is continuously recycled. Replication is performed by a number of nuclear encoded proteins. MtDNA polymerase gamma (POLG) is the only DNA polymerase present in mammalian mitochondria. It is a heterodimer with a catalytic subunit (coded by POLG) and two identical accessory subunits (coded by POLG2). The accessory subunit is a DNA binding factor required to increase the affinity of the heterotrimer for template DNA. POLG together with other nuclear encoded proteins is central to the synthesis of mtDNA. Other proteins involved in mtDNA maintenance and associated with human disease are tabulated (Table 4).

POLG mutations

Autosomal dominant disease

POLG came to attention when pathogenic mutations were discovered to cause autosomal dominant PEO (adPEO). It is now apparent that POLG defects account for about half of adPEO. In such patients, multiple defects are found in mtDNA with muscle biopsy findings indicating a mitochondrial myopathy. PEO is accompanied by a variable number of other clinical manifestations. Clinically patients with adPEO may have a similar phenotype to patients with PEO due to a single deletion or a mtDNA mutation. Some additional features such as deafness and neuropathy are common accompaniments of mitochondrial disease, but

some families show an excess of cataracts, parkinsonism and psychiatric disorders. POLG or other nuclear mutations may be suspected if autosomal inheritance is present, or if mtDNA analysis raises the possibility of multiple deletions or depletion.

Autosomal recessive disease

Compound heterozygotes for POLG mutations may also show ophthalmoparesis. However, neuropathy and ataxia are common additional features, giving rise to the acronym SANDO (sensory ataxic neuropathy with dysarthria and ophthalmoparesis). Recessive POLG mutations may also cause adult onset ataxia without ophthalmoplegia (mitochondrial recessive ataxia syndrome or MIRAS).

POLG mutations are not a rare cause of mitochondrial disease and the phenotype is expanding, ranging from fatal childhood hepatopathy (Alpers syndrome) to milder clinical syndromes in later life.⁸ Whereas the diagnosis is often suggested by the muscle biopsy, biochemical and mtDNA findings, clinicians may suspect the diagnosis when autosomal inheritance is evident or clinical features match those tabulated (Table 5).

The phenotype of POLG gene variants is still expanding. There are several reports of deterioration on initiation of valproate, and it is prudent to avoid this medication if POLG associated disease is suspected.

Other nuclear mutations causing mtDNA deletions and depletion

This is currently an active area of research, with an increasing number of genes implicated (Table 4). The latest gene investigated is RRM2B, which accounts for the depletion syn-

drome in further families.⁹ More causative genes are likely to be identified as some families with deletions/depletion do not have a known causative gene.

Thymidine phosphorylase mutations

Mitochondrial neurogastrointestinal encephalopathy (MNGIE) is an autosomal recessive disorder caused by mutations in the thymidine phosphorylase gene. The phenotype is relatively stereotyped with onset often in late childhood with PEO, and cachexia secondary to gastrointestinal dysmotility. A demyelinating neuropathy may be initially asymptomatic or may be the presenting feature, mimicking CIDP.¹⁰ A striking leucoencephalopathy seen on magnetic resonance imaging of the brain remains asymptomatic.¹¹

Disability results from the neuropathy as well as myopathy, but the gastrointestinal manifestations cause cachexia and life threatening complications. As a consequence of loss of function of thymidine phosphorylase, blood levels of thymidine and deoxyuridine are raised. Alterations of nucleoside metabolism cause preferential impairment of mtDNA replication, leading to both depletion and deletions on mtDNA.

Elucidation of the biochemical disturbance has led to the possibility of therapeutic intervention, by restoring thymidine phosphorylase function. Allogeneic stem cell transplantation is one approach, already shown to be capable of improving the plasma levels of nucleosides, but it is not clear whether this will have a beneficial effect on cellular function. This approach has not yet been shown to produce significant clinical improvement.

Co-enzyme Q10 deficiency

Coenzyme Q10 is a respiratory chain cofactor. Deficiency states would be predicted to cause respiratory chain dysfunction. Co-enzyme Q10 deficiency has been reported in association with diverse presentations from early onset encephalomyopathy often with fatal renal involvement, to later onset and milder myopathic presentations. This heterogeneous condition is now being elucidated. Nine enzymes are involved in Co-enzyme Q10 biosynthesis. To date pathogenic mutations have been described in three of these enzymes, PDSS1, PDSS2, and COQ2.¹² It has been shown that what has been described as the myopathic form of co-enzyme Q10 deficiency is a presentation of late onset glutaric aciduria. Recessive mutations in the electron transferring flavoprotein dehydrogenase gene (ETFDH) have been identified.¹³

The importance of these disorders is the potential response to Co-enzyme Q10 supplementation, including the secondary deficiency seen in glutaric aciduria. High doses may be needed, and the case of the myopathic presentations additional riboflavin may be required. Whereas the myopathic presentation may be suggested by lipid accumulation and mitochondrial changes on muscle biopsy, the primary deficiencies showed no such clues, and Co-enzyme Q10 assay on muscle or fibroblasts may be advisable in suggestive phenotypes in order to detect therapeutic possibilities.

Conclusion

Classical mitochondrial disorders affecting the nervous system are often diagnosed promptly by neurologists, but this article has shown that mitochondrial disease may not always be associated with expected patterns of inheritance, muscle histochemistry, and molecular genetic abnormalities. Consequently, mitochondrial disease must be considered beyond the classical mitochondrial phenotypes. Recent therapeutic attempts with respect to MNGIE and coenzyme Q10 deficiency are still being evaluated and may herald the start of a new era of therapeutic options in mitochondrial disease.

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