
Editorial

Clinical features, investigation, and management of patients with defects of mitochondrial DNA

Over the past decade we have seen a surge of interest in patients with mitochondrial disease.¹ More than 100 pathological defects of mitochondrial DNA (mtDNA) have been characterised in patients with a wide range of different disorders,^{2–3} and we are only just beginning to recognise the impact of mitochondrial disease on both neurological and general medical practice.⁴ These disorders often present in early adult life, with a progressive disabling neurological syndrome which is responsible for considerable morbidity and premature death.⁵ Although the actual prevalence of mtDNA disorders is not known, over the past five years we have accumulated about 100 patients with probable mitochondrial disease in the Northern Region. We have identified a pathogenic mtDNA defect in over 50 of these, and on this basis we calculate the point prevalence of established mtDNA disease to be around 1 in 50 000. This figure is likely to be a highly conservative estimate as it is dependent on the recognition and referral of these patients to only one centre, but in practical terms, it means that each United Kingdom neurologist will have at least four patients with mtDNA defects within their own district.

Mitochondrial dysfunction and mtDNA mutations seem to have infiltrated every branch of medicine, and as a consequence, the field has become increasingly complex. In this editorial we discuss the relevance of these recent findings on adult neurological practice. We focus on three major clinical issues: which patients should we investigate for possible mitochondrial disease, how should we investigate them, and what are the management options?

Which patients should we investigate for possible mitochondrial disease?

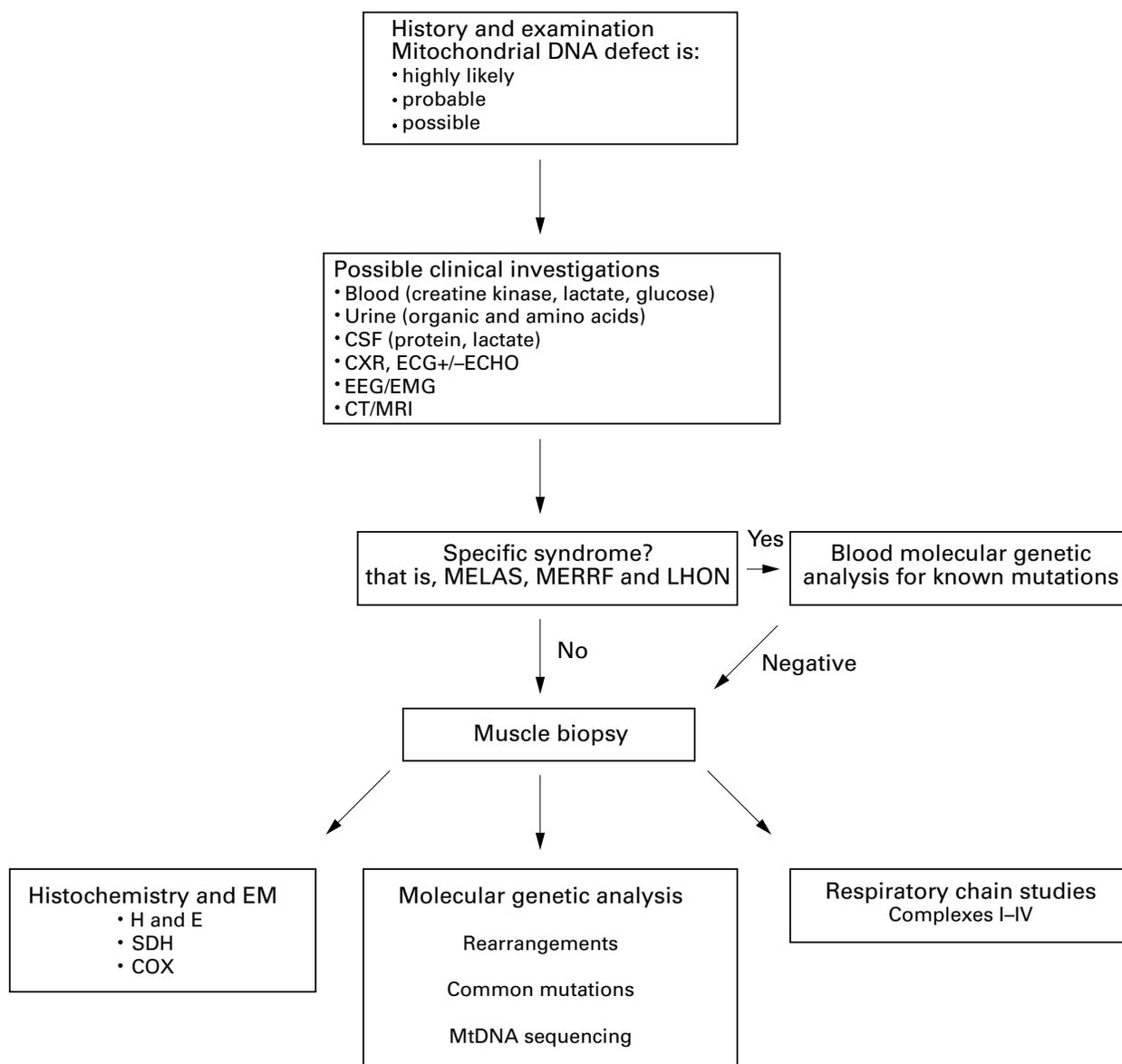
Patients with suspected mitochondrial disease fall into three groups: those with a clearly recognised syndrome which is associated with specific mtDNA abnormality, those who have a cluster of clinical features which suggests that they have a mtDNA defect, and those with an unusual clinical presentation who may have pathological mtDNA mutation.

The most common presentation of mitochondrial disease in older patients is with an asymmetric ptosis and proximal limb muscle weakness which progress insidiously over many years. On examination they have an asymptomatic external ophthalmoplegia and a mild proximal myopathy. Most of these patients have a deletion of mtDNA,⁶ although a proportion are associated with point

mutations.^{7,8} In patients with onset at a younger age, these features may be associated with ataxia, pigmentary retinopathy, and cardiac conduction defects—features compatible with the Kearns-Sayre syndrome which is usually due to a large mtDNA rearrangement.⁶

Another characteristic clinical syndrome is MELAS (mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes). Hirano *et al* proposed three diagnostic criteria for MELAS:⁹ (1) stroke before the age of 40; (2) an encephalopathy characterised by seizures, dementia, or both; and (3) a blood lactic acidosis, or ragged red fibres in skeletal muscle, or both. They also suggested that normal early development, recurrent headaches, or recurrent vomiting added further weight to the clinical diagnosis. These features are often due to a point mutation in the mitochondrial leucine (UUR) transfer RNA gene at position 3243.¹⁰ Myoclonic epilepsy with ataxia, encephalopathy, and a myopathy would favour the MERRF syndrome (myoclonic epilepsy with ragged red fibres), which is usually due to a point mutation in the mitochondrial lysine transfer RNA gene at position 8344.¹¹ Although there is considerable overlap in the clinical presentation of the A3243G and A8344G mutations, they do seem to be distinct syndromes.¹² The NARP syndrome consists of neurogenic weakness, ataxia, and retinitis pigmentosa, and is due to a point mutation in the mitochondrial ATPase 6 gene at position 8993,¹³ and the same mutation may also cause a subacute encephalopathy in childhood with prominent brain stem signs (Leigh's syndrome).¹⁴ Bilateral visual failure in younger patients, particularly if they are male, suggests a diagnosis of Leber's hereditary optic neuropathy (LHON) and is usually due to one of three point mutations in the complex I protein coding region of mtDNA (A11778G, A3460G, and T14484C).¹⁵

Unfortunately, we now realise that many mitochondrial patients do not present in this way. Most new cases of mtDNA disease are sporadic and clinically they do not fit neatly into any particular category.⁴ However, certain clinical features, particularly when in combination, are strongly suggestive of mitochondrial disease. A myopathy, which may be either slowly progressive¹⁶ or fluctuating in severity, in association with lactic acidosis,¹⁷ is a common presenting feature. The combination of a myopathy and CNS involvement, such as deafness or ataxia, is highly suggestive of a metabolic defect. Patients with mitochondrial disease often have a short stature, bilateral sensorineural deafness, and ptosis. Fundoscopy may disclose a pigmentary retinopathy



(the “bone spicule” appearance of classic retinitis pigmentosa is unusual), and optic atrophy. Birth and development may be normal before the onset of recurrent metabolic crises which are often associated with intercurrent viral illnesses. They may present with a subacute encephalopathy leading on to seizures and dementia, recurrent stroke-like episodes, or a myopathy affecting the limbs or external ocular muscles. They may have pyramidal tract signs and ataxia, myoclonus, diabetes, and a peripheral neuropathy. A history of migraine is relatively common as is a maternal family history of neurological disease (for reviews see^{14 18 20}).

The third group is the most difficult to define. As neurologists become more aware of mitochondrial disease, and investigations become more widely available, we are seeing increasing numbers of patients with otherwise unexplained neurological disease who are referred for mitochondrial studies. Mitochondrial disease may present with stroke in patients under 45 years of age. Estimates of the frequency of mtDNA defects in the young stroke population vary greatly (between 0.5 and 8%^{21 22}), presumably because of differences in study populations. Mitochondrial stroke is often (but not exclusively) associated with migraine, and other features such as a raised blood or

CSF lactic acidosis (as discussed in the next section), and a maternal family history of neurological disease may support a mitochondrial aetiology. Mitochondrial disease should certainly be considered in young stroke cases after thrombophilia, vasculitis, and structural cardiovascular disease have been excluded.

Recurrent strokes in the older patient,²³ chorea,²⁴ focal dystonia,²⁵ an isolated spinocerebellar syndrome,²⁶ and peripheral neuropathy^{26 27} may be the only manifestation of a pathological mtDNA mutation. Furthermore, the non-neurological range of organ involvement in mitochondrial disease continues to increase, and patients with renal tubular disorders,^{28 29} gastrointestinal dysmotility (dysphagia and pseudo-obstruction),³⁰ endocrinopathy (particularly hypoparathyroidism and including diabetes mellitus),³¹ and hypertrophic cardiomyopathy³² may harbour a pathogenic mtDNA mutation. It is most difficult to decide when to investigate this third group of patients. The presence of unexplained multisystem neurological disease, coupled with one or more of the extraneurological features, would warrant further investigation. However, for many of these patients, the specific investigation of mitochondrial disease usually begins after the exclusion of other diagnoses.

How should we investigate patients with suspected mitochondrial disease?

The investigation of possible mitochondrial disease requires an integrated approach, incorporating clinical, histochemical, biochemical, and molecular biological investigations (figure). Serum creatine kinase is often normal, and random and fasting lactate and pyruvate concentrations are often within the normal range. The CSF protein may be raised, as may the fasting CSF lactate. However, both seizures and strokes on their own may increase serum and CSF lactate concentrations, and these results should always be interpreted with caution. Mitochondrial patients may have a raised fasting glucose and an abnormal urinary amino acid profile. Every patient should have an ECG looking for evidence of a conduction defect, cardiomyopathy, or an accessory pathway. Chest radiography and echocardiography should be considered if there is clinical evidence of cardiorespiratory involvement. Nerve conduction studies may disclose an axonal²⁷ or a mixed axonal-demyelinating³³ peripheral sensorimotor neuropathy, and an EMG may show evidence of an active myopathy. However, as with other metabolic myopathies, even in the presence of clinical myopathy, the EMG may be normal.¹⁷ An EEG may show subclinical seizures or generalised slow waves consistent with a subacute encephalopathy. Cerebral imaging may be helpful in patients with either cognitive impairment, central neurological signs, a movement disorder, or an abnormal EEG. Under these circumstances, CT may demonstrate scattered hypodensities, calcification of the basal ganglia, and generalised atrophy, and MRI may show regions of high signal on T2 images either deep in the white matter or at the grey-white matter interface. Functional imaging in mitochondrial patients may disclose abnormalities of cerebral metabolism or perfusion, but it rarely adds useful discriminatory information.

If the clinical picture is highly suggestive of one of the classic mitochondrial syndromes such as MELAS, MERRF, or LHON, blood should be sent for molecular genetic analysis at this stage. Screening the blood for known mutations may prevent further more invasive and expensive tests. Negative results should, however, be interpreted with caution. We recently investigated a 34 year old patient with recurrent strokes. Blood screening for the A3243G MELAS mutation was negative. The patient was subsequently found to have very high levels of the A3243G mutation in skeletal muscle, but levels in blood were below the limit of detection for the conventional screening method. In addition, recent studies have also shown that recognised mtDNA mutations only account for a small proportion of cases of mitochondrial disease. Therefore, if clinical suspicion is sufficiently strong, patients who do not have recognised mutations in blood should be investigated further.

Patients who do not have a recognised mtDNA mutation in blood should have a muscle biopsy. A needle biopsy is usually adequate, and fresh muscle should be examined for histochemical evidence of a mitochondrial defect. The presence of ragged red fibres or cytochrome c oxidase (COX) negative fibres in a young person, or more than a few per cent in an older person supports a clinical diagnosis of mitochondrial disease. Patients with mtDNA defects typically exhibit a mosaic staining pattern for COX. A uniform low level of COX staining is suggestive of a nuclear genetic defect (although this is far from absolute), and normal COX staining does not rule out a specific defect of other respiratory chain complexes.³⁴ Electron microscopy may add supplementary information, but the morphological changes associated with mitochondrial disease (such as abnormal mitochondria and paracrystalline inclusions) are

relatively non-specific and in our experience they are not particularly helpful in making a diagnosis.

Mitochondrial respiratory chain studies should be performed in tandem with the molecular genetic analysis. It is preferable to carry out mitochondrial studies on fresh (not frozen) skeletal muscle. Multiple complex deficiencies are suggestive of a mtDNA defect affecting intramitochondrial protein synthesis (such as a transfer RNA gene mutation). Isolated complex deficiencies point to a pathogenic mutation within the appropriate polypeptide encoding region, and isolated complex II deficiency must always have a nuclear genetic basis (although this is extremely rare).³⁵

Although all patients with abnormal muscle histochemistry should have molecular genetic analysis of skeletal muscle mtDNA, it is important to perform mtDNA analysis on patients with a normal muscle biopsy if there is a strong clinical suspicion of mitochondrial disease.³⁶ Deletions and duplications of mtDNA are detected either by long range polymerase chain reaction (PCR) or Southern blotting. If these studies are negative, the muscle DNA should be screened for the A3243G MELAS, A8344G MERRF, and T8993G/C NARP mutations.

If there is clinical, histochemical, or biochemical evidence to support a pathogenic mtDNA defect, we routinely sequence the mitochondrial genome. We begin by sequencing the transfer RNA genes as most recognised pathogenic mtDNA point mutations occur in these regions. If initial results are negative then we continue to sequence the rest of the mitochondrial genome. Over the past five years there have been huge technological advances in automated DNA sequencing. For example, we have developed a system which allows us to amplify the whole mtDNA molecule with 20 parallel PCR reactions, and to sequence all 16 569 mitochondrial nucleotide base pairs within three days.

MtDNA is highly polymorphic and it is often difficult and time consuming to establish whether a mutation is pathogenic or not, particularly if the base change has not been reported before. The basic principle is to show that the mutation is disease specific. If the patient has a mosaic histochemical pattern of COX activity in muscle, then it is possible to compare the level of the mutation in COX positive and COX deficient muscle fibres by single fibre radioactive PCR. If the COX deficient fibres contain a higher level of mutant mtDNA than the COX positive fibres, then it is highly likely that the mutation is responsible for the disease.^{8, 37} These investigations form part of the routine diagnostic investigation for patients with mitochondrial disease. Further investigations enter the realms of laboratory research and are not the focus of this article.

How should we treat patients with mitochondrial disease?

Although the diagnosis of a mtDNA defect has profound implications on patient management, unfortunately there is little evidence that we can alter the course of mitochondrial disease. Pharmacological treatments are of limited benefit and in our experience the symptomatic effects are often short lived, suggesting a strong placebo effect. Most studies looking at different agents have been hampered by few patients and diverse clinical features.³⁸ Although objective improvements in plasma lactate concentrations and intracerebral metabolism have been documented, it has not always been possible to detect a functional improvement,³⁹ and in the only randomised controlled trial, the outcome was non-conclusive.⁴⁰ Having said this, there is anecdotal evidence supporting the efficacy of ubiquinone (coenzyme Q10),^{41, 42} and the antioxidant

vitamins C and E⁴³ in the treatment of patients with mitochondrial disease.

Dichloroacetate has been shown to reduce cerebral lactate concentrations and it may be of use in patients whose principal symptoms stem from the metabolic acidosis.⁴⁴ Patients with a profound acidosis as part of a metabolic crisis should be treated with sodium bicarbonate, but paradoxically, lactate production may, in part, compensate for impaired respiratory chain activity. It is currently uncertain whether we should treat lactic acidosis in the non-acute state, and as side effects are common with dichloroacetate, we rarely use this drug in our patients. Riboflavin,⁴⁵ thiamine,⁴⁶ and vitamin K³⁴³ may be of benefit in individual cases, and there is a rationale for replacing L-carnitine if there is a secondary carnitine deficiency.⁴⁷ In general the benefits of these vitamins and cofactors are minimal.³⁹ Although high dose prednisolone has been reported to be beneficial in a few patients with MELAS,⁴⁸ in one case it proved fatal,⁴⁹ and we would not advocate the use of steroids for mitochondrial disease alone.

Supportive care forms the mainstay for patients with mtDNA defects. A team of specialists involving the neurologist, nurses, physiotherapists, and speech therapists should be involved.³⁸ A broad knowledge of the potential complications of mitochondrial disease is essential for any physician managing these patients.⁴ Effective seizure control and the treatment of spasticity are essential, and diabetes should be carefully managed. Ocular complications such as ptosis and cataracts are amenable to surgery, and the early detection of heart block may be life saving. Patients often ask how much should they exercise? Recent studies have shown that mitochondrial function in elderly athletes is no different from younger subjects matched for the level of fitness,⁵⁰ and exercise in patients with mitochondrial myopathies may be more effective at preventing future disability than for other muscle diseases.⁵¹ There is, therefore, a good argument for these patients maintaining a moderate level of exercise to prevent further decline in respiratory chain function. Although many patients prefer to moderate their own level of exercise, hospital and community based physiotherapy can be very helpful.

Patients with mitochondrial disease are more susceptible to complications from general anaesthesia. Anaesthetists should be aware of potential cardiac conduction defects, and increased sensitivity to etomidate and thiopentone have been documented in the Kearns-Sayre syndrome.⁵² Chronic hypoxia and CO₂ insensitivity may also cause problems on reversal of anaesthesia⁵³ or in the intensive care unit.⁵⁴

Prognostic and genetic counselling is difficult for these diseases. MtDNA is inherited exclusively down the maternal line. As a consequence, we can confidently say that a male cannot pass on a mtDNA mutation to his offspring. Most rearrangements are sporadic and are not transmitted from mother to child. Unfortunately the situation is a little more complex for mtDNA point mutations. In a recent study of over 200 cases of the A3243G MELAS and A8344G MERRF mutations, higher levels of mutant mtDNA in the muscle were associated with an increased frequency of major neurological features,¹² and higher levels of mutant mtDNA in the blood of females were associated with an increased risk of having affected offspring.⁵⁵ It is possible, however, that other mutations will behave in very different ways, making it very difficult to give advice in the clinic.

Over the past few years there have been important advances in novel treatments for mtDNA defects. Seibel *et al* have successfully delivered a self replicating loop of mtDNA into mitochondria in vitro.⁵⁶ In theory this could

be used to synthesise deficient respiratory chain proteins and thus correct the biochemical defect. In contrast to nuclear DNA, mtDNA is under constant turnover, with a half life of about 10 days. Taylor *et al* have shown that the replication of mutant mtDNA can be specifically inhibited in vitro,⁵⁷ potentially allowing the concentration of normal mtDNA to increase with time and thus correcting the mitochondrial defect. If a similar effect were seen in vivo, this may have a direct clinical application. Finally, recent studies have shown that in some patients a low concentration of mutant mtDNA is present in the satellite precursor cells, despite pathologically high concentrations in adjacent mature muscle fibres. Clark *et al*⁵⁸ induced focal damage to mature muscle, stimulating the proliferation of satellite cells (which contained low concentrations of mutant mtDNA). After three weeks, the regenerated mature muscle had low levels of mutation and normal COX activity on histochemical analysis.

In conclusion, mitochondrial diseases are far more common than was anticipated only five years ago. Apart from the well recognised syndromes, mitochondrial disease should be considered in any patient with an unexplained, progressive multisystem neurological disorder, particularly if there is evidence of other organ dysfunction. The investigation of mitochondrial disease is difficult, but a structured approach will result in rapid and efficient diagnosis. Recent advances in automated mtDNA sequencing mean that it is now possible to confirm the presence or absence of a mtDNA mutation in patients in whom a diagnosis is difficult. Although there is no cure for these diseases at present, mitochondrial patients have many problems which require specific treatments. Over recent years there have been great advances in our understanding of the pathophysiology of these diseases and hopefully new treatments will become available.

We thank Dr NEF Cartridge for his comments on the manuscript. PFC is a Wellcome Trust Clinical Research Training Fellow.

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