

Mitochondrial medicine

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Introduction

Mitochondrial genetic defects were first associated with disease in 1988.^{1,2} Since then, we have seen an exponential increase in the number of mitochondrial DNA (mtDNA) abnormalities linked to a wide variety of human disorders.³ At the last count, over 100 different mutations have been associated with disease, many resulting in profound disability and premature death.^{4,5} Pathogenic mtDNA defects are far more common than was previously anticipated, and patients may present to physicians in any medical specialty. The diagnosis and management of these disorders is a challenge. Although their clinical features may initially be restricted to one organ, they often develop complications affecting many different systems, requiring an integrated approach from clinicians of many different disciplines. At present we have no cure for mtDNA disorders, but effective management can minimize the burden of disease in both patients and their families. In this article we will discuss the relevance of mitochondrial disease to general medicine and paediatrics, placing particular emphasis on the clinical features which should prompt further investigation. We will then describe the most helpful investigations before discussing the management of individual patients. Finally, we will mention some of the more recent advances in the treatment of mtDNA disease, providing some hope for the future.

Are mtDNA mutations an important cause of disease?

At present we do not know the frequency of pathogenic mtDNA mutations. From a neurological point of view, we have confirmed about 50 cases from within the Northern Region, giving a point prevalence

of around 1 in 50 000. This figure is likely to be a gross underestimate because it reflects clinically-affected patients referred to one centre, many of whom have neurological features suggestive of mitochondrial disease. Many patients with disease-causing mtDNA defects do not present with, or develop a specific neurological problem, and it is likely that many of these individuals remain undetected. It is estimated that 1.5% of all diabetics harbour a specific mtDNA defect,⁶ and between 0.5 and 1% of strokes in the under-45 age group are due to a point mutation at position 3243 of the mtDNA L-strand.⁷ Furthermore, mtDNA abnormalities have been associated with common diseases such as idiopathic Parkinson's disease and Alzheimer's disease,⁸ and they are present with an increased frequency in elderly subjects.⁹ Therefore, although the classical mitochondrial encephalomyopathies are rare, mtDNA defects are relatively common, and probably contribute to the pathogenesis of many different diseases, many non-neurological, and they may also be involved in the ageing process itself.

Which clinical features suggest the possibility of mitochondrial disease?

The mitochondrial respiratory chain is essential for aerobic metabolism. Each mitochondrion contains numerous copies of mtDNA which encodes 13 polypeptides which are necessary for the optimal function of the mitochondrial respiratory chain.¹⁰ Pathogenic mutations of mtDNA thus affect organs which are highly dependent upon oxidative metabolism—the order of frequency being determined by

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bioenergetic demand:¹¹ central and peripheral neurons, skeletal muscle, pancreatic β -cells and endocrine organs, myocardium and the cardiac conduction system, renal tubular cells, hepatocytes, and the gastrointestinal tract. Haematological and dermatological features are also seen, but much less frequently. It should also be noted that many adults with mitochondrial disease are below the 10th percentile for height, although we do not fully understand why this is the case.

Neurological

Patients with mitochondrial disease often present with neurological features (recently reviewed¹² and see the large series in reference 13). A minority of patients fit neatly into well-defined syndromes such as MELAS (Mitochondrial Encephalomyopathy with Lactic Acidosis and Stroke-like episodes),¹⁴ MERRF (Myoclonic Epilepsy with Ragged-Red Fibres),¹⁵ CPEO (Chronic Progressive External Ophthalmoplegia) or the Kearns-Sayre syndrome (external ophthalmoplegia, ataxia, sensorineural deafness, heart block and an elevated CSF protein beginning before the age of 20).¹⁶ Likewise, subacute bilateral visual loss in young males is highly suggestive of Leber hereditary optic neuropathy (LHON).¹⁷ However, many patients do not present in this way, and mitochondrial disease should be considered in any patient with an unexplained multi-system neurological disorder (see reviews^{12,18,19}).

Mitochondrial DNA defects can affect almost every component of the nervous system, but certain signs, particularly when in combination, strongly suggest a mitochondrial aetiology. Eye signs are frequently seen in patients with mitochondrial disease.²⁰ External ophthalmoplegia is common but evolves slowly. As a result, individuals with a gross restriction of eye movements and loss of conjugate gaze rarely complain of diplopia, except briefly in the very early stages. Optic atrophy is common, a peripheral pigmentary retinopathy may be apparent, and cataracts may be a problem. Bilateral sensorineural deafness is common,²¹ as is a mild proximal myopathy.¹³ Gross muscle wasting and weakness are rare, and a (usually asymptomatic) peripheral neuropathy may be present.^{22,23} Patients with mitochondrial disease may have a normal early development before the onset of a subacute encephalopathy leading on to seizures and dementia, and both spontaneous and stimulus sensitive myoclonus may be observed. Cerebellar ataxia is not uncommon and pyramidal and extrapyramidal tract signs may be apparent.

There is undoubtedly an association between migraine, stroke and mitochondrial disease.^{24,25} Over 50% of patients with mitochondrial disease complain

of migraine-like headaches, and in a small proportion of cases, severe migraine can lead on to stroke-like episodes.²⁶ It has been suggested that mitochondrial dysfunction plays an important role in the aetiology of a significant proportion of cases of migraine,²⁷ but the link is far from certain.^{28,29} MtDNA abnormalities have been found in between 0.5 and 8% of young strokes—depending upon the study group.^{7,30} We would consider investigating possible mitochondrial disease in any individual under the age of 50 with a single stroke and no evidence of structural cardiovascular disease, thrombophilia or vasculitis.

Diabetes

On the basis of currently available evidence, diabetes mellitus is the most common mtDNA disease phenotype. By general consensus, mtDNA defects cause approximately 1.5% of cases of diabetes, giving a prevalence of 200 000 in the European population.³¹ Mitochondrial diabetics typically present in young-to-middle age (mean age of onset 22–35), in between the peak age of onset for young- and maturity-onset diabetes (see review⁶). Mitochondrial diabetes is principally a defect of insulin secretion. The majority are thin (BMI < 25 kg/m²), and although most require insulin relatively rapidly, ketoacidosis is rare. Cardiac and renal complications have been documented in patients with mitochondrial diabetes, but these may be a consequence of the mtDNA defect itself and not necessarily a complication of the diabetes.

How do mtDNA mutations cause diabetes? Pancreatic β cells are highly dependent on oxidative metabolism,¹¹ and in particular, glucose-induced insulin secretion is dependent on the intracellular ATP/ADP ratio.⁶ Histopathologically, patients with mitochondrial diabetes have islet-cell atrophy and it is interesting that patients with mitochondrial diabetes may have circulating islet-cell antibodies.³² Whether the autoimmune response is secondary to β -cell damage due to the mtDNA defect, or whether both pancreatic autoimmunity and a mitochondrial defect contribute to the pathogenesis of the diabetes, is not known. Despite having inherited a mtDNA mutation, the diabetes often presents in middle adult life. This is thought to reflect the age-dependent accumulation of mutant mtDNA in post-mitotic β -cells.³³

Clearly this begs the question: which diabetics have a mitochondrial defect? Although not necessarily clinically apparent, the majority of patients with mitochondrial diabetes have sensorineural deafness.⁶ The presence of any other neurological signs, or other organ involvement suggest a mitochondrial aetiology, and a maternal family history of diabetes or deafness would raise the suspicion of a mtDNA

defect. Blood lactate levels may be elevated but are usually normal in our experience. We would look for the A3243G MELAS mutation in the blood of these patients, but a negative blood test does not exclude the diagnosis (see the investigations section).

Endocrine and gonads

Hypoparathyroidism may be the presenting feature of mitochondrial disease and is often associated with rearrangements of mtDNA.^{34,35} Hypothyroidism is not uncommon, and, like mitochondrial diabetes, is often associated with significant titres of organ specific autoantibodies. Growth hormone deficiency has also been documented in isolated cases, and anterior pituitary failure has been documented. Infertility is common in both males and females with mitochondrial disease.³⁶ For example, there are very few successful pregnancies documented in females with high levels of the heteroplasmic A3243G MELAS mutation (>60% in blood—see later for a definition of heteroplasmy), despite many in females with <60% in their blood.³⁷ This is probably a result of multiple interacting factors such as primary gonadal failure,³⁶ endocrine dysfunction (also at the pituitary level),³⁶ the metabolic and general effects of chronic illness, and loss of gametes due to high levels of mutation load.³⁸

Cardiac

MtDNA defects may cause cardiac disease in isolation³⁹ or as part of a recognized syndrome (such as the Kearns-Sayre syndrome due to a large rearrangement of the mtDNA molecule).⁴⁰ Both dilated⁴¹ and hypertrophic cardiomyopathy⁴² have been described, and conduction abnormalities are common.⁴³ These usually take the form of an insidiously progressive heart block, but aberrant A-V pathways have been documented in a number of patients with Leber hereditary optic neuropathy,⁴⁴ and in one patient with the MELAS syndrome (perhaps a chance association).⁴⁵ Ischaemic heart disease is associated with the accumulation of mtDNA mutations in myocardial cells,⁴⁶ but these abnormalities are probably secondary to free-radical-induced oxidative damage, and are of dubious significance.

Respiratory

Lung sepsis is probably the most common respiratory complication of mitochondrial disease and it is often the terminal event.⁴⁷ Mitochondrial dysfunction may, however, present to the chest physicians in a number of other ways. Lactic acidosis may lead to hyperventilation. Patients with a chronic lactic acidemia, by definition do not have a systemic acidosis, and in

the presence of a low serum bicarbonate they may be labelled as having an hysterical hyperventilation syndrome. In contrast, central hypoventilation is common in patients with a severe encephalopathy, particularly children during an acute exacerbation of Leigh syndrome, who often have a mixed respiratory and metabolic acidosis.⁴⁸ Sleep apnoea is well recognized in adults with mitochondrial disease,⁴⁹ with both central⁵⁰ and peripheral components.⁵¹ Chronic hypoxia⁴⁷ and CO₂ insensitivity⁵⁰ have also been documented within this context. Finally, chronic musculoskeletal hypoventilation⁵¹ may lead to pulmonary hypertension and right heart failure.

Renal

Aminoaciduria (particularly involving lactate, pyruvate and alanine) is common in patients with mitochondrial disease and may reflect the systemic metabolic disturbance and not renal dysfunction *per se*. Glomerular dysfunction has been documented but is rare. In contrast, a significant proportion of mitochondrial patients have renal tubular dysfunction⁵² and some have tubulointerstitial disease.⁵³

Approximately 90% of renal oxygen consumption is used to generate ATP for the Na⁺/K⁺ ATPase in the proximal tubules and ascending loop of Henle. With this in mind, it is not surprising that mtDNA defects can lead to renal tubular disease. Children with mitochondrial disease often have severe renal dysfunction with a non-selective loss of amino acids, glucose, phosphate and bicarbonate (the Toni-Fanconi-Debre syndrome).^{13,54,55} In adults, the defect is usually mild and is rarely clinically significant. A generalized aminoaciduria is the most common adult disorder,⁵² but isolated renal tubular acidosis has been described in a patient with the Kearns-Sayre syndrome⁵⁶ and the A3243G MELAS mutation,⁴⁵ and Bartter's syndrome⁵⁷ has been documented in a number of cases. Adults may also develop the Toni-Fanconi-Debre syndrome, but overt renal failure is rare.

From a practical point of view, renal presentations of adult mitochondrial disease are few and far between, and most renal complications of mitochondrial disease are a consequence of ascending sepsis and/or obstruction in association with an upper-motor-neuron bladder.

Gastrointestinal

Patients with mitochondrial disease may present to gastroenterologists with episodic nausea and vomiting.⁵⁸ In mitochondrial patients this may be due to a systemic lactic acidosis, but often this is not the case, and by excluding other causes, these symptoms

are assumed to be something akin to migraine, with a central neurological origin.

The MNGIE (Mitochondrial Neurogastro/Intestinal Encephalopathy) or POLIP (Polyneuropathy, Ophthalmoplegia, Leukoencephalopathy and Intestinal Pseudo-obstruction) syndrome as the various acronyms suggest, is characterized by CPEO, and subacute encephalopathy, a mitochondrial myopathy, a neuropathy and gut dysmotility.⁵⁹ Although these patients have a mtDNA abnormality (multiple deletions), the primary genetic defect is nuclear, and the inheritance pattern autosomal-dominant. Both a gut myopathy and a myenteric plexus neuropathy have been implicated in different cases of MNGIE, but at present we do not know the cause. Gut dysmotility has been described in association with other mtDNA mutations⁴⁵ which may result in bacterial overgrowth and malabsorption. Malabsorption due to exocrine pancreatic failure has also been documented in patients with mitochondrial disease, as has chronic diarrhoea and villous atrophy.⁶⁰ Although MNGIE and pancreatic exocrine failure are rare, over 15% of our patients with mitochondrial disease complain of either dysphagia and/or chronic constipation. The dysphagia is progressive and often leads to a gastrostomy, and in a minority of cases the constipation can lead to pseudo-obstruction.

Hepatic failure is common in neonates and young children with mitochondrial disease, leading to a worsening of the metabolic acidosis.⁶¹ In adults, abnormal serum transaminase enzymes are more likely to be due to muscle disease (ALT) or secondary to anticonvulsant therapy (AST, ALT, γ GT) and are rarely clinically significant. Like the kidney, considerable functional reserve protects the liver against the clinical expression of mtDNA defects, despite being heavily dependent upon oxidative metabolism.

Haematological

Sideroblastic anaemia has been described in adults with mitochondrial disease.⁶² In children, it may be associated with pancytopenia and exocrine pancreatic failure (Pearson's syndrome). This will be discussed in the paediatric section.

Dermatological

Cervical lipomatosis has been associated with myoclonic epilepsy and a myopathy (Ekblom's syndrome) and may be due to a point mutation of mtDNA.⁶³

Rheumatological

Patients may present to rheumatologists with muscle pain and weakness due to a mitochondrial myopathy,

and rheumatological complications may result from mitochondrial hypoparathyroidism.

Adverse drug reactions

Azidothymidine (AZT) produces a reversible mitochondrial myopathy with mtDNA depletion.⁶⁴ Recent trials with the new anti-viral drug fialuridine were complicated by hepato-encephalopathy and myopathy with associated mtDNA abnormalities.⁶⁵ Being nucleotide analogues, both of these drugs affect mtDNA replication and produced mitochondrial toxicity when used for over 6 weeks. Short-term use of anti-viral drugs is unlikely to be associated with clinically significant mitochondrial toxicity.

Psychiatric

Psychiatric complications of mitochondrial disease are common. These usually take the form of a reactive depression associated with a progressive, incurable neurological disease. We have also seen a number of cases of severe depression and attempted suicide *before* diagnosis. These individuals had breathlessness (metabolic acidosis), muscle weakness and chronic fatigue (due to a mitochondrial respiratory chain defect). For many years these symptoms had been dismissed as being functional, despite considerable disability, and the recognition of organic disease significantly improved each individual's outlook.

In an extended pedigree, multiple mtDNA deletions were associated with external ophthalmoplegia and depression.⁶⁶ The mtDNA abnormalities appeared to be inherited in an autosomal-dominant fashion and have been linked to different autosomal loci in different families.⁶⁷

Chronic fatigue

There is no doubt that the symptoms of mitochondrial disease can mimic the chronic fatigue syndrome (CFS). In our experience, there are usually additional clinical features to suggest a mitochondrial aetiology. An abnormal creatine kinase, fasting lactate or electromyogram should prompt further investigation, but all three of these investigations may be normal in a patient with a mitochondrial myopathy. This poses a difficult problem—individuals who rest for long periods will develop histological and histochemical abnormalities in skeletal muscle, and respiratory chain function may be below the lower limit of normal for age matched controls. At present we do not know what percentage of patients with CFS have mitochondrial dysfunction, but we suspect that they are rare.

Paediatric

Many patients with pathogenic mtDNA mutations present in childhood, usually in one of four ways. Firstly, mitochondrial disease may present in the neonatal period with a metabolic encephalopathy and systemic lactic acidosis, often associated with hepatic and cardiac failure.⁶¹ Despite maximal intervention, the majority of these babies die. It is, however, essential to make an accurate diagnosis as soon as possible. Mitochondrial disease may mimic eminently treatable childhood metabolic disorders such as biotinidase deficiency, and without obtaining the correct tissue samples *in vivo* (including skin fibroblasts for culture), it may not be possible to counsel the parents who may be keen to try for another child. Secondly, mtDNA mutations may present in infancy and childhood with Leigh syndrome (also known as subacute necrotizing encephalomyopathy).⁶⁸ Leigh syndrome is characterized by a relapsing encephalopathy with prominent brainstem and cerebellar signs. Children with Leigh syndrome often have bilateral basal ganglia hypodensities on the CT scan, and often have a raised blood and CSF lactate in between the subacute episodes.¹³ It is often possible to identify a respiratory chain complex deficiency in patients with Leigh syndrome. Complex IV deficiency (cytochrome *c* oxidase) is the most common defect, followed by (and often in combinations with) complex I.⁴⁸ In the majority of cases, these abnormalities have a nuclear genetic basis, but a significant proportion will have a mtDNA mutation (usually a point mutation at position 8993 in one of the two mitochondrially-encoded ATPase genes).^{68,69} Finally, pyruvate dehydrogenase deficiency is also common cause of Leigh syndrome and is usually due to a defect on the X-chromosome.⁷⁰ As for the earlier presentations, making a precise diagnosis can have major implications in predicting the risks of having affected offspring in the future. Thirdly, children with mtDNA deletions may present with Pearson's syndrome of pancreatic exocrine failure, sideroblastic anaemia and marrow panhypoplasia.⁷¹ With advances in supportive care, these children are now living into adulthood, and there are well-documented cases of Pearson's syndrome who develop the Kearns-Sayre phenotype in late teenage years.⁷² Fourthly, patients with mitochondrial disease may present as failure to thrive and hypotonia leading on to developmental delay and then regression.^{13,48} Identifying mitochondrial pathology in these patients is a challenge since they present in a non-specific way.⁷³ A high index of suspicion, a family history of neurological disease, and a history of recurrent abortions or early neonatal death (particularly in association with a systemic acidosis) suggest an inherited disorder due to either an autosomal

dominant or mitochondrial genetic defect. The investigations outlined in the next section will ultimately lead to a diagnosis. Finally, children may present with any of the recognized adult clinical features of mitochondrial disease—although often in a modified form.

In conclusion, mitochondrial disease should be considered in any patient with a combination of apparently unrelated symptoms and signs, particularly if there are neurological features (however subtle). Well-defined syndromes are not always seen, and multi-organ involvement is common.

The investigation of patients with suspected mitochondrial disease

The investigation of mitochondrial disease is difficult (see Figure 1). In a minority of cases, the clinical evidence is sufficiently strong to warrant molecular genetic studies which lead to a diagnosis (such as in LHON and overt MELAS syndrome).⁷⁴ In most patients, however, an integrated diagnostic approach is essential, incorporating clinical, histochemical, biochemical and molecular genetic data. Mitochondrial investigations often begin after a thorough multi-system diagnostic work-up excluding other more common diseases. If this has not been the case, then it is essential to look for common features of mitochondrial disease (cardiac involvement, diabetes, etc.) which may not be clinically apparent. Investigations can be divided into two groups: those designed to accumulate evidence of different tissue and organ involvement in a pattern suggestive of mitochondrial disease, and those designed to give a direct answer to the question—does this patient have mitochondrial dysfunction?

To deal with the former group, patients with a defect of mitochondrial oxidative phosphorylation may have an elevated blood lactate.¹³ An overnight fast increases the chances that the lactate may be abnormal, but it is important to remember that there are many other causes of a raised blood lactate and that many patients with a severe mitochondrial defect have normal blood lactate levels.⁷⁵ The serum creatine kinase may be mildly elevated and suggest a myopathy, but again this is often normal.¹³ When looking for neurological features, a raised CSF protein and lactate suggest central neurological involvement, and an electroencephalogram (EEG) may show diffuse slow-wave activity consistent with a subacute encephalopathy or evidence of a seizure disorder. Cerebral imaging should be performed on all patients with central neurological signs, a cognitive defect, or an abnormal EEG. CT may reveal infarcts and hypodensities or calcification in the basal ganglia, and MRI may show patches of high signal in both

Management

Investigation

Clinical Features

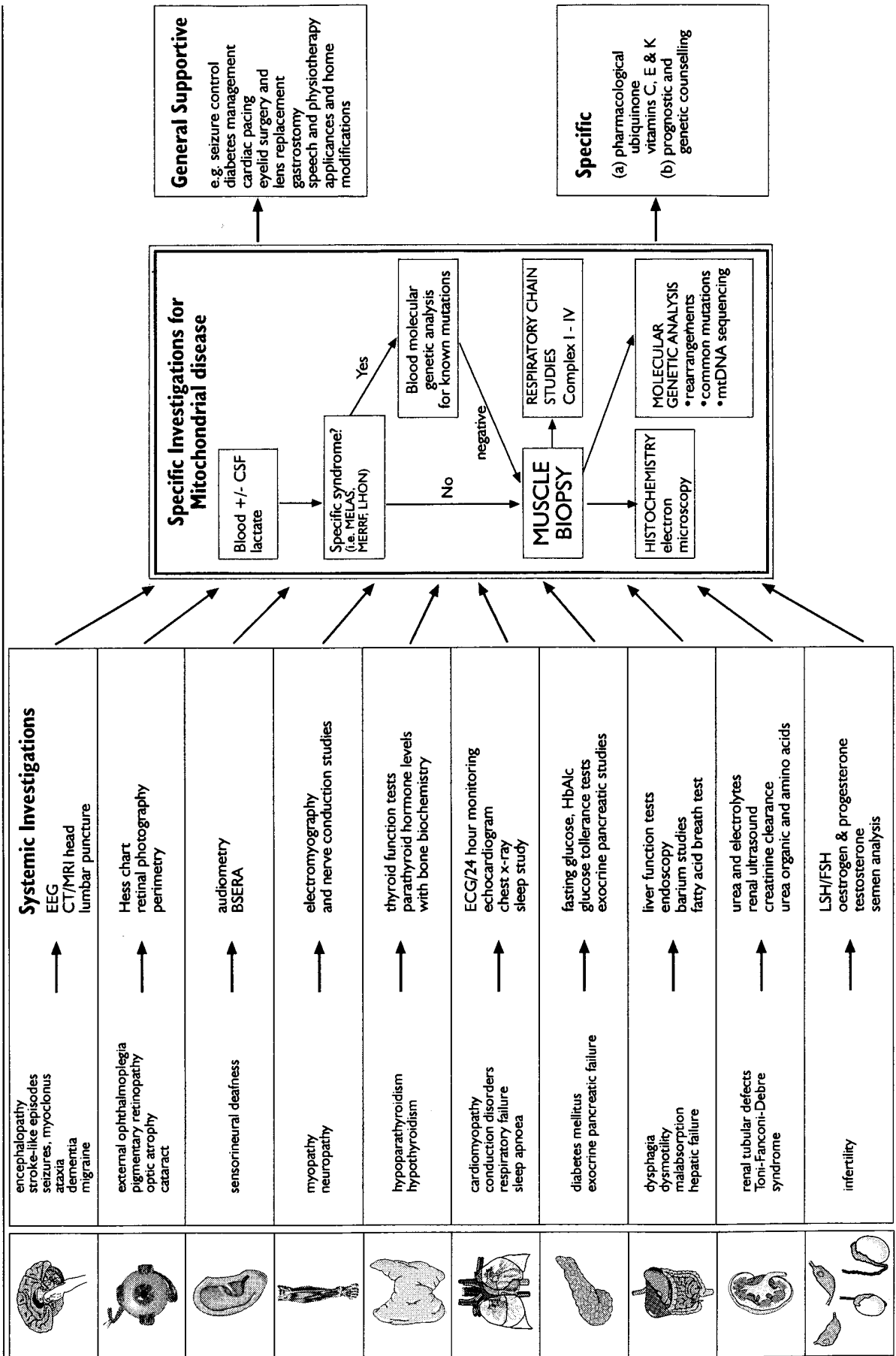


Figure 1. The clinical features, investigation and management of mitochondrial disease.

grey and white matter in T2-weighted images.^{76,77} Mitochondrial patients often have subclinical neuropathy, and even with clinical evidence of muscle involvement, the electromyogram (EMG) may be normal.¹³ All patients with suspected mitochondrial disease should have their fasting glucose measured and a glucose tolerance test if appropriate. They should all have an ECG, and if there are symptoms and signs suggestive of cardiac involvement, an echocardiogram should be performed. An ionized calcium and serum alkaline phosphatase are also indicated, along with a full urea and electrolyte profile. Urine organic and amino acids may be abnormal in patients with mitochondrial disease even in the absence of overt tubular dysfunction.⁷⁴

In all but a few cases, patients with suspected mitochondrial disease will need to have a muscle biopsy. A needle biopsy is usually adequate, and fresh muscle yields optimal results. Histochemical analysis is essential, looking for subsarcolemmal accumulations of mitochondria (ragged-red fibres) or cytochrome *c* oxidase deficiency, but the muscle may be both morphologically and histochemically normal.⁷⁸ Respiratory chain studies should be carried out in tandem with molecular genetic analysis of mtDNA isolated from the muscle biopsy. Individual respiratory chain complexes are measured by spectrophotometry, and mtDNA is screened for rearrangements (deletions and duplications) and common point mutations. Although a number of mtDNA mutations can be detected in blood, molecular genetic analysis of muscle mtDNA leads to fewer false-negatives. Individuals harbouring pathogenic mtDNA mutations usually have a mixture of both mutant and wild-type (normal) mtDNA within each cell (*intracellular heteroplasmy*). The level of a heteroplasmic mtDNA mutation is one of the principal factors which determines whether a mtDNA mutation is expressed clinically. This level can vary greatly from cell to cell, and organ to organ, within the same individual. Tissue levels are usually the highest in post-mitotic tissues such as skeletal muscle, and low in rapidly-dividing tissues such as blood. As a consequence, it is possible to 'miss' patients whose blood level falls below the lower limit of detection by conventional techniques, despite high levels in muscle.²⁶

Further investigations enter the realm of research, but it is now possible to sequence the entire mitochondrial genome in few days—giving an absolute answer as to whether a patient has a mtDNA defect. It is interesting that although the majority of respiratory subunits are encoded by the nuclear genome, only a small proportion of adults with clinical mitochondrial disease do not have an identifiable mtDNA defect.

Current management options

Although we currently have no cure for mitochondrial disease, the accurate genetic diagnosis of a mtDNA defect has major implications for patient management (see Figure 1). A detailed knowledge of the potential complications of a particular mtDNA mutation can prevent sudden death, using cardiac pacing, and subclinical diabetes can be diagnosed and treated appropriately. It is important to be aware of the various stages in progressive disability and complications that usually develop in these patients. This requires an integrated approach involving the primary physician, other specialist physicians, nurses, physiotherapists and speech therapists.

A number of different vitamins and co-factors have been used in patients with mitochondrial disease (for a recent review see reference 79). Subjective and objective improvement has been documented in isolated cases, but the results of the only randomized clinical trial were inconclusive.⁸⁰ Ubiquinone (co-enzyme Q10) has been shown to improve cerebral metabolism in patients with mitochondrial disease,^{81,82} but an objective improvement in function has not always been documented.⁸³ Antioxidant vitamins C and K have been of benefit in some cases,⁸⁴ and riboflavin⁸⁵ and thiamine⁸⁶ have also been helpful. Dichloracetate is effective in reducing cerebral lactate levels,⁸⁷ but this was not associated with a clinical improvement, and this drug often has unpleasant side-effects.

We are often asked whether patients with mitochondrial disease require any 'special medical treatment'. In general, we prescribe ubiquinone and vitamins to our patients because of the low side-effect profile and efficacy in isolated cases. There are theoretical reasons why sodium valproate should be avoided,⁸⁸ but we have seen many patients on this drug without adverse effects. Mitochondrial patients have been reported to be sensitive to certain anaesthetic agents (etomidate and thiopentone),⁸⁹ but in general, the management of mitochondrial patients is the same as that of any chronic neurological disorder. Good nursing and home support are of paramount importance. Epilepsy and spasticity should be managed effectively, and dietary requirements should be closely monitored, particularly if the patient complains of dysphagia.

Genetic counselling for mitochondrial DNA defects is a contentious area. It is important to identify a causative mtDNA defect because, in contrast to a nuclear gene defect, mtDNA is exclusively inherited down the maternal line. We can therefore say with confidence that a male with a mtDNA defect cannot pass the mutation on to his offspring. If we identify a pathogenic mtDNA deletion then it is most unlikely that this will be passed by a mother

to her offspring. The inheritance pattern of mtDNA point mutations is, however, much more difficult to predict. A particular mother with a heteroplasmic mtDNA point mutation can have offspring with either very low levels of mutant mtDNA (who may not be affected) or very high levels of mutant mtDNA (who may be severely affected). This variability has profound implications: for example, a mother with mitochondrial diabetes due to the A3243G MELAS mutation may have children who suffer from a severe encephalopathy with stroke-like episodes in childhood. In a retrospective study, at least for the A3243G MELAS and A8344G MERRF mutations, the level of mutant mtDNA in the mother was related to the incidence of affected offspring, but the inheritance pattern for each specific mutation was quite different.^{37,79} Extensive prospective studies will be necessary before we can give detailed counselling to mothers harbouring point mutations. Making a precise diagnosis in Leigh syndrome is important for genetic counselling. Many children with Leigh syndrome die in the first few years of life, and parents usually ask about the risk of having any subsequent affected offspring. Many Leigh syndrome patients have a histochemical pattern which suggests a nuclear genetic basis, which is usually autosomal-recessive.⁶⁸ The most common cause of pyruvate dehydrogenase deficiency is X-linked, but females may be affected through varied Lyonization in different tissues,⁷⁰ and identifying a causative mtDNA mutation has the implications outlined above. Sporadic cases do occur⁹⁰ and it is important to look for the mutation in maternal relatives to assess whether any future offspring are at risk.

Prospects for the future

Over the last few years, a number of new treatment approaches for mtDNA disease have been under development. Although two of these are very much at the *in vitro* stage, one has involved a patient. Siebel and colleagues successfully delivered a self-replicating loop of DNA into isolated human mitochondria.⁹¹ This approach which could, in theory, be used to synthesize deficient respiratory chain subunits in diseased mitochondria. More recently, Taylor and co-workers⁹² were able to specifically inhibit the replication of point-mutated mtDNA *in vitro* using a peptide nucleic acid, leaving wild-type (normal) mtDNA to continue replicating unabated. These novel anti-genomic agents are readily taken up by human cells in culture, and over a short period of time they have the potential to correct the pathogenic genetic defect. Clark *et al.*^{93,94} have taken advantage of a unique characteristic seen in a number of patients with mtDNA disease. Despite

high levels of mutant mtDNA in mature skeletal muscle, the mutation may not be detectable in dormant muscle precursor cells (satellite cells). In a patient with a severe mitochondrial myopathy and cytochrome *c* oxidase deficient muscle fibres, they used bupivacaine to kill the mature muscle fibres containing a high mutation load. After 6 weeks, they observed a low level of mutant mtDNA in the new regenerating fibres which had normal cytochrome *c* oxidase activity.

Conclusions

Over the last decade we have come to realize that mitochondrial DNA defects are far more common than was previously anticipated. Many mtDNA defects present to general physicians and paediatricians with symptoms and signs which at first glance are indistinguishable from other more common diseases. The presence of unusual patterns of multi-organ involvement, although subtle, should lead to a high index of suspicion of mitochondrial disease. Further investigations may be helpful in defining the extent of the problem, but most patients should undergo a muscle biopsy, with fresh muscle being taken for histochemical, biochemical and molecular genetic analysis. Recent advances in automated molecular biology mean that within 3 days it is now possible to give a definitive answer to the question: does this patient have a mtDNA defect? Identifying a mitochondrial mutation has profound implications on the future management of the individual and their family, particularly for genetic counselling. Although in their infancy, recent advances in the laboratory are providing new hope for the treatment of these hitherto untreatable genetic diseases.

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