HOW TO UNDERSTAND IT

The *mitochondrion* and its *disorders*

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Mitochondria are ubiquitous intracellular organelles that play a pivotal role in cellular energy metabolism. It therefore should come as no surprise that mitochondrial dysfunction can cause neurological disease. These disorders are not rare; each UK neurologist will have at least 20 patients with mitochondrial disease within their catchment area of about 200 000 people. This short article will focus on the basic science that underpins our current understanding of mitochondrial disease. It will stick to the bare essential facts that will help the busy neurologist to identify, investigate and manage these fascinating and challenging patients.

The tables should serve as a useful reference. Table 1 illustrates the clinical relevance of the basic science described in this article, but the reader should be aware that it will soon be out of date. Table 2 lists the features associated with well–recognized mitochondrial 'syndromes'.

MITOCHONDRIA AND MITOCHONDRIAL DISORDERS: WHAT ARE THEY?

Rather than thinking of mitochondria as discrete membrane-bound structures, they probably form a budding and fusing reticulum that is integrated into the cellular network. Mitochondria have a number of interrelated functions. They are involved in intracellular calcium signalling and apoptosis (programmed cell death), and they have a crucial role in metabolism. Many metabolic enzyme systems are contained within mitochondria, including components of tricarboxylic acid (Krebs) cycle enzymes, and the fatty acid β-oxidation pathway. However, the term 'mitochondrial disorder' usually refers to a primary abnormality of the mitochondrial respiratory chain.

Secondary mitochondrial dysfunction is seen as part of normal ageing and also in neurodegenerative disorders such as Alzheimer's disease, but the significance of these changes is not clear. Mitochondrial dysfunction also plays an important part in the pathophysiology of a group of inherited neurological diseases that includes Friedreich's ataxia, Wilson's disease and hereditary spastic paraparesis. Although related, these are not thought of as 'primary mitochondrial disorders' and will not be considered here.

WHAT DOES THE MITOCHONDRIAL RESPIRATORY CHAIN DO?

The mitochondrial respiratory chain is a group of five large enzyme complexes that sit within the inner mitochondrial membrane (Fig. 1). Each enzyme complex contains multiple subunits, and the largest is complex I with over 70 components. The metabolism of carbohydrates, fats and proteins generates intermediary metabolites that feed electrons in to the respiratory chain. These electrons are passed from complex to complex, and this energy is used to pump protons out of the mitochondrial matrix. This generates the mitochondrial membrane potential, which is harnessed by complex V to synthesize adenosine triphosphate (ATP), the principal intracellular energy source. The detailed biochemistry of the respiratory chain is not important clinically, but it is worth remem-

Table 1 Mitochondrial disorders

Nuclear genetic disorders	Inheritance pattern
Disorders of mtDNA maintenance	
Autosomal dominant external ophthalmoplegia (with 2° multiple mtDNA deletions)	
Ant 1 mutations	AD
POLG mutations	AD or AR
Twinkle (C10orf2) mutations	AD
Mitochondrial neuro-gastrointestinal encephalomyopathy (with 2° multiple mtDNA deletions)	
Thymidine phosphorylase gene	AR
Myopathy with mtDNA depletion	
Thymidine kinase deficiency	AR
Encephalopathy with liver failure	
Deoxyguanosine kinase deficiency	AR
Disorders of mitochondrial protein import	
Dystonia-deafness	
DDP1/TIMM8a mutations	XLR
Primary disorders of the respiratory chain	
Leigh's syndrome	
Complex I deficiency – mutations in NDUFS2,4,7,8 and FV1 complex I subunits	AR
Complex II deficiency – mutations in Fp subunit of complex II	AR
Leukodystrophy and myoclonic epilepsy	
Complex I deficiency – mutations in NDUFV1 complex I subunit (Scheulke *et al.* 1999)	AR
Cardioencephalomyopathy	
Complex I deficiency – mutations in *NDUFS2*	AR
Optic atrophy and ataxia	
Complex II deficiency – mutations in Fp subunit of complex II	AD
Disorders of assembly of the respiratory chain	
Leigh's syndrome	
Complex IV deficiency – mutations in *SURF* I	AR
Complex IV deficiency – mutations in *COX* 10	AR
Cardioencephalomyopathy	
Complex IV deficiency – mutations in *SCO* 2	AR
Hepatic failure and encephalopathy	
Complex IV deficiency – mutations in *SCO* 1	AR
Tubulopathy, encephalopathy and liver failure	
Complex III deficiency – mutations in BCS1L	AR
Mitochondrial genetic disorders (mtDNA nucleotide positions refer to the L-chain)	**Inheritance pattern**
Rearrangements (deletions and duplications)	
Chronic progressive external ophthalmoplegia (CPEO)	S
Kearns–Sayre syndrome	S
Diabetes and deafness	S
Point mutations	
Protein-encoding genes	
LHON (G11778A, T14484C, G3460A)	M
NARP/Leigh syndrome (T8993G/C)	M
tRNA genes	
MELAS (A3243G, T3271C, A3251G)	M
MERRF (A8344G, T8356C)	M
CPEO (A3243G, T4274C)	M
Myopathy (T14709C, A12320G)	M
Cardiomyopathy (A3243G, A4269G)	M
Diabetes and deafness (A3243G, C12258A)	M
Encephalomyopathy (G1606A, T10010C)	M
rRNA genes	
Non-syndromic sensorineural deafness (A7445G)	M
Aminoglycoside induced nonsyndromic deafness (A1555G)	M

AD, autosomal dominant; AR, autosomal recessive; M, maternal; S, sporadic; XLR, X-linked recessive.

bering that complex II is also called succinate dehydrogenase (SDH) and complex IV is usually called cytochrome *c* oxidase (COX).

MITOCHONDRIAL BIOGENESIS: A TALE OF TWO GENOMES

The respiratory chain has a dual genetic basis. The vast majority of the respiratory chain complex subunits are synthesized within the cytoplasm from nuclear gene transcripts (messenger RNA molecules transcribed from genes within the cell nucleus). These are delivered into mitochondria by a targeting sequence that enters through the mitochondrial protein import machinery. Thirteen of the complex subunits are synthesized within the mitochondria themselves from small circles of DNA called the mitochondrial genome (mtDNA; Fig. 2). In addition to the protein coding genes, mtDNA also encodes for 24 RNA molecules that are needed for intramitochondrial protein synthesis. As a result, genetic mutations of nuclear DNA (nDNA) or mtDNA can affect respiratory chain activity. MtDNA defects fall into two groups: rearrangements (large chunks of deleted or duplicated mtDNA) and point mutations (single base changes). These mutations can affect the RNA genes and lead to a general defect of protein synthesis within the mitochondria, or they can affect the protein-encoding genes themselves. MtDNA duplications are often found in patients harbouring mtDNA deletions, but the duplications are not thought to be pathogenic.

Mitochondria cannot survive on their own, and there are many nuclear-encoded factors that play a crucial role in maintaining a healthy respiratory chain. Over recent years we have learned that these factors are important clinically. The nucleus maintains healthy mtDNA thoughout human life. Disruption of the mtDNA polymerase-γ (POLG), or the balance of nucleotides (DNA building blocks) within the mitochondrial matrix, leads to the formation of many different secondary mutations of mtDNA throughout life, or the loss (depletion) of mtDNA (Table 1, disorders of mtDNA maintenance). Finally, specific proteins are needed to assemble the various components of the respiratory chain into the complete complexes, and disruption of these processes can also lead to severe respiratory chain deficiencies that usually present in childhood (Table 1, disorders of assembly of the respiratory chain).

Two other mitochondrial components also need mentioning. Co-enzyme Q10

Table 2 Clinical syndromes associated with mitochondrial disease

DISORDER	PRIMARY FEATURES	ADDITIONAL FEATURES
Chronic progressive external ophthalmoplegia (CPEO)	External ophthalmoplegia and bilateral ptosis	Mild proximal myopathy
Infantile myopathy and lactic acidosis (fatal and nonfatal forms)	Hypotonia in the first year of life Feeding and respiratory difficulties	Fatal form may be associated with a cardiomyopathy and/or the Toni–Fanconi–Debre syndrome
Kearns–Sayre syndrome (KSS)	PEO onset before age 20 with pigmentary retinopathy plus one of the following: CSF protein greater than 1 g/L, cerebellar ataxia, heart block	Bilateral deafness Myopathy Dysphagia Diabetes mellitus Hypoparathyroidism Dementia
Leber's hereditary optic neuropathy (LHON)	Subacute painless bilateral visual failure Males:females approx. 4 : 1 Median age of onset 24 years	Dystonia Cardiac pre–excitation syndromes
Leigh's syndrome (LS)	Subacute relapsing encephalopathy with cerebellar and brain-stem signs presenting during infancy	Basal ganglia lucencies
Mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes (MELAS)	Stroke-like episodes before age 40 years Seizures and/or dementia Ragged-red fibres and/or lactic acidosis	Diabetes mellitus Cardiomyopathy (hypertrophic leading to dilated) Bilateral deafness Pigmentary retinopathy Cerebellar ataxia
Myoclonic epilepsy with ragged-red fibres (MERF)	Myoclonus Cerebellar ataxia Myopathy	Dementia Seizures Optic atrophy Bilateral deafness Peripheral neuropathy Spasticity Multiple lipomata
Neurogenic weakness with ataxia and retinitis pigmentosa (NARP)	Late childhood or adult onset peripheral neuropathy with associated ataxia and pigmentary retinopathy	Basal ganglia lucencies Abnormal electroretinogram Sensorimotor neuropathy
Pearson's Syndome	Sideroblastic anaemia of childhood Pancytopenia Exocrine pancreatic failure	Renal tubular defects

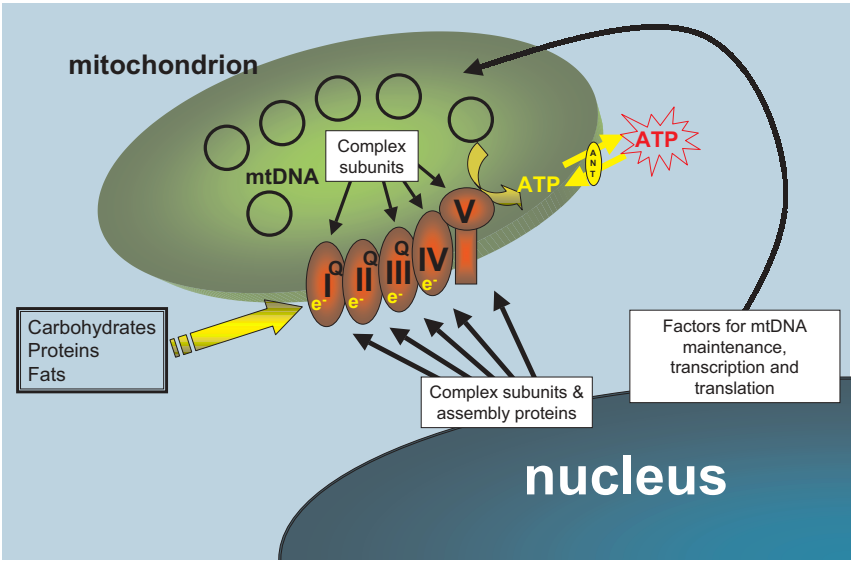

Figure 1 Nuclear – mitochondrial interactions and the respiratory chain. The mitochondrial respiratory chain consists of five enzyme complexes that use the products of intermediary metabolism (of proteins, carbohydrates and fats) to synthesize adenosine triphosphate (ATP) which is shuttled out of the mitochondria by adenine nucleotide transferrase (ANT). MtDNA is maintained by a number of nuclear encoded factors. Nuclear factors also regulate the transcription of mtDNA (forming a messenger RNA template) and the translation of the transcripts into proteins within the mitochondrion. Nuclear DNA also codes for most of the respiratory chain subunits and the complex assembly factors. MtDNA codes for 13 essential respiratory chain subunits and part of the machinery needed for protein synthesis within the mitochondrial matrix. e^-, electrons; Q, coenzyme Q10 (ubiquinone).

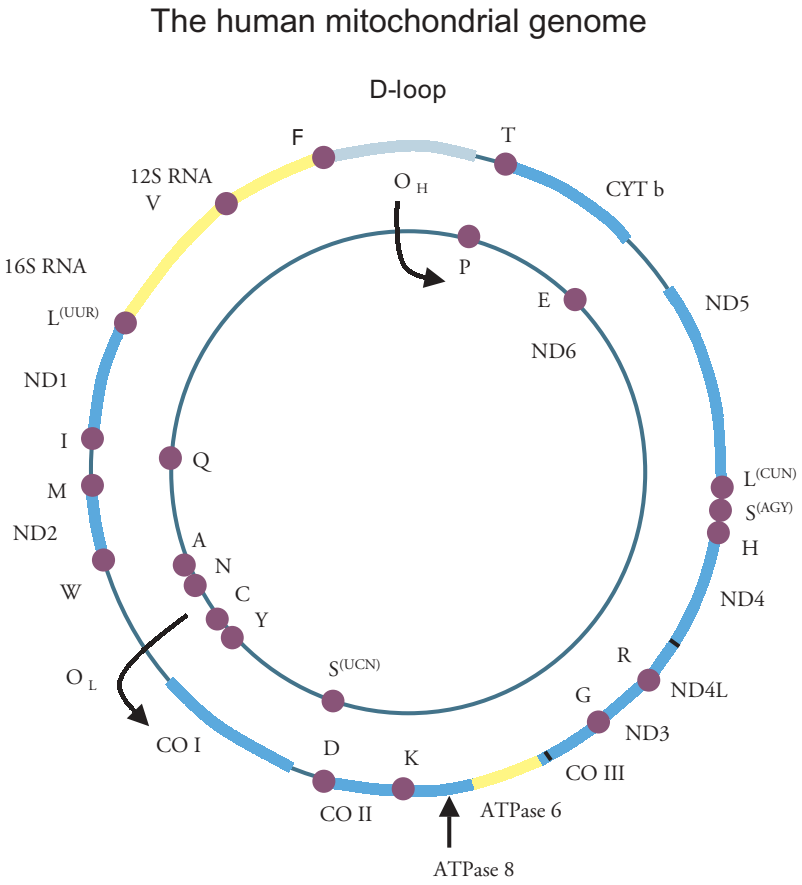

Figure 2 The human mitochondrial genome. The human mitochondrial genome (mtDNA) is a small 16.5kb molecule of double stranded DNA. The D-loop is the 1.1kb noncoding region that is involved in the regulation of transcription and replication of the molecule, and is the only region not directly involved in the synthesis of respiratory chain polypeptides. MtDNA encodes for 13 essential components of the respiratory chain. ND1-ND6, and ND4L encode seven subunits of complex I. Cyt b is the only mtDNA encoded complex III subunit. COX I to III encode for three of the complex IV (cytochrome *c* oxidase, or COX) subunits, and the ATPase 6 and ATPase 8 genes encode for two subunits of complex V. Two ribosomal RNA genes (12S and 16S rRNA), and 22 transfer RNA genes are interspaced between the protein-encoding genes. These provide the necessary RNA components for intramitochondrial protein synthesis. O_H and O_L are the origins of heavy and light strand mtDNA replication.

(ubiquinone) has an important role shuttling electrons between different respiratory chain complexes, and which adenine nucleotide transferrase (ANT) exchanges ATP and ADP across the mitochondrial membrane.

MITOCHONDRIAL DNA, HETEROPLASMY AND THE THRESHOLD EFFECT

Mononuclear human cells contain only two copies of each nuclear gene, but many thousands of copies of mtDNA. At birth all the mtDNA molecules are identical (a situation called homoplasmy). Patients with pathogenic mtDNA defects usually harbour a mixture of mutant and wild-type (normal) mtDNA within each cell (heteroplasmy). The proportion of mutant mtDNA can vary between 1 and 99%, and the cell only expresses a biochemical defect when the proportion of mutant mtDNA exceeds a critical threshold (typically 50–80% mutant, depending on the exact genetic defect).

TISSUE SPECIFICITY...GETTING MORE COMPLICATED

Perhaps the most difficult thing to explain is the relative selectivity of clinical involvement. Although the traditional view is that mitochondrial disorders can affect any organ system this is not strictly true, and certain tissues seem to be preferentially involved. In simple terms, tissues and organs that are heavily dependent upon ATP appear to be preferentially involved in patients with mitochondrial diseases. Neurones appear to be particularly vulnerable (including the retina and optic nerve), followed by skeletal and cardiac muscle, and endocrine organs (particularly the endocrine pancreas). However, a wide range of tissues may be involved including the cochlea, the gastrointestinal tract, the skin and haematological tissues.

Patients with mtDNA disease have the added complexity of mtDNA heteroplasmy. Different levels of mutant mtDNA in different tissues, coupled with tissue specific thresholds, partly explains the pattern of clinical involvement. However, if it were that simple then all patients with mitochondrial disease would look the same in the clinic – something that is clearly not the case.

PHENOCOPIES AND PHENOTYPIC VARIATION

To pick an example, the A3243G point mutation of mtDNA characteristically causes mitochondrial encephalopathy with lactic acidosis and

stroke-like episodes (MELAS). This mutation affects a mitochondrial tRNA gene that impairs the synthesis of respiratory chain proteins within mitochondria and it is invariably heteroplasmic. The A8344G point mutation of mtDNA causes myoclonic epilepsy with ragged-red fibres (MERRF) and also affects a mitochondrial tRNA gene with a consequent reduction in intramitochondrial protein synthesis, and it is also invariably heteroplasmic. Although there is some clinical overlap in the phenotypes associated with these two mutations, they often cause quite different disorders (Table 2), despite the fact that the molecular defects are strikingly similar.

Unfortunately there is a poor relationship between the clinical features of mitochondrial disease and the underlying genetic and biochemical abnormalities. Different genetic defects can cause a similar clinical phenotype (for example, a mutation in mtDNA or nDNA can cause clinically indistinguishable Leigh's syndrome), and yet the same genetic defect can also cause very different clinical features even within the same family (for example A3243G can cause MELAS, but more often causes a milder disorder with deafness, diabetes or external ophthalmoplegia with ptosis). It is becoming clear that additional mitochondrial and nuclear genetic factors modulate the expression of the primary mtDNA defects, and that these interact with the environment. We clearly do not have all the answers at present.

Despite the complexities, there are, however, a number of well recognised syndromes that are usually due to mitochondrial disease (Table 2). However, mitochondrial dysfunction should be considered in any patient with an unexplained neurological disorder along with multiple additional organ involvement.

PROBLEMS WITH MAKING A MOLECULAR DIAGNOSIS

If a patient has a suspected nuclear mitochondrial disorder, then DNA analysis can be carried out on a blood sample. In some patients with a mtDNA mutation it may be possible to identify the genetic defect in a blood sample (for example, in patients with Leber's hereditary optic neuropathy where most patients have *only* mutant mtDNA – homoplasmic mutant). Unfortunately a simple blood sample may not be sufficient when investigating every patient with suspected mitochondrial disease. This is because the level of mutant mtDNA may be very low in blood (below the threshold of detection), or not present at all. This is almost always the case in patients with sporadic chronic progressive external ophthalmoplegia or the Kearns–Sayre syndrome, which is usually due to a mtDNA deletion, but it also applies to point mutations such as the common A3243G mutation. Thus, whilst it is entirely reasonable to send off a blood sample for molecular analysis in patients with suspected mitochondrial disease, if the blood mtDNA test is negative the patient should then have a muscle biopsy.

CLUES FROM THE HISTOCHEMISTRY

Routine muscle histochemisty includes a reaction for COX and SDH (Fig. 3). COX has both nuclear and mtDNA encoded subunits. A generalized decrease in COX in all muscle fibres suggests that the genetic defect is in the nuclear genome, probably involving a COX

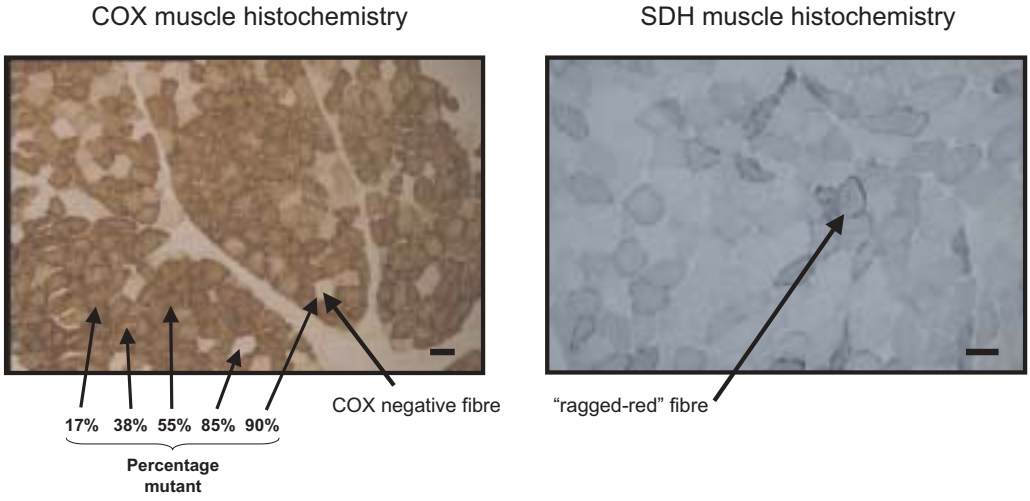

Figure 3 Skeletal muscle histochemistry from a patient with a pathogenic mtDNA defect. COX (cytochrome c oxidase; complex IV) showing a mosaic distribution of COX negative fibres. The COX negative fibres contain a high percentage of mutant mtDNA (above the critical threshold level, 80% for the mutation in this patient). SDH (succinate dehydrogenase; complex II) showing mitochondrial proliferation. The subsarcolemmal proliferation corresponds to the 'ragged-red' appearance of muscle fibres with the Gomori trichrome stain. Scale bar = 70 μm.

assembly gene. Patients with mtDNA defects often have a mosaic COX deficiency (Fig. 3) because different muscle fibres contain different amounts of mutant mtDNA and only some fibres contain supra-threshold levels. SDH is the only respiratory chain complex that is entirely encoded by nuclear genes. Patients with mtDNA mutations usually show up-regulation of SDH in affected fibres (thought to be a compensatory mechanism) and proliferation of mitochondria (corresponding to ragged red-fibres, Fig. 3). Unfortunately some patients have a biochemical defect that does not involve SDH or COX, and these patients have *normal* muscle histochemistry. As luck would have it, the most common heteroplasmic mtDNA point mutation (the A3243G 'MELAS' mutation) does just this. So, normal muscle histochemistry does *not* mean no mitochondrial disease. If a mitochondrial disease is still high on the list of differential diagnoses, then the next step is to measure individual respiratory chain complexes in muscle. This is only done well in a few specialist centres.

INHERITANCE

The nuclear genetic mitochondrial disorders are inherited as autosomal dominant, autosomal recessive or rarely as X-linked recessive traits (Table 1). By contrast, mtDNA is inherited down the maternal line. This means that a male *cannot* transmit the defect to their offspring – information that is always received by families with great relief. The offspring of women with a mtDNA defect are at risk of inheriting the disorder. Approximately one-third of pathogenic mtDNA defects are deletions, and the risk of women transmitting mtDNA deletions is low (< 1%, the reasons for this are not known). Approximately one-third of pathogenic mtDNA defects are homoplasmic (i.e. all of the mtDNA is mutant). In this situation, a woman will pass on the defect to all her offspring who will also be homoplasmic (this is usually the case for Leber's hereditary optic neuropathy, where about 40% of male offspring and 10% of female offspring become affected). For the remaining one-third, the mutation is heteroplasmic and a varying proportion of mutant mtDNA may be passed on to the offspring. The inherited mutation load roughly correlates with the likelihood of becoming clinically affected. There are no robust counselling guidelines available for women with heteroplasmic mtDNA mutations. This is an area of current study.

MITOCHONDRIAL DISORDERS – SOME FACTS TO REMEMBER

- Mitochondrial disorders are primary disorders of the respiratory chain.
- Mitochondrial disorders can be due to genetic defects in either the nuclear genome or the mitochondrial genome.
- Nuclear genetic mitochondrial disorders are inherited as autosomal dominant, recessive or rarely as X-linked traits.
- Most adults with mitochondrial disease have an underlying defect of mitochondrial DNA (mtDNA).
- In many patients there is a mixture of mutant and wild-type (normal) mtDNA (heteroplasmy).
- The level of mutant mtDNA heteroplasmy may be undetectable in blood, and patients with suspected mtDNA disease should have a muscle biopsy if blood DNA tests are negative.
- MtDNA disorders are transmitted down the maternal line – males cannot transmit the genetic defect.
- The recurrence risks for most mtDNA disorders are not well established. Some are sporadic and some are transmitted.
- There is no effective treatment for mitochondrial disorders – management is supportive.

ANY TREATMENTS?

There are currently no established disease modifying treatments for mitochondrial disease. Patients are often given ubiquinone (coenzyme Q10) because it is innocuous and there have been reports of improvement in some cases. Various vitamins and cofactors have also been used, and there is a clinical trial of dichloracetate currently in progress. Management is largely supportive, with particular attention being paid to genetic counselling.

CONCLUSION

Although mitochondrial disease is confusing, it is relatively straightforward to avoid the clinical pitfalls if a few basic facts are kept in mind (see text in box). We may have no treatments, but a precise diagnosis has important implications for the management of these patients who are being increasingly recognized.

ACKNOWLEDGEMENTS

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FURTHER READING

Genetests: a medical genetics information resource. http://www.geneclinics.org/

Mitomap: a human mitochondrial genome database. http://www.mitomap.org/

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