Genetics

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The clinical spectrum of mitochondrial genetic disorders

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Mitochondrial disorders are increasingly being recognised as a major cause of chronic morbidity,[1,2] with an estimated minimum prevalence of one in 10,000 in the UK and a further one in 5,000 at-risk carriers.[3] The clinical manifestations of this group of disorders are extremely varied and the initial presentation can be to any medical subspecialty. This brief overview describes the salient features that might alert physicians to a possible mitochondrial aetiology and the specialist investigations currently available to diagnose these diseases.

The mitochondrion

Mitochondria are present in every nucleated cell and are the major source of adenosine triphosphate obtained through the process of oxidative phosphorylation (OXPHOS) involving five respiratory chain complexes situated on the inner mitochondrial membrane. Mitochondrial dysfunction results in an energy deficit detrimental to normal biological activities and, if severe, ultimately leads to cell death, organ failure and overt disease.

Mitochondria contain their own genome – the only source of DNA found outside the nucleus in mammalian cells. Mitochondrial DNA (mtDNA) is a small, circular molecule 16,569 base pairs in length containing a limited number of genes which code for two ribosomal RNAs (rRNAs), 22 transfer RNAs (tRNAs) and 13 essential OXPHOS polypeptides (Fig 1). All the remaining mitochondrial structural and respiratory chain subunits (>100) are encoded by nuclear DNA

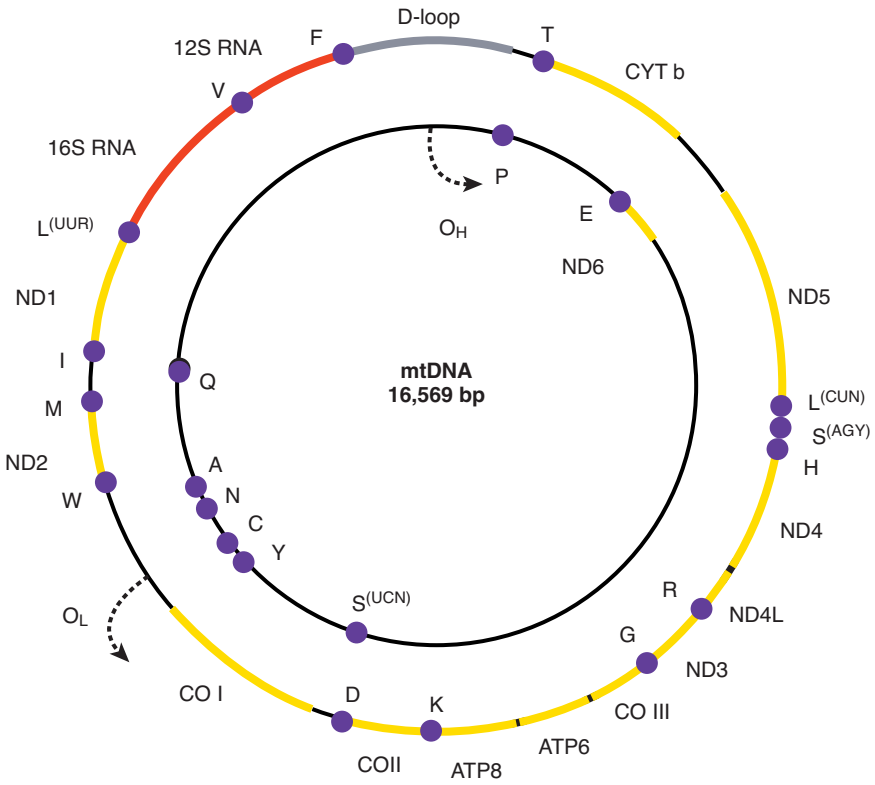

Fig 1. The human mitochondrial genome. Heavy (H-, outer) and light (L-, inner) strands are depicted with their protein coding genes. Protein coding (yellow), ribosomal RNA (rRNA)(red) and transfer RNA (tRNA) (purple) genes are shown on the strand which serves as template during their transcription. The 22 tRNAs are indicated by their cognate amino acid letter code and the two rRNAs by their sedimentation coefficient (12S and 16S). The origins of DNA replication and the direction of synthesis are denoted by O_H for the H-strand and O_L for the L strand. mtDNA = mitochondrial DNA.

(nDNA). This highlights the key concept that mitochondrial disorders can arise from mutations in both the mitochondrial genome (primary mtDNA disorders) and the nuclear genome (nuclear mitochondrial disorders) (Table 1).

Table 1. Primary mitochondrial (mtDNA) and nuclear DNA mutations causing mitochondrial disease.

	Inheritance pattern
Mitochondrial genetic disorders*	
Rearrangements (large-scale partial deletions and duplications):	
CPEO	S/M
Kearns-Sayre syndrome	S/M
Diabetes and deafness	S
Pearson marrow-pancreas syndrome	S/M
Point mutations:	
Protein-encoding genes	
LHON (11778G>A, 14484T>C, 3460G>A)	M
NARP/Leigh syndrome (8993T>G/C)	M
tRNA genes	
MELAS (3243A>G, 3271T>C, 3251A>G)	M
MERRF (8344A>G, 8356T>C)	M
CPEO (3243A>G, 4274T>C)	M
Cardiomyopathy (3243A>G, 4269A>G, 4300A>G)	M
Diabetes and deafness (3243A>G, 12258C>A)	M
rRNA genes	
Aminoglycoside-induced non-syndromic deafness (1555A>G)	M
Nuclear genetic disorders	
Primary disorder of the respiratory chain:	
Leigh syndrome (complex I deficiency, mutations in *NDUFS1, 4, 7, 8 and NDUFV1*; complex II deficiency, *SDHA*)	AR
Cardiomyopathy and encephalopathy (complex I deficiency, mutations in *NDUFS2*)	AR
Optic atrophy and ataxia (complex II deficiency, mutations in *SDHA*)	AD
Disorders of assembly of the respiratory chain:	
Leigh syndrome (mutations in *SURF* I and the mRNA binding protein *LRPPRC*)	AR
Hepatopathy and ketoacidosis (mutations in *SCO1*)	AR
Cardiomyopathy and encephalopathy (mutations in *SCO2*)	AR
Leukodystrophy and renal tubulopathy (mutations in *COX10*)	AR
Encephalopathy, liver failure, renal tubulopathy (with complex III deficiency, mutations in *BCS1L*)	AR
Disorders of mtDNA maintenance:	
Autosomal progressive external ophthalmoplegia (mutations in *POLG, POLG2, PEO1* and *SLC25A4*)	AD/AR
Mitochondrial neurogastrointestinal encephalomyopathy (thymidine phosphorylase deficiency, mutations in *ECGF1*)	AR
Alpers syndrome (mutations in *POLG* and *MPV*)	AD/AR
Infantile myopathy/spinal muscular atrophy (mutations in TK2)	AR
Encephalomyopathy and liver failure (mutations in *DGUOK*)	AR
Hypotonia, movement disorder and/or Leigh syndrome with methylmalonic aciduria (mutations in *SUCLA2*)	AR
Optic atrophy, ophthalmoplegia, ataxia, peripheral neuropathy (mutations in *OPA1*)	AD

* mtDNA nucleotide positions refer to the L-chain, and are taken from the Cambridge reference sequence.
AD = autosomal dominant; Alpers syndrome = epilepsy, cortical blindness, micronodular hepatic cirrhosis, episodic psychomotor regression; AR = autosomal recessive; COX= cytochrome C oxidase; CPEO = chronic progressive external ophthalmoplegia; LHON = Leber hereditary optic neuropathy; M = maternal; MELAS = mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes; MERRF = myoclonic epilepsy with ragged red fibres; NARP = neurogenic weakness with ataxia and retinitis pigmentosa; POLG = polymerase gamma; rRNA = ribosomal RNA; S = sporadic; tRNA = transfer RNA.

Primary mitochondrial DNA disorders

Primary mtDNA disorders obey two important rules:

1 Strict maternal inheritance, important for genetic counselling.
2 A critical mutational threshold must be exceeded before the energy production within the cell becomes compromised.

Depending on their metabolic requirements, cells can contain anywhere between 100 and 10,000 mitochondria. As each mitochondrion contains 2–10 mtDNA molecules, this results in a high mtDNA copy number. Individuals with mtDNA disease often harbour both mutant and wild-type mtDNA, known as heteroplasmy.[4] This is important since individual cells express an OXPHOS defect only when the proportion of the mutant species exceeds a critical threshold, which varies between tissues. In general, post-mitotic (non-dividing) tissues such as neurones and muscle fibres accumulate much higher levels of mutant mtDNA and tend to be affected preferentially. Over 100 primary mtDNA defects have been identified (point mutations or deletions) resulting in a heterogeneous group of diseases, both defined clinical syndromes and overlapping multisystemic phenotypes.

Nuclear mitochondrial disorders

Mutations in a growing number of nuclear genes are now known to disrupt the maintenance of the mitochondrial genome, resulting in secondary mtDNA deletions or depletion. The most important nuclear genes implicated in mitochondrial disease code for mtDNA polymerase gamma (*POLG*), mtDNA helicase Twinkle (*PEO1*) and the outer mitochondrial membrane translocase ANT-1 (*SCL25A4*). Although relatively rare, nuclear defects in the respiratory chain complex I subunits (*NDUFS 1,2,4,7* and *8*, and *NDUFV1*) are associated with Leigh syndrome and leukodystrophy, and mutations in complex II subunits with Leigh syndrome, paragangliomas and phaeochromocytomas.[1] Leigh syndrome can result from muta-

tions in all five respiratory chain complexes, either through mutations in the subunits themselves or in the proteins involved in the assembly of these subunits (*BSCL1L*, *COX10* and *15*, *SCO2* and *SURF1*). Nuclear mitochondrial disorders have a wide phenotypic spectrum similar to primary mtDNA diseases.[5]

Clinical features

The diverse, non-specific and overlapping clinical features of mitochondrial disorders often result in incorrect and delayed diagnoses (Fig 2).[6,7] As a general rule, mitochondrial diseases should be considered in all cases with a complex, multisystemic presentation, especially those with neuromuscular, ocular and endocrine involvement. Although the list of mitochondrial disorders is extensive and difficult to remember in routine clinical practice, patients with mitochondrial diseases can be divided into one of four categories.[8]

Classical mitochondrial phenotype

In a small minority of patients a mitochondrial aetiology is relatively straightforward to identify, for example, mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes (MELAS). Patients with the 3243A>G mtDNA point mutation usually achieve normal developmental milestones, but go on to develop bilateral deafness in late childhood, diabetes in early adulthood and progressive neurological disabilities from the third decade of life with migraines, seizures, stroke-like episodes and encephalopathy. However, not all MELAS patients will follow this clinical pattern. The 3243A>G mutation can lead to a more limited phenotype, with only diabetes and deafness (maternally inherited diabetes and deafness, MIDD), or an isolated cardiomyopathy.

Another primary mtDNA disease with a fairly typical presentation is Leber hereditary optic neuropathy (LHON). This predominantly affects young adult males, with a history of subacute bilateral visual failure, both eyes becoming affected within 2–3 months. Over 95% of LHON cases carry one of three mtDNA point mutations: 3460G>A, 11778G>A or 14484T>C.[9,10] Two other typical features present in up to 20% of mitochondrial disorders are ptosis and the gradual limitation of eye movements (chronic progressive external ophthalmoplegia (CPEO)). Over 95% of patients with CPEO are sporadic cases due to mtDNA point mutations or deletions, but it can also be inherited as either an autosomal dominant or recessive trait secondary to mutations in *POLG*, *PEO1* and *SCL25A4*.[11,12]

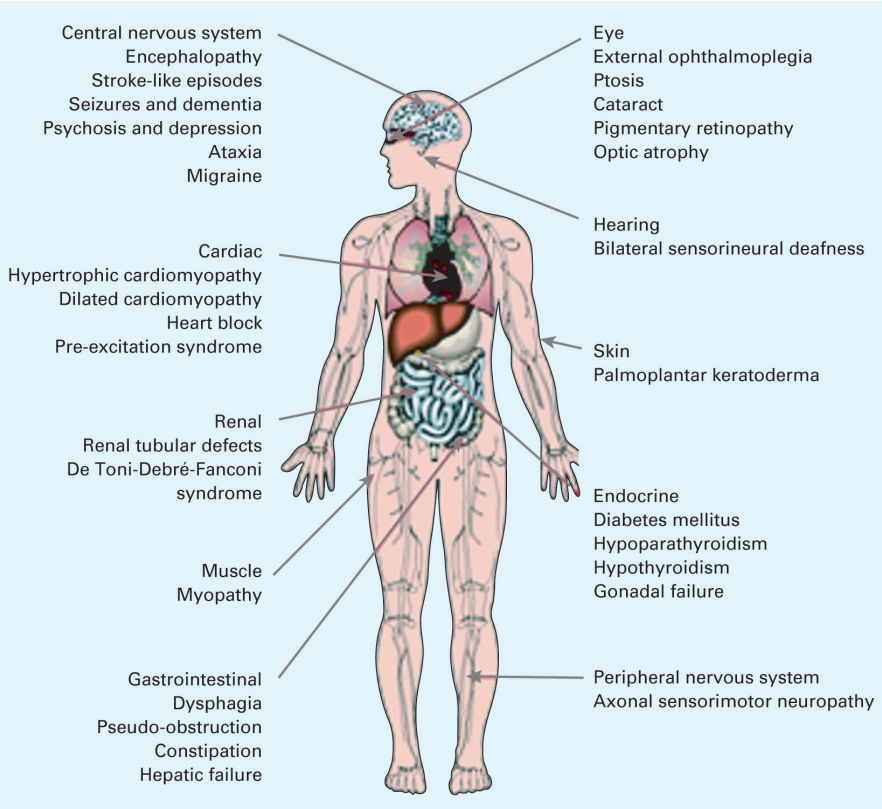

Fig 2. The clinical features of mitochondrial disorders. Reproduced with permission from BMJ Publishing Group.[7]

Suspected mitochondrial aetiology

A second group of patients presents with a cluster of clinical features which do not fit into a specific syndrome category, but, when viewed together, are highly suggestive of mitochondrial disease (Fig 2). These include:

- short stature
- impaired glucose tolerance
- exercise intolerance
- sensorineural deafness
- premature cataracts.

Non-specific clinical presentations

With molecular genetic testing becoming widely available for an increasing array of pathogenic mtDNA and nDNA mutations, the phenotypes linked to mitochondrial disorders have expanded considerably and now include various non-specific presentations. This group is the most challenging for clinicians. It is the hardest to define and includes disparate symptoms and signs not previously associated with this group of diseases. For example, mtDNA defects cause a small, yet significant proportion of strokes in young adults (<40 years). Other mitochondrial diseases will present only with isolated, non-neurological features, all of which are relatively common in the general population, such as:

- hypoparathyroidism
- adrenal insufficiency

- gastrointestinal dysfunction (vomiting, dysphagia, constipation and diarrhoea)
- renal tubular disease
- psychiatric disturbance
- infertility.

In these situations it is often the family history that provides the clue to the diagnosis, with other affected family members having evidence of multisystem involvement.

Paediatric group

Children are less likely to present with a 'classical' mitochondrial syndrome and have a much higher prevalence of nuclear genetic defects than adults.[8] Mitochondrial disorders in the paediatric population are invariably severe and result in premature death as a result of subacute necrotising encephalomyopathy (Leigh syndrome), hepatorenal failure (mtDNA depletion syndromes), cardiomyopathy and severe lactic acidosis. Children with Pearson syndrome, caused by the accumulation of mtDNA deletions, typically present with pancytopenia, sideroblastic anaemia and exocrine pancreatic failure.

Investigations

The diagnostic approach to mitochondrial disorders (Table 2) starts with a detailed clinical history for the whole family, looking specifically for a possible mode of inheritance and a pattern of organ involvement consistent with the more common mitochondrial disease variants. General clinical investigations should not be overlooked and are important in the detection of subclinical disease and the prevention of secondary complications. These include:

- cardiac assessment (ECG, echocardiography)
- endocrine studies (eg oral glucose tolerance test and thyroid function tests)
- measurement of urinary organic and amino acids, fasting plasma lactate and creatine kinase levels
- analysis of cerebrospinal fluid (including lactate)

Table 2. Investigation of suspected mitochondrial disease.

General investigations	Looking for:
Detailed personal and family history	Common phenotypes (including recurrent miscarriages, premature neonatal deaths, diabetes and blindness)
Neurological and general clinical examination	Features supporting mitochondrial dysfunction (eg myopathy, ataxia, peripheral neuropathy)
Cardiac assessment (ECG, echocardiography)	Cardiomyopathy or conduction defect (ECG) Assessment of myocardial thickness and function (echocardiography)
Ophthalmological assessment	Ptosis, restriction of eye movement, optic atrophy, pigmentary retinopathy
Endocrine studies (eg oral GTT, TFT)	Diabetes and other signs of metabolic dysfunction
Fasting plasma lactate	Raised blood lactate (more likely in children)
CSF analysis	Levels of protein and lactate*
CK levels	Raised levels (more likely if proximal myopathy)
Neuroelectrophysiology (EMG)	Neuropathy or myopathy
Electroencephalography (EEG)	Monitoring of encephalopathy or seizure activity
Neuroimaging (CT, MRI)	Infarcts, MS-like lesions (MRI), bilateral hypodensities and atrophy, basal ganglia calcification (CT)
Metabolic studies: Urinary organic and amino acid levels, plasma and urine carnitine, blood ammonia, full blood count, urinalysis and CXR	Abnormalities in urinary organic and amino acid levels, plasma and urinary carnitine, blood ammonia
Specific investigations	
mtDNA analysis (Southern blot, point mutations)	Specific defects indicating a primary mtDNA disease
Nuclear DNA sequencing	Nuclear genetic defects causing (secondary) mitochondrial diseases
Muscle biopsy: Histochemical analysis	Subsarcolemmal accumulations of mitochondria (ragged red fibres – non-specific) COX-deficient fibres (mosaic COX defect)
Biochemical assays (eg measurement of individual respiratory chain complexes (I–V)	Deranged mitochondrial enzyme activity (isolated or multiple complex defects)

* More specific than plasma lactate, especially if neurological involvement, but other non-mitochondrial neurological conditions result in raised cerebrospinal fluid (CSF) lactate (eg seizures, thromboembolic stroke).
CK = creatine kinase; COX = cytochrome C oxidase; CT = computed tomography; CXR = chest X-ray; EMG = electromyography; GTT= glucose tolerance test; MRI = magnetic resonance imaging; MS = multiple sclerosis; mtDNA = mitochondrial DNA; nDNA = nuclear DNA; TFT= thyroid function test.

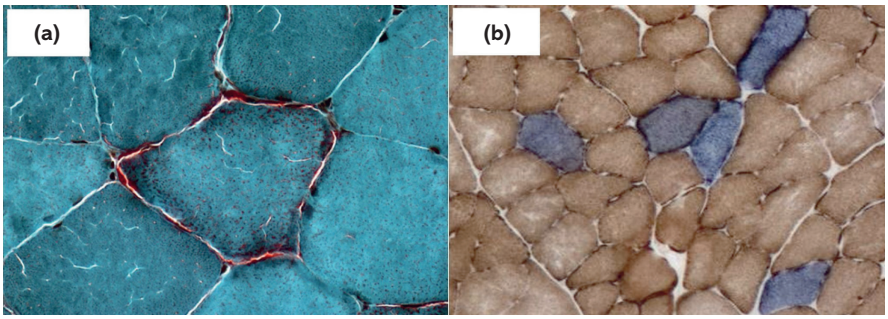

Fig 3. The pathological hallmarks of mitochondrial disorders. These muscle biopsy sections (20 μm thick) demonstrate the characteristic histochemical features of mitochondrial dysfunction: (a) the modified Gomori trichome stain is selectively sequestered by abnormal clusters of mitochondria at the sarcolemma, giving rise to the irregular red outline (ragged-red muscle fibre); (b) sequential cytochrome C oxidase (COX) and succinate dehydrogenase staining showing normal COX-positive (brown) and energy-deficient, COX-negative (blue) muscle fibres (with thanks to Professor Robert Taylor).

- neurophysiological evaluation for neuropathy or myopathy
- EEG for monitoring encephalopathy or seizure activity
- neuroimaging.

In cases where a specific clinical syndrome is identifiable (eg MELAS, CHON or CPEO), or molecular genetic studies looking for the known pathogenic mtDNA or nDNA defects are appropriate and a blood DNA sample is adequate. In other (especially sporadic) cases where the clinical features are non-specific, 'blind' DNA testing can be misleading and additional specialist investigations are required.

A muscle needle biopsy (a relatively minor procedure, performed under local anaesthetic) can provide valuable information when investigating a possible mitochondrial disorder.[13] Specific histochemical stains will reveal pathological hallmarks of mitochondrial dysfunction such as the subsarcolemmal accumulation of mitochondria, so-called 'ragged-red fibres' and cytochrome C oxidase-negative muscle fibres (Fig 3). These results should always be interpreted within the clinical context because a normal histochemical appearance does not exclude mitochondrial disease.

Various biochemical assays are also used in assessing mitochondrial enzyme activities. Measurement of the individual respiratory chain complexes (I–V) can also help determine whether a patient has isolated or multiple complex defects. These highly specialised procedures should be undertaken only in specialist centres and are sometimes carried out on other tissues (eg cultured skin fibroblasts).

The diagnosis of mitochondrial disorders is often complex and clinicians need to liaise closely with specialist diagnostic services to co-ordinate the appropriate investigations. The investigation of complex mitochondrial disorders in the UK is provided by three centres (Newcastle upon Tyne, Oxford and London) funded by the National Commissioning Group.

Key Points

Mitochondrial disorders constitute one of the most important groups of inherited human diseases, affecting at least one in 10,000 of the UK population

Mitochondrial DNA and nuclear DNA mutations can both result in mitochondrial disorders

Mitochondrial disorders should be considered in all cases with a complex, multisystemic presentation, especially those with neuromuscular, ocular and endocrine involvement

The non-specific nature of this heterogeneous group of diseases can often result in diagnostic delays; these can be avoided by using a few clinical 'rules of thumb' and by taking a structured investigative approach when a mitochondrial disorder is suspected

Treatment is currently limited and management mainly supportive, with an important role for genetic counselling

KEY WORDS: heteroplasmy, mitochondrial disorders, mitochondrial DNA, neurodegenerative diseases, respiratory chain defect

Treatment

There is no effective treatment for mitochondrial diseases and management is mainly supportive.[14] Based on small, uncontrolled studies, standard doses of vitamins C and K, thiamine, riboflavin and ubiquinone (Coenzyme Q_{10}) have shown varying degrees of benefit in individual cases. Many patients will require long-term follow-up to prevent secondary complications (eg related to cardiac and endocrine dysfunction) and a multidisciplinary approach is essential.[4] Specific therapeutic interventions include ptosis correction, squint and cataract surgery, cardiac pacing and percutaneous gastrostomy. These often result in significant improvements in quality of life.

Novel therapeutic strategies are currently under investigation, such as exercise therapy.[15] Other approaches include pre-implantation embryonic screening and the transfer of pro-nuclear DNA from the oocyte of a mother carrying a pathogenic mtDNA defect to an enucleated donor oocyte with only wild-type mtDNA.[16]

Genetic counselling

Genetic counselling for mitochondrial disorders is complex, partly because a precise molecular diagnosis is not always possible for patients and their families. Even when the underlying genetic defect is known, the situation is further complicated by incomplete penetrance and the

often marked inter- and intrafamilial variability in clinical manifestations. In primary mtDNA disorders, males cannot transmit the pathogenic mtDNA defect to their offspring and the mtDNA defect in the proband may have been maternally inherited or sporadic. For nuclear mitochondrial disorders, genetic counselling is based on the laws of Mendelian inheritance, but the clinical penetrance of most nuclear genetic mitochondrial disorders is not yet established.

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