NEUROSCIENCE FOR NEUROLOGISTS

Mitochondria

P F Chinnery, E A Schon

Following the discovery in the early 1960s that mitochondria contain their own DNA (mtDNA), there were two major advances, both in the 1980s: the human mtDNA sequence was published in 1981, and in 1988 the first pathogenic mtDNA mutations were identified. The floodgates were opened, and the 1990s became the decade of the mitochondrial genome. There has been a change of emphasis in the first few years of the new millennium, away from the “magic circle” of mtDNA and back to the nuclear genome. Various nuclear genes have been identified that are fundamentally important for mitochondrial homeostasis, and when these genes are disrupted, they cause autosomally inherited mitochondrial disease. Moreover, mitochondrial dysfunction plays an important role in the pathophysiology of several well established nuclear genetic disorders, such as dominant optic atrophy (mutations in OPA1), Friedreich’s ataxia (FRDA), hereditary spastic paraplegia (SPG7), and Wilson’s disease (ATP7B). The next major challenge is to define the more subtle interactions between nuclear and mitochondrial genes in health and disease.

HISTORICAL INTRODUCTION

It is over 40 years since the first human mitochondrial disease was described in a patient with non-thyroidal hypermetabolism (Luf disease). Although this disorder is exceptionally rare (only two cases have been described), the clinical description, and while some studies paved the way for three decades of clinical and pathological research on patients with suspected mitochondrial disease. Patients were classified into groups based upon the pattern of clinical involvement, histological and ultrastructural abnormalities of mitochondria, and biochemical assays of mitochondrial function. It was clear that there were clinical similarities among some patients, allowing the definition of syndromes such as the Kearn–Sayre syndrome (KSS) or chronic progressive external ophthalmoplegia (CPEO), but it was recognised that there was considerable phenotypic diversity and that many patients did not fit neatly into a specific diagnostic group.

The inheritance pattern also varied. Some patients appeared to be sporadic cases, whereas others were clearly familial. It was known for some time that mitochondrial DNA (mtDNA) was maternally inherited, and while some families displayed a clear maternal inheritance pattern, others did not. There were attempts to classify based upon the number and size of mitochondria in skeletal muscle, leading to terms such as pleoclonal or megaclonal myopathies, and also on the pattern of respiratory chain involvement. There were those who wanted to subdivide suspected mitochondrial disease into discrete categories (the “splitters”) and those who thought of all mitochondrial disease as a single, if wide, spectrum of disorders (the “lumpers”). At this early stage it was apparent that mitochondrial disorders were a heterogeneous group—clinically, histologically, biochemically, and probably genetically.

Following the discovery in the early 1960s that mitochondria contain their own DNA (mtDNA), there were two major advances, both in the 1980s: the human mtDNA sequence was published in 1981, and in 1988 the first pathogenic mtDNA mutations were identified. The floodgates were opened, and the 1990s became the decade of the mitochondrial genome. Over 150 different pathogenetic point mutations and a larger number of different rearrangements (that is, partial deletions and duplications) of mtDNA were associated with disease, and there were major advances in our understanding of the molecular pathophysiology. There has been a change of emphasis in the first few years of the new millennium, away from the “magic circle” of mtDNA and back to the nuclear genome. Various nuclear genes have been identified that are fundamentally important for mitochondrial homeostasis, and when these genes are disrupted, they cause autosomally inherited mitochondrial disease. Moreover, mitochondrial dysfunction plays an important role in the pathophysiology of several well established nuclear genetic disorders, such as dominant optic atrophy (mutations in OPA1), Friedreich’s ataxia (FRDA), hereditary spastic paraplegia (SPG7), and Wilson’s disease (ATP7B). The next major challenge is to define the more subtle interactions between nuclear and mitochondrial genes in health and disease. It is likely that these mechanisms will have broader relevance for our understanding of many inherited and sporadic neurological disorders.

In this article we will review the basic scientific principles that underpin our understanding of mitochondrial pathology. Rather than giving a comprehensive description of mitochondrial biology, we will focus on the bare essential facts that will help the practising general neurologist to understand, identify, investigate, and manage patients with primary mitochondrial disease (by which we mean disorders that result directly from mutations either in mtDNA or in nuclear genes affecting the respiratory chain or mtDNA homeostasis). Mitochondrial abnormalities have been identified in many common neurological disorders, including Alzheimer’s disease and Parkinson’s disease, and they also occur as part of
normal aging. The role of these secondary mitochondrial abnormalities is uncertain, and they will be discussed in other articles in this series.

WHAT ARE MITOCHONDRIA AND WHAT DO THEY DO?

Mitochondria are a subcompartment of the cell bound by a double membrane. Although some mitochondria probably do look like the traditional cigar shaped structures that appear in standard textbooks, it is more accurate to think of them as a budding and fusing network similar to the endoplasmic reticulum (fig 1). Mitochondria are intimately involved in cellular homeostasis. Among other functions they play a part in intracellullar signalling and apoptosis, intermediary metabolism, and in the metabolism of amino acids, lipids, cholesterol, steroids, and nucleotides. Apoptosis is discussed in other articles of this series and will not be considered here. Perhaps most importantly, mitochondria have a fundamental role in cellular energy metabolism. This includes fatty acid β oxidation, the urea cycle, and the final common pathway for ATP production—the respiratory chain.

The mitochondrial respiratory chain is a group of five enzyme complexes situated on the inner mitochondrial membrane (fig 2). Each complex is composed of multiple subunits, the largest being complex I with over 40 polypeptide components. Reduced cofactors (NADH and FADH2) generated from the intermediary metabolism of carbohydrates, proteins, and fats donate electrons to complex I and complex II. These electrons flow between the complexes down an electrochemical gradient, shuttled by complexes III and IV and by reduced cofactors (NADH and FADH2) generated from the intermediary metabolism of carbohydrates, proteins, and fats donate electrons to complex I and complex II. These electrons flow between the complexes down an electrochemical gradient, shuttled by complexes III and IV and by reduced cofactors (NADH and FADH2). The electron transfer function of complexes I–IV is accomplished through subunits harbouring prosthetic groups (for example, iron–sulphur groups in complexes I, II, and III, and haem iron in cytochrome c and complex IV). The liberated energy is used by complexes I, III, and IV to pump protons (H+ ) out of the mitochondrial matrix into the intermembrane space. This proton gradient, which generates the bulk of the mitochondrial membrane potential (the asymmetrical distribution of ions, such as Na+, K+, and Ca2+), across the inner membrane makes up the "chemical" portion of the gradient), is harnessed by complex V to synthesise adenosine triphosphate (ATP) from adenosine diphosphate (ADP) and inorganic phosphate. The overall process is called oxidative phosphorylation (OXPHOS). ATP is the high energy source used for essentially all active metabolic processes within the cell, and it must be released from the mitochondrion in exchange for cytosolic ADP. This is carried out by the adenine nucleotide translocator (ANT), which has various tissue specific isomers.

Thus the respiratory chain is an elaborate system that must respond to the energy requirements of the cell. While these requirements may be constant (for example, in hepatocytes), they may also change dramatically over short periods of time (as in skeletal muscle). We are only just beginning to understand the mechanisms that maintain and regulate a healthy respiratory chain, and it is likely that many additional unknown genetic and environmental factors will be involved.

THE GENETIC BASIS OF MITOCHONDRIAL BIOGENESIS

Two distinct genetic systems encode mitochondrial proteins: mitochondrial DNA (mtDNA) and nuclear DNA (nDNA). mtDNA is a small 16.6 kb circle of double stranded DNA that codes for 13 respiratory chain polypeptides and 24 nucleic acids (two ribosomal RNAs (rRNAs) and 22 transfer RNAs (tRNAs)) that are needed for intramitochondrial protein synthesis (fig 3). Nuclear genes code for the majority of mitochondrial respiratory chain polypeptides. These polypeptides are synthesised in the cytoplasm with a mitochondrial targeting sequence that directs them through the translocation machinery spanning the outer and inner membranes. The targeting sequence is then cleaved before the subunit is assembled with its counterparts on the inner mitochondrial membrane. The components of the import machinery ("TIM" and "TOM" proteins), the importation processing enzymes, and the respiratory chain assembly proteins are all the products of nuclear genes.

Nuclear genes are also important for maintaining the mitochondrial genome, including those encoding the mitochondrial DNA polymerase γ (POLG) and products that maintain an appropriate balance of free nucleotides within the mitochondrion (TP, TK, DGK, and ANT1). Nuclear genes code for the majority of mitochondrial respiratory chain polypeptides and 24 nucleic acids (two ribosomal RNAs (rRNAs) and 22 transfer RNAs (tRNAs)) that are needed for intramitochondrial protein synthesis (fig 3). Nuclear genes code for the majority of mitochondrial respiratory chain polypeptides. These polypeptides are synthesised in the cytoplasm with a mitochondrial targeting sequence that directs them through the translocation machinery spanning the outer and inner membranes. The targeting sequence is then cleaved before the subunit is assembled with its counterparts on the inner mitochondrial membrane. The components of the import machinery ("TIM" and "TOM" proteins), the importation processing enzymes, and the respiratory chain assembly proteins are all the products of nuclear genes.

MITOCHONDRIAL DISEASE: GENOTYPE AND PHENOTYPE

A neurologist who has seen a few patients with mitochondrial disease will be puzzled by a number of questions. If mitochondria are so important, why don’t mitochondrial diseases affect every tissue in the body, and if primary mitochondrial disorders are all genetically determined, why do they have such a varied clinical phenotype, even within the same family? Recent advances in our understanding of the molecular pathology of mitochondrial disease have provided us with some explanations and also raised new questions.

The clinical features of mitochondrial disease have been discussed widely elsewhere27–29 and they will not be considered in detail here (an overview is given in table 2 and fig 4). In general terms, tissues and organs that are heavily dependent upon oxidative phosphorylation bear the brunt of the pathology. This means that neurological features are common, but cardiac, endocrine, and ophthalmological features are often prominent. Other tissues are less dependent upon sustained oxidative phosphorylation so are less likely to be involved in mitochondrial disease, but there are some notable exceptions.

While this general rule is helpful, it can only be part of the explanation. Mitochondrial disease ultimately reflects a defect of oxidative phosphorylation within a cell, but the pattern of
MITOCHONDRIAL GENETIC FACTORS

mtDNA has unusual properties that are important for our understanding of mitochondrial disease caused by mtDNA mutations.

Heteroplasm and the threshold effect

While most human cells contain two copies of nuclear DNA, they contain many more copies of mtDNA (from 1000 to 100 000, depending on the cell type). These are all identical in a healthy individual at birth (homoplasmy). By contrast, patients harbouring pathogenic mtDNA defects often have a mixture of mutated and wild-type mtDNA (heteroplasmy). The percentage of mutated mtDNA can vary widely among different patients, and also from organ to organ, and even between cells within the same individual. In vitro studies using “transmitochondrial cytoplasmic hybrid (cybrid)” cells containing different amounts of mutated mtDNA have shown that most mtDNA mutations are highly recessive. In other words, the cells were able to tolerate high percentage levels of mutated mtDNA (typically 70–90%) before they developed a biochemical respiratory chain defect. The precise threshold for biochemical expression varies from mutation to mutation, and from tissue to tissue. Large retrospective studies have shown that the percentage level of mutated mtDNA in clinically relevant tissues does correlate with the severity of disease.

Figure 2. Schematic diagram of the respiratory chain. Reduced cofactors (NADH and FADH₂) are produced from the intermediary metabolism of carbohydrates, proteins, and fats. These cofactors donate electrons to [e⁻] to complex I (NADH-ubiquinone oxidoreductase) and complex II (succinate-ubiquinone oxidoreductase). These electrons flow between the complexes down an electrochemical gradient [black arrow], shuttled by ubiquinone [Q] and cytochrome c [C] involving complex III (ubiquinol-cytochrome c oxidase reductase) and complex IV (cytochrome c oxidase, or COX). Complex IV donates an electron to oxygen which results in the formation of water. Protons (H⁺) are pumped from the mitochondrial matrix into the intermembrane space (red arrows). This proton gradient generates the mitochondrial membrane potential which is harnessed by complex V to synthesize adenosine triphosphate (ATP) from adenosine diphosphate (ADP) and inorganic phosphate (Pi). ANT, adenine nucleotide translocator, which exchanges ADP for ATP across the mitochondrial membrane.

Maternal inheritance and the genetic bottleneck

Although it has been known for some time that mtDNA is transmitted from mother to offspring, the mechanisms are only just becoming clear. Sperm contain around 100 mtDNAs which enter the zygote on fertilisation before being actively degraded. There has been a recent report of a pathogenic mtDNA microdeletion in a patient with a sporadic muscle specific mitochondrial disorder. The mutated mtDNA arose on a mitochondrial genome that was paternal in origin, bringing into question the traditional dogma of strict maternal inheritance. However, many families with mtDNA disease have been studied in detail over the last decade, and there are no other reports of paternal mtDNA transmission. Based upon the available evidence, paternal leakage is unlikely to be clinically significant.

One of the most remarkable features of mitochondrial disease caused by mtDNA defects is the clinical variability among siblings. This is thought to reflect the mitochondrial “genetic bottleneck”.

Our understanding of the transmission of mtDNA heteroplasm has been greatly advanced by detailed studies of heteroplasmic mice generated by karyoplast transfer. These mice transmit heteroplasmic mtDNA polymorphisms (table 3). By measuring the variation in heteroplasm between the offspring of a single female, and...
Relaxed replication

Unlike nuclear DNA which replicates only once during each cell cycle, mtDNA is continuously recycled, even in non-dividing tissues such as skeletal muscle and brain.\(^7^\) mtDNA replication is therefore independent of the cell cycle (that is, it is relaxed). In a heteroplasmic cell, it is possible that mutated and wild-type mtDNA replicate at subtly different rates—either because one type was selected for destruction or replication by chance, or because of a subtle selective effect in favour of one particular type. In theory, this mechanism could lead to changes in the proportion of mutated mtDNA that have been described in patients with mtDNA disease, providing an explanation for the late onset and progression of some mtDNA disorders.\(^8^\)

Mitotic (vegetative) segregation

When a heteroplasmic cell divides, subtle differences in the proportion of mutated mtDNA may be passed on to the daughter cells, leading to changes in the level of mutated mtDNA within a dividing tissue.\(^9^\) The unequal partitioning may be a purely random process, independent of any selection caused by an effect of the mutation on mitochondrial function. On the other hand, presumed shifts due to functional selection may explain why the level of some pathogenic mtDNA mutations decreases in blood during life (for example, 0.5% to 1% per annum for A3243G\(^+\)).

mtDNA “background,” nuclear genes, and the environment

While there are a great many different heteroplasmic mtDNA mutations, in epidemiological terms most patients with a pathogenic mtDNA defect harbour only mutated mtDNA (that is, they are homoplasmic mutated).\(^10^\) The most common example is LHON (Leber hereditary optic neuropathy).\(^11^\) LHON is a mitochondrial genetic disorder that is primarily caused by mutations in mtDNA complex I (ND) genes and is characterised by subacute bilateral visual failure presenting in early adult life.\(^12^\) LHON is intriguing because it is essentially an organ specific disease that principally affects the retinal ganglion cells and the optic nerve.\(^13^\) LHON also has a markedly reduced penetrance with a clear sex bias, with only around 50% of men and around 10% of women developing visual failure.\(^14^\) Most patients with LHON are homoplasmic mutated for one of three mtDNA ND gene mutations (fig 4),\(^15^\) so heteroplasmia cannot explain the varied disease penetrance, and certain unknown additional factors appear to be important.

Wild-type (normal) mtDNA can be subdivided into different genetic groups (haplogroups) based upon a characteristic pattern of polymorphism that occurs within the normal population.\(^16^\) Two of the three principal LHON mtDNA mutations (T14484C in the ND6 gene and G11778A in the ND4 gene) are preferentially associated with haplogroup J, which is found in around 13% of northern Europeans.\(^17^\) The reason for this association is not known, but it seems likely that haplogroup J increases the penetrance of the T14484C and G11778A mutations.\(^18^\) It therefore appears that the mitochondrial genetic background can influence disease expression, but this cannot explain the sex bias in LHON.

The segregation pattern of disease in some LHON families suggests that there may be a nuclear genetic modifier locus modulating the clinical expression of the LHON mtDNA mutations. A recessive visual loss susceptibility locus on the X chromosome would explain the sex bias in LHON,\(^19^\) but attempts to identify the locus have not been successful.\(^20^\) Environmental factors may also play a part in LHON. There are many anecdotal reports of visual failure following alcohol intoxication, starvation, heavy smoking, and head trauma,\(^21^\) but large studies have yielded conflicting results.\(^22^\)
In many ways LHON is best considered as a complex trait, where the disease phenotype arises through multiple genetic factors (both mitochondrial and nuclear) interacting with the environment. A similar mechanism might explain the variable penetrance of other homoplasmic mtDNA mutations that cause organ specific disease, such as the A1555G mtDNA mutation in the 12S rRNA gene that causes maternally inherited susceptibility to aminoglycoside induced deafness, and possibly the A4300G mtDNA mutation in tRNA\(_{\text{Ile}}\) that causes maternally inherited cardiomyopathy (see table 1). Similar nuclear–mitochondrial interactions are also likely to contribute to the varied phenotype seen in other mitochondrial disorders, be they caused by primary nDNA defects or primary mtDNA defects.

### Nuclear genetic disorders

#### Disorders of mtDNA maintenance

- **Autosomal dominant progressive external ophthalmoplegia (with 2° multiple mtDNA deletions)**
  - Mutations in adenine nucleotide translocator (ANT1) AD
  - Mutations in DNA polymerase \(\gamma\) (POLG) AD or AR
  - Mutations in Twinkle helicase (C10orf2) AD

- **Mitochondrial neuro-gastrointestinal encephalomyopathy (with 2° multiple mtDNA deletions)**
  - Mutations in thymidine phosphorylase (TP) AR
  - Mutations in thymidine kinase (TK2) AR
  - Encephalopathy with liver failure
    - Mutations in deoxyguanosine kinase (DGK) AR

#### Primary disorders of the respiratory chain

- **Leigh syndrome**
  - Complex I deficiency: mutations in complex I subunits (NDUFS2, 4, 7, 8, and NDUFV1) AR
  - Complex II deficiency: mutations in complex II flavoprotein subunit (SDH) AR
  - Leukodystrophy and myoclonic epilepsy
    - Complex I deficiency: mutations in complex I subunit (NDUFV1) AR
  - Cardioencephalomyopathy
    - Complex I deficiency: mutations in complex I subunit (NDUFS2) AR

- **Optic atrophy and ataxia**
  - Complex II deficiency: mutations in complex II flavoprotein subunit (SDH) AR

#### Disorders of mitochondrial protein import

- **Dystonia-deafness**
  - Mutations in deafness-dystonia protein (DDP1) (TIMM8A) XLR

- **Disorders of assembly of the respiratory chain**

- **Leigh syndrome**
  - Complex IV deficiency: mutations in COX assembly protein (SURF1) AR
  - Complex IV deficiency: mutations in COX assembly protein (COX10) AR

- **Cardioencephalomyopathy**
  - Complex IV deficiency: mutations in COX assembly protein (SCO2) AR

- **Hepatic failure and encephalopathy**
  - Complex IV deficiency: mutations in COX assembly protein (SCO1) AR

- **Tubulopathy, encephalopathy, and liver failure**
  - Complex III deficiency: mutations in complex III assembly (BCS1L) AR

*mtDNA nucleotide positions refer to the L-chain, and are taken from the standard Cambridge reference sequence*. AD, autosomal dominant; AR, autosomal recessive; M, maternal; S, sporadic; XLR, X linked recessive.

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**Table 1 Genetic classification of human mitochondrial disorders**

<table>
<thead>
<tr>
<th>Type of disorder</th>
<th>Inheritance pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mitochondrial genetic disorders</strong></td>
<td></td>
</tr>
<tr>
<td>Rearrangements (large scale partial deletions and duplications)</td>
<td>S</td>
</tr>
<tr>
<td>Chronic progressive external ophthalmoplegia (CPEO)</td>
<td>S</td>
</tr>
<tr>
<td>Kearns-Sayre syndrome</td>
<td>S</td>
</tr>
<tr>
<td>Diabetes and deafness</td>
<td>S</td>
</tr>
<tr>
<td>Pearson marrow-pancreas syndrome</td>
<td>S</td>
</tr>
<tr>
<td>Sporadic tubulopathy</td>
<td>S</td>
</tr>
<tr>
<td><strong>Point mutations</strong></td>
<td></td>
</tr>
<tr>
<td>Protein encoding genes</td>
<td></td>
</tr>
<tr>
<td>• LHON (G11778A, T14484C, G3460A)</td>
<td>M</td>
</tr>
<tr>
<td>• NARP/Leigh syndrome (T8993G/C)</td>
<td>M</td>
</tr>
<tr>
<td>tRNA genes</td>
<td></td>
</tr>
<tr>
<td>• MELAS (A3243G, T3271C, A3251G)</td>
<td>M</td>
</tr>
<tr>
<td>• MERRF (A8344G, T8356C)</td>
<td>M</td>
</tr>
<tr>
<td>• CPEO (A3243G, T4274C)</td>
<td>M</td>
</tr>
<tr>
<td>• Myopathy (T14709C, A12320G)</td>
<td>M</td>
</tr>
<tr>
<td>• Cardiomyopathy (A3243G, A4269G, A4300G)</td>
<td>M</td>
</tr>
<tr>
<td>• Diabetes and deafness (A3243G, C12258A)</td>
<td>M</td>
</tr>
<tr>
<td>• Encephalomyopathy (G1606A, T10010C)</td>
<td>M</td>
</tr>
<tr>
<td>rRNA genes</td>
<td></td>
</tr>
<tr>
<td>• Non-syndromic sensorineural deafness (A7445G)</td>
<td>M</td>
</tr>
<tr>
<td>• Aminoglycoside induced non-syndromic deafness (A1555G)</td>
<td>M</td>
</tr>
</tbody>
</table>

**Nuclear genetic disorders**

- Disorders of mtDNA maintenance

- Disorders of mitochondrial protein import

- Disorders of assembly of the respiratory chain

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genome was favoured in some tissues, and the other mitochondrial genome was favoured in others. Detailed experiments showed that this selective effect was not a result of detectable differences in respiratory chain activity or rates of mtDNA replication, and that the selection appeared to be controlled at the level of the mtDNA molecule itself. Recent work has identified three specific nuclear genetic loci that influence this process. This has important implications for our understanding of mtDNA diseases because the equivalent genes in humans might influence the level of heteroplasmy in different tissues and organs, and therefore modulate the clinical phenotype.

Table 2  Clinical syndromes associated with mitochondrial disease

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Primary features</th>
<th>Additional features</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic progressive external ophthalmoplegia (CPEO) and lactic acidosis (fatal and non-fatal forms)</td>
<td>External ophthalmoplegia and bilateral ptosis</td>
<td>Mild proximal myopathy</td>
</tr>
<tr>
<td>Kearns-Sayre syndrome (KSS)</td>
<td>PEO onset before age 20 with pigmentary retinopathy, plus one of the following: CSF protein &gt; 1 g/l, cerebellar ataxia, heart block</td>
<td>Bilateral deafness; myopathy; dysphagia; diabetes mellitus and hypoparathyroidism; dementia</td>
</tr>
<tr>
<td>Leber hereditary optic neuropathy (LHON)</td>
<td>Subacute painless bilateral visual failure; male:female approx 4:1; median age of onset 24 years</td>
<td>Dystonia; cardiac pre-excitation syndromes</td>
</tr>
<tr>
<td>Leigh syndrome (LS)</td>
<td>Subacute relapsing encephalopathy with cerebellar and brain stem signs presenting during infancy</td>
<td>Basal ganglia lucencies</td>
</tr>
<tr>
<td>Mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes (MELAS)</td>
<td>Stroke-like episodes before age 40 years; seizures and/or dementia; ragged-red fibres and/or lactic acidosis</td>
<td>Diabetes mellitus; cardiomyopathy (hypertrophic leading to dilated); bilateral deafness; pigmentary retinopathy; cerebellar ataxia</td>
</tr>
<tr>
<td>Myoclonic epilepsy with ragged-red fibers (MERRF)</td>
<td>Myoclonus; seizures; cerebellar ataxia; myopathy</td>
<td>Dementia; optic atrophy; bilateral deafness; peripheral neuropathy; spasticity; multiple lipomata</td>
</tr>
<tr>
<td>Neurogenic weakness with ataxia and retinitis pigmentosa (NARP)</td>
<td>Late childhood or adult onset peripheral neuropathy with associated ataxia and pigmentary retinopathy</td>
<td>Basal ganglia lucencies; abnormal electroretinogram; sensorimotor neuropathy</td>
</tr>
<tr>
<td>Pearson syndrome</td>
<td>Sideroblastic anaemia of childhood; pancytopenia; exocrine pancreatic failure</td>
<td>Renal tubular defects</td>
</tr>
</tbody>
</table>

PEO, progressive external ophthalmoplegia.

Figure 4  Clinical features of mitochondrial disease. Mitochondrial disease may present with single organ involvement (sensorineural deafness, diabetes, visual failure, myopathy, or cardiomyopathy), or multisystem involvement. It may be possible to recognise a specific clinical syndrome (table 2), but often patients do not fit neatly into one particular category. The combination of neurological disease and extraneurological involvement should raise the suspicion of a mitochondrial disorder.
The last five years have seen major advances in our understanding of mitochondrial genetics and how mtDNA mutations cause disease. Clinical expression is influenced by heteroplasmy, mtDNA background, nuclear genes, and their interaction with the environment. Evolutionary studies are casting light on this complex relation. For example, in the char (a fish), different environments, and particularly the water temperature, have selected in favour of a particular mitochondrial genotype. Recent work on humans suggests that the same phenomenon may have occurred during population migrations throughout the world. Understanding these processes is of fundamental importance for the clinical management of patients—from genetic counselling to developing new treatments.

### CONFIRMING SUSPECTED MITOCHONDRIAL DISEASE

Our understanding of mitochondrial biochemistry and genetics has important implications for the investigation of suspected mitochondrial disease. In patients with a clearly defined clinical syndrome it may be possible to confirm the diagnosis with a simple molecular genetic test carried out on DNA extracted from blood. A good example of this is Leber hereditary optic neuropathy, where over 97% of cases are caused by one or more known mtDNA point mutations that are usually homoplasmic in blood. A similar approach may also be possible for nuclear genetic mitochondrial disorders (see Table 1, although most of these genetic tests are still within the realms of research and are not part of a routine diagnostic service). Investigating the remaining patients is more complex, partly because many disorders may mimic mitochondrial disease and also because there is no single test that will prove or disprove whether a patient has a mitochondrial disorder. Many different genetic defects in both mitochondrial and nuclear DNA can cause similar neurological disorders, so rather than carry out a series of random genetic tests, it is better to approach the problem systematically to identify and characterise the underlying metabolic defect.

Heteroplasmy is the main problem when investigating mtDNA disorders. Pathogenic mtDNA mutations may not be detectable in blood using conventional techniques, and, almost counterintuitively, direct sequencing of mtDNA is the least robust technique of all. This means that a negative blood test result does not exclude a particular genetic diagnosis (for example, see Chinnery et al., 1997). If mitochondrial disease is suspected and the blood DNA tests are negative, the patient should have a muscle biopsy (usually the first choice in adults) or a skin biopsy (usually the first choice in children). Urine sediment, and to a lesser degree hair follicles, are excellent sources for non-invasive mtDNA testing.

Fresh muscle can be analysed histologically and historically for evidence of mitochondrial disease. Characteristic features include ragged red fibres which can be seen with the Gomori-trichrome stain or with succinate dehydrogenase histochemistry. The ragged red appearance is caused by the subsarcolemmal accumulation of mitochondria and is thought to be a response to metabolic stress within a diseased muscle cell. There may also be a reduction in cytochrome c oxidase activity (COX, complex IV) either within some of the fibres (a mosaic defect, suggestive of a mtDNA disorder), but see Sasarman et al., 2002 or affecting all the fibres within the entire biopsy (suggesting a nuclear genetic defect).

Specialist centres carry out measurements of the individual respiratory chain complexes, which may also provide a clue to the underlying genetic defect. These can be done on fresh muscle or cultured fibroblasts grown from a skin biopsy. If a single complex is deficient, this points to a genetic defect in the relevant coding region of mtDNA or nuclear DNA, or a gene involved in the assembly of that particular complex. If there are multiple complex defects, that suggests a generalised defect of protein synthesis, and an underlying mtDNA defect involving a tRNA gene (including deletions that remove tRNA genes), or perhaps a nuclear gene defect with secondary effects on mtDNA. It is worth remembering that mitochondrial biochemical tests carried out on muscle and fibroblasts in the laboratory measure mitochondrial function under optimal conditions. It is therefore possible that there is a functional defect of mitochondrial metabolism that is not detectable in the laboratory (mutations in the ATPase 6 gene causing NARP (neuropathy, ataxia, and optic atrophy) are a good example of this pitfall). Evidence of impaired mitochondrial function may only be apparent on

Table 3 Animal models of mitochondrial disease (adapted from Larsson and Rustin)

<table>
<thead>
<tr>
<th>Type</th>
<th>Mouse model</th>
<th>Gene (reference)</th>
<th>Biochemical abnormality</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nuclear gene knockouts</td>
<td>Adenine nucleotide translocase</td>
<td>ANT1 [48]</td>
<td>Defect of coupled respiration</td>
<td>Myopathy and cardiomyopathy</td>
</tr>
<tr>
<td></td>
<td>Mitochondrial superoxide dismutase</td>
<td>SOD2 [49]</td>
<td>Mitochondrial superoxide deficiency</td>
<td>Myopathy and cardiomyopathy</td>
</tr>
<tr>
<td></td>
<td>Mitochondrial transcription factor A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Germ line</td>
<td>TFAM, germ line [25]</td>
<td></td>
<td>Respiratory chain defect</td>
<td>Embryonic lethality</td>
</tr>
<tr>
<td>Heart specific</td>
<td>TFAM, tissue specific [50]</td>
<td></td>
<td>Respiratory chain defect</td>
<td>Abnormal development with absence of heart and optic disc</td>
</tr>
<tr>
<td>Pancreatic β cell specific</td>
<td>COX assembly protein SURF-1</td>
<td>SURF1 [52]</td>
<td>Respiratory chain defect</td>
<td>Dilated cardiomyopathy and cardiac conduction block</td>
</tr>
<tr>
<td>Thymidine phosphorylase</td>
<td>TF [53]</td>
<td></td>
<td>Reduction in liver TP activity</td>
<td>Diabetes</td>
</tr>
<tr>
<td>Mitochondrial DNA</td>
<td>BALB/NZB heteroplasmic</td>
<td>NZB/BALB mtDNA [44]</td>
<td>None</td>
<td>Random drift during transmission.</td>
</tr>
<tr>
<td></td>
<td>Chloramphenicol resistance</td>
<td>CAP &amp; T2443C mtDNA [54]</td>
<td>None</td>
<td>Tissue specific selection of different genotypes,</td>
</tr>
<tr>
<td></td>
<td>ΔmtDNA</td>
<td>4.7 kb mtDNA deletion [46]</td>
<td>None</td>
<td>Myopathy, cardiomyopathy, perinatal death</td>
</tr>
<tr>
<td>Spontaneous mutants</td>
<td>Defect of nuclear–mitochondrial communication</td>
<td>Not known [55]</td>
<td>None</td>
<td>Deafness</td>
</tr>
</tbody>
</table>

Outlook

The last five years have seen major advances in our understanding of mitochondrial genetics and how mtDNA mutations cause disease. Clinical expression is influenced by heteroplasmy, mtDNA background, nuclear genes, and their interaction with the environment. Evolutionary studies are casting light on this complex relation. For example, in the char (a fish), different environments, and particularly the water temperature, have selected in favour of a particular mitochondrial genotype. Recent work on humans suggests that the same phenomenon may have occurred during population migrations throughout the world. Understanding these processes is of fundamental importance for the clinical management of patients—from genetic counselling to developing new treatments.

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clinical testing using techniques such as exercise testing with lactate measurements," magnetic resonance spectroscopy, 50 or infrared spectroscopy. 51

A structured approach to investigation allows targeted genetic analysis, which often means a Southern blot of muscle mtDNA looking for a mtDNA rearrangement, a series of allele specific assays looking for common point mutations of mtDNA or nuclear DNA, and direct sequencing of the relevant genes. A significant proportion of adults have rare or unique mtDNA defects (that is, “private” mutations). These are identified by mtDNA sequencing which should also be carried out on DNA extracted from muscle (but with the caveat noted above).

Proving a mtDNA mutation is pathogenic

mtDNA is highly polymorphic, with any two individuals differing at up to 60 base pairs (see the mtDNA sequence databases in table 4). The variation is so great that it is not unusual to find unique base changes in control individuals. This presents a particular problem when investigating patients with suspected mtDNA disorders—when is the base change a neutral polymorphism and when is it pathogenic?

Five “canonical” criteria suggest that a novel base change is pathogenic: 39:

- The mutation must not be a known polymorphism (as described on one of the established sequence data bases, see table 4).
- The base change must affect a site that has been conserved during evolution. If the site is conserved across species then it implies that it is functionally important, and a mutation at this site is likely to be deleterious. The mutation must also be in a region that is functionally important. This essentially means anywhere in the tRNA genes, certain regions of the rRNA genes, or causing an amino acid change in the protein encoding genes.
- Deleterious mutations are usually (but not exclusively) heteroplasmic. This implies that the mutation occurred recently and it has not had time to “fix” in the female line, or that there has been selection against fixation acting at the level of the organism.
- The mutation segregates with the disease clinically. For heteroplasmic mutations this means that severely affected individuals have a high percentage level of mutated mtDNA, and unaffected individuals have a lower percentage level of mutated mtDNA.
- The mutation segregates with the disease biochemically. This is usually achieved by single cell mtDNA analysis. 46

Individual muscle fibres are microdissected from thick cross sections of muscle and the percentage level of mutated mtDNA is measured in histochemically normal and abnormal muscle fibres (either because they are ragged red or COX deficient). For a pathogenic mutation, the percentage level of mutated mtDNA will be higher in the pathologically abnormal fibres.

MANAGING MITOCHONDRIAL DISEASE—THE FUTURE

Making a specific genetic diagnosis is helpful in various ways. It allows a comparison of that individual with other patients described in published reports, providing some guide to prognosis and highlighting complications that may evolve over time (table 2). It also has implications for genetic counselling (table 1). Nuclear defects may be autosomal recessive, autosomal dominant, or sex linked. mtDNA defects may be sporadic or maternally transmitted. There are no statistically based robust counselling guidelines for mtDNA disease, 99 but data collection is underway, and they should become available over the next five years. Retrospective studies suggest that measuring the percentage level of mutated mtDNA in the mother will provide some guidance. 92–94

At present the management of mitochondrial disease is largely supportive and aimed at identifying, preventing, and treating complications wherever possible. Pharmacological treatments have been used with varying degrees of success (recently reviewed by Chinnery & Turnbull). 96 Limited clinical trials have been carried out, but no consistent clinical improvements have been demonstrated. A multicentre trial is currently under way for dichloracetate to reduce lactic acidosis in MELAS patients.

Our understanding of the basic biology of mitochondrial disease provides a basis for developing new treatments. Several strategies have been employed to try and correct the underlying genetic defect. The overall aim is to reduce the proportion of mutated mtDNA to subthreshold levels. This could be achieved by adding more wild-type mtDNA, or by removing mutated mtDNA.

Adding wild-type mtDNA

Despite initial promise, attempts to deliver synthetic wild-type mtDNA into cells have not been successful. A more attractive strategy is to move wild-type mitochondrial genomes from one compartment to another—an approach called “gene shifting” 39, 50. Healthy skeletal muscle contains small precursors called satellite cells. Satellite cells proliferate and fuse with the juxtapositionary mature skeletal fibres in
response to stress and exercise. In some patients with mtDNA myopathy, the percentage level of mutated mtDNA in satellite cells is lower that the level in affected skeletal muscle. It is possible to induce satellite cell proliferation by injecting a toxin into muscle (such as bupivacaine)" or by exercising the muscle.\textsuperscript{5} Both techniques have been shown to deliver wild-type mtDNA from the satellite cell compartment into mature muscle fibres, to reduce the proportion of mutated mtDNA within affected tissues, and to correct the biochemical defect. Exercise also improves the strength and stamina of patients with mtDNA myopathy\textsuperscript{6}—but there are concerns that it may also increase the amount of mutated mtDNA in the muscle, leading to short term improvements that may be detrimental in the longer term.

**Removing mutated mtDNA**

Two strategies have been employed to remove mutated mtDNA. Both are at the experimental stage, and both require considerable development before they can be used on patients. One approach has been to develop synthetic molecules that bind to mutated mtDNA molecules and prevent them from replicating, but allowing wild-type mtDNA replication to continue unimpeded.\textsuperscript{7} While this strategy works in vitro, and it appears that the “antigenomic” molecules can be delivered into mitochondria,\textsuperscript{8} so far it has not been possible to influence the level of heteroplasmy in living cells. An alternative approach is to use drugs that select against mutated mtDNA in dividing cells, allowing wild-type mtDNA levels to increase.\textsuperscript{9} All of these approaches have the same drawback—even if they are effective, how can the treatments be delivered to the nervous system and alter the mtDNA levels in non-dividing cells? For this reason perhaps the best strategy is to remove all mutated mtDNA at an early stage in development, by nuclear transfer. By removing the nucleus from an affected zygote with a mtDNA mutation and inserting it into a healthy enucleated donor with normal mtDNA, it should be possible to form healthy offspring that do not harbour the mtDNA defect, thereby preventing the disease in that individual, and also preventing further transmission of the disease. This approach is currently at an experimental stage, but provides some hope for the future.

**ANIMAL MODELS**

Various mouse models for mitochondrial disease have been developed over the last five years. It is hoped that these models will advance our understanding of the pathobiology and will also be useful for developing new treatments (table 3). Most of the models were produced by nuclear gene manipulation, and some bear clinical or pathological resemblances to human mitochondrial diseases. Developing a good model for human mtDNA disorders is proving difficult because it is currently not possible to transfected mammalian mitochondria with exogenous DNA. To get around this problem, Inoue and colleagues fused synaptic nerve terminals (synaptosomes) from aged mice harbouring low levels of mtDNA deletions with cybrid cells.\textsuperscript{10} They screened the cybrid clones for detectable levels of mtDNA deletions and fused one with a mouse zygote that was implanted into a foster mother. The offspring contained a mixture of wild-type mtDNA and mtDNA with a 4.7 kb deletion. These mice share some clinical features with human mtDNA deletion disorders, but they were remarkable in two respects. First, the mice developed a nephropathy (a feature not typically found in KSS), and second, the female offspring also harboured deleted mtDNA (also not typical of KSS). Thus, like many other mouse models, there is not a complete correspondence between the human and the murine phenotype. To some extent this is inevitable—human mtDNA disorders are late onset diseases, and it is difficult to mimic the effects of aging in other shorter lived mammals. Attempts to generate mice transmitting mtDNA point mutations similar to those found in humans have not yet been forthcoming.

**CONCLUSIONS**

Mitochondria have an essential role in maintaining cellular homeostasis, and their many functions integrate closely with the cellular and clinical consequences will depend on many other genetic and environmental factors. Precisely how the jigsaw fits together will vary from person to person. This presents a unique challenge to neurologists wanting to identify, diagnose, and manage patients and families with mitochondrial disease. In this respect, areas for future development are highlighted in box 1.

These are not rare disorders. Recent epidemiological studies have shown that as a group primary mitochondrial disorders affect at least one in 8000 of the general population,\textsuperscript{11} and that number is likely to increase as the genotypic and phenotypic spectrum expands. It is also becoming clear that mitochondrial dysfunction occurs in many common sporadic neurological disorders, and there may be common mitochondrial mechanisms associated with certain neurological phenotypes (a good example being the various forms of hereditary spastic paraplegia). Our grasp of primary mitochondrial disorders will therefore have a much broader relevance, helping us to understand many other diseases and hopefully leading to novel generic treatments for neurological diseases.

**ACKNOWLEDGEMENTS**

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GLOSSARY

ANT: Adenine nucleotide translocator. A transporter protein that exchanges ADP for ATP across the mitochondrial inner membrane. There are various tissue specific isoforms of ANT. Mutations in the nuclear gene ANT1 cause autosomal dominant chronic progressive external ophthalmoplegia with secondary mtDNA deletion formation (see table 1). There is a mouse knockout model for the cardiac isoform of ANT (see table 3).

Apoptosis: Programmed cell death. A critical process essential for normal development and cell turnover. The release of cytochrome c from the inner mitochondrial membrane is one mechanism that can trigger apoptosis. This is discussed in detail in other articles in this series.

ATP: Adenosine triphosphate. A high energy phosphate molecule produced by the respiratory chain from ADP. ATP is required for all active cellular processes.

Cybrid cell: Cultured cell generated by fusing the cytoplasm of one cell (cytoplast) with that of another. This technique can be used to generate cell lines with different levels of heteroplasmy on a specific nuclear genetic background.

Cytoplasmic transfer: Technique used to generate heteroplasmic mice. Cytoplasm containing mitochondria from one inbred mouse strain (for example, NZB) are transferred and fused with an early embryonic cell from another cell inbred mouse strain (for example, BALB).

Genetic bottleneck (mitochondrial): A restriction in the number of mitochondrial genomes during early development of the female germ line causes a “sampling effect” which results in great variation in the level of heteroplasmy among the offspring of a heteroplasmic mother.

Haplogroup (mtDNA): mtDNA is highly polymorphic within the general population. There are certain groups of polymorphisms that reflect the maternal ancestry of a particular individual. These large groups are called haplogroups, and they contain individual haplotypes.

Haploype (mtDNA): A specific mitochondrial genotype defined by a characteristic collection of mtDNA polymorphisms.

Heteroplasmy: Mammalian cells contain many copies of mtDNA. Patients with mtDNA disease often harbour a mixture of mutated and wild-type mtDNA—a situation known as heteroplasmy. The proportion of mutated mtDNA can vary between zero and 100%.

Homoplasmy: Mammalian cells contain many copies of mtDNA. Usually all of these copies are identical—a situation known as homoplasmy.

Knockout: Type of mouse model where a specific nuclear gene is removed.

Mitotic (vegetative) segregation: When a heteroplasmic cell divides, the daughter cells may receive different amounts of mutated mtDNA by chance.

mtDNA: Mitochondrial DNA. The 16 569 base pair circular mitochondrial genome. Each cell contains thousands of copies.

Oxidative phosphorylation (OXPHOS): Electrochemical process carried out by the respiratory chain. Electrons received from reduced cofactors are passed between the different respiratory chain complexes; protons are pumped from the mitochondrial matrix into the inter-membrane space generating the mitochondrial membrane potential. The influx of protons through complex V is used to generate ATP from ADP and inorganic phosphate.

POLG: Polymerase γ—the only mitochondrial DNA polymerase. Mutations in the nuclear gene POLG1 are a common cause of autosomal dominant and recessive chronic progressive external ophthalmoplegia with secondary mtDNA deletions (see table 1).

Polymorphism: A phenotypically neutral natural variation in DNA code at a particular site. In strict terms a particular sequence change must be present in 1% of the population to be a polymorphism, but the term is often used to describe any phenotypically neutral sequence variant.

Relaxed replication: Unique nuclear DNA which is only copied during cell division, mtDNA is continuously recycled within the cell (while the overall amount is maintained at roughly constant levels).

RESPIRATORY chain: A group of four enzyme complexes (I–IV) situated on the inner mitochondrial membrane. Together with ATP synthase (complex V), the respiratory chain is the final common pathway for aerobic energy metabolism and the production of ATP.

Satellite cell: Muscle cell precursor situated adjacent to the mature muscle fibre. Satellite cells proliferation in response to muscle stress and fuse with the mature muscle fibre.

Threshold effect: Most commonly a biochemical defect of the respiratory chain when the proportion exceeds a critical threshold level. This threshold varies from tissue to tissue, and from mutation to mutation.

Wild-type: Normal DNA found in the outbred population.

REFERENCES

Mitochondria play a crucial role in the function of tissues and organs. Disruptions in mitochondrial function can lead to various diseases, including genetic disorders and age-related degenerative conditions. Research on mitochondrial DNA (mtDNA) and its interaction with nuclear DNA has revealed that mitochondrial diseases can be caused by mutations in mtDNA or the nuclear genome. These disorders can be transmitted through the maternal line, affecting both sex and survival. Studies have shown that the power of mtDNA can vary, influencing disease susceptibility and severity.

For example, mutations in the mtDNA 3243 position associated with the MELAS mutation can lead to mitochondrial disease and cardiomyopathy. Similarly, the 8344 position is also associated with mitochondrial disease and deafness. The interaction between mtDNA and nuclear DNA is crucial for the proper functioning of these organelles, and disruptions can lead to a range of clinical manifestations.

The presence of a single copy of mtDNA in somatic cells suggests that mtDNA is not randomly distributed. Instead, it appears to follow a pattern where the copy number is influenced by multiple factors, including nuclear gene expression and the environment. This non-random distribution is significant for understanding the inheritance and progression of mitochondrial diseases.

In conclusion, research on mitochondrial biology is essential for developing new treatments and therapies for mitochondrial diseases. Understanding the complex interactions between mtDNA and nuclear DNA, as well as the factors that influence the power of mtDNA, will be critical for advancing our knowledge and improving patient outcomes.