

# Batteries not included: diagnosis and management of mitochondrial disease

■ R. McFarland & D. M. Turnbull

From the Mitochondrial Research Group, School of Neurology, Neurobiology and Psychiatry, Newcastle University, Newcastle-upon-Tyne, UK

**Abstract.** McFarland R, Turnbull DM (Newcastle University, Newcastle-upon-Tyne, UK). Batteries not included: diagnosis and management of mitochondrial disease (Review). *J Intern Med* 2009; **265**: 210–228.

In 1998, Wallace *et al.* (*Science* 1988; **242**: 1427–30) published evidence that the mutation m.11778G>A was responsible for causing Leber's hereditary optic neuropathy. This was the first account of a mitochondrial DNA mutation being irrefutably linked with a human disease and was swiftly followed by a report from Holt *et al.* (*Nature* 1988; **331**: 717–9) identifying deletions in mitochondrial DNA as a cause for myopathy. During the subsequent 20 years there has

been an exponential growth in 'mitochondrial medicine', with clinical, biochemical and genetic characterizations of a wide range of mitochondrial diseases and evidence implicating mitochondria in a host of many other clinical conditions including ageing, neurodegenerative illness and cancer. In this review we shall focus on the diagnosis and management of mitochondrial diseases that lead directly or indirectly to disruption of the process of oxidative phosphorylation.

**Keywords:** mitochondrial disease, neurogenetics, respiratory chain disorders.

## Introduction

Even in the resting metabolic state humans require an abundant source of readily available energy for tissues with high metabolic demands such as brain, liver and muscle. Daily human energy requirements vary with age, sex, physiological status and levels of activity, but all of this expended energy must be recouped from ingested foodstuffs. The mitochondrion, and in particular the mitochondrial respiratory chain (MRC), plays a key role in maintaining this energy homeostasis with oxidative phosphorylation (OXPHOS) being the principal means of generating adenosine triphosphate (ATP) – the energy currency of the cell. Faults in this system of ATP generation can occur at many different stages, but the focus of this article is the diagnosis and management of diseases that affect the integrity of the MRC. Before discussing the diagnosis, investigation and pathological consequences of mitochondrial disease, we review the basic structure and function of mitochondria, as well as some basic concepts of mitochondrial genetics.

## Mitochondrial morphology

These diminutive intracellular organelles have a dynamic morphology that is only just beginning to be understood. Their origins date back to autonomous, primitive, bacteria-like organisms that developed a successful endosymbiotic relationship with eukaryotic cells. These humble beginnings belie the enormous importance mitochondria subsequently assumed in the vital energy and waste management functions of the eukaryotic cell [1]. The capacity of mitochondria to readily generate ATP by OXPHOS has led to this becoming the principal intracellular energy source, and normal eukaryotic cell function is entirely dependent on its supply. The relationship is however reciprocal, with mitochondria relying on the import of cytosolic proteins for a variety of specialized purposes [2]. Indeed this import of cytosolic proteins and the loss of the earliest bacterial pathways have been so extensive that only 14–16% of modern mitochondrial protein content (or proteome) can be traced back to the original bacterial endosymbiont [3].

The mitochondrial matrix is separated from the cytosol by two lipid membranes: the inner membrane housing the complexes of the MRC. This same membrane also provides a highly efficient barrier to ionic diffusion: a crucial factor in generating the proton gradient necessary to produce ATP. The mitochondrial matrix, also enveloped by this inner membrane, is a hostile environment containing a large number of enzymes involved in the tricarboxylic acid cycle and  $\beta$ -oxidation necessary for the metabolism of carbohydrates and fats respectively. An outer porous membrane allows passive diffusion of low molecular weight substances between the cytosol and the intermembrane space. Historically, mitochondria have been considered as discrete, noncommunicative entities, but recent evidence indicates that quite the opposite is true with frequent fission and fusion events allowing exchange of genetic material between mitochondria [4] (Fig. 1). Indeed, mutations in genes related to these interactive processes have now been associated with human disease [5].

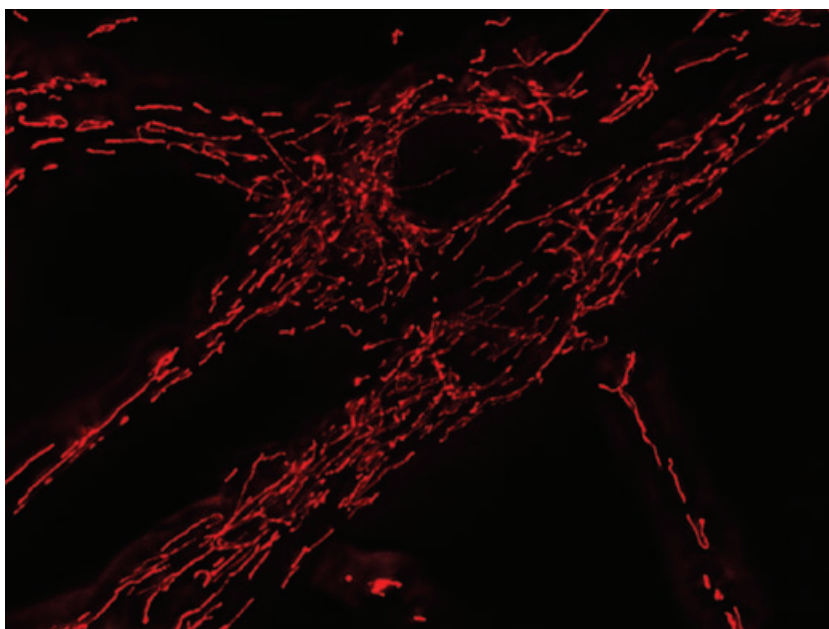
#### *Oxidative phosphorylation*

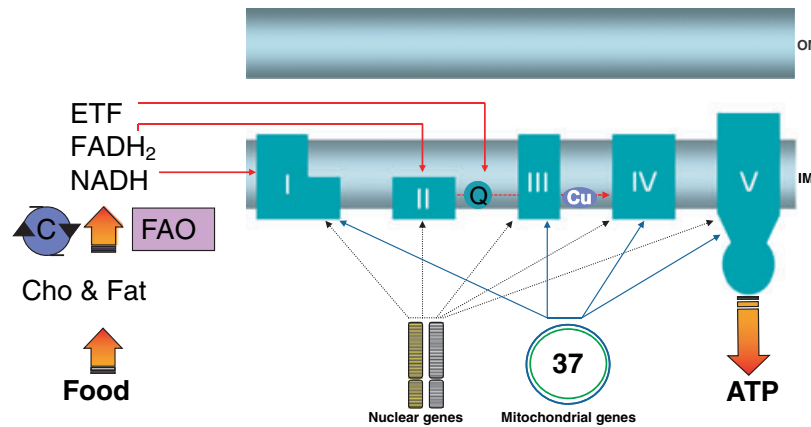
The process of mitochondrial OXPHOS is dependent on five multi-subunit polypeptide complexes (I–V)

located within the inner mitochondrial membrane, and ultimately results in the condensation of inorganic phosphate and adenosine diphosphate (ADP) to produce ATP (Fig. 2). Complex II is the only one of the MRC complexes that is entirely encoded by the nuclear genome; the others comprise subunits encoded by the nuclear and mitochondrial genomes. Electron transfer is possible through a series of oxido-reduction reactions, which take place on each of these complexes in turn and utilize a variety of adjuvants including flavins, nicotinamides, cytochromes, iron–sulphur centres and copper ions. Electrons pass along the MRC through complexes I–IV in succession. Simultaneously protons are extruded from the matrix at complexes I, III and IV generating an electrochemical gradient across the inner mitochondrial membrane. Dissipation of the generated proton gradient occurs through complex V (ATP synthase), fuelling the condensation of inorganic phosphate and ADP to form ATP.

Mitochondria occupy an exclusive evolutionary niche in the metabolism of the eukaryotic cell and have fostered an absolute dependence on ATP derived from OXPHOS. Interruption of the supply of this ATP has dire consequences for the cell, and even small reductions in the efficiency of ATP production may be sufficient to cause

**Fig. 1** Confocal image of fibroblasts stained with Mitotracker Red. This image demonstrates the reticular pattern of interconnecting mitochondria that result from the dynamic processes of fission and fusion. Long filamentous networks of fused mitochondria can be observed. Mutations in OPA1 and MFN2 genes have been shown to impair fusion and result in stunted networks. Image courtesy of Miss Jo Stewart.





**Fig. 2** The process of oxidative phosphorylation in mitochondria. A schematic representation of the process of oxidative phosphorylation. Complex I (NADH: ubiquinone oxidoreductase) accepts electrons from substrates such as glutamate, pyruvate and  $\beta$ -hydroxybutyrate, whilst succinate donates an electron at complex II (succinate: ubiquinone oxidoreductase) via  $\text{FADH}_2$ . Ubiquinone and electron transfer factor (ETF) then 'shuttle' electrons to complex III (ubiquinol-cytochrome-*c* reductase) where reduction of cytochrome *c* (Cu) enables transfer of electrons to complex IV (cytochrome *c* oxidase). In this way electrons pass along the 'chain' of complexes (I–IV) and in doing so, provide sufficient energy to fuel proton pumping from the matrix across the membrane at complexes I, III and IV. The electrochemical gradient generated by the extrusion of protons is then utilized by complex V, adenosine triphosphate (ATP) synthase, to generate ATP from the condensation of inorganic phosphate ( $\text{P}_i$ ) and adenosine diphosphate (ADP). The double circle and arrows indicate which complexes have mtDNA-encoded subunits, whilst the two chromosomes and arrows indicate that all of the complexes have nDNA-encoded components.

symptoms. The aim of this review is to discuss how such 'power failures' lead to human disease, and how these conditions can be diagnosed and managed.

## Basic concepts of mitochondrial genetics

### Structure of mitochondrial DNA

The mitochondrial genome is a 16 569 base pair closed circular loop of double stranded deoxyribonucleic acid (DNA) found in multiple copies within the mitochondrial matrix. Mitochondrial DNA (mtDNA) encodes the genetic information for the 13-polypeptide subunits essential for the process of OXPHOS. In addition, the mitochondrial genome encodes two ribosomal RNA genes and 22 tRNA genes necessary for the intramitochondrial synthesis of these 13 polypeptides. The genome was first sequenced in its entirety in 1981 [6], and this 'Cambridge Sequence' was subject to minor revision in 1999 [7]. The mitochondrial genome is remarkably concise, containing little noncoding capacity and no introns. This reductive evolution of the mitochondrial genome has come at a price, and mitochondria are no longer autonomous

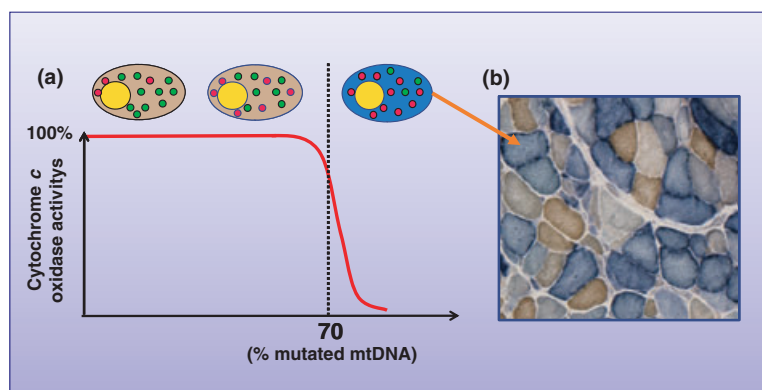
organelles, relying heavily for infrastructure and maintenance on the nuclear genome.

### Inheritance of mitochondrial DNA

For many years it was accepted that mtDNA was inherited exclusively through the maternal lineage and whilst for the purposes of genetic counselling, evolutionary and epidemiological studies this remains true, a single report of paternal inheritance has been documented [8]. In this case paternal mtDNA was identified in skeletal muscle through discrepancies in mtDNA sequence between blood and muscle tissues. Despite subsequent re-analyses of multiple cohorts of patients where blood and muscle tissues were available, no further cases of paternal inheritance have been identified [9, 10].

### Heteroplasmy, homoplasmy and threshold

Although errors occur during replication and repair of nuclear DNA, such errors are thought to be much more frequent in the hostile environment inhabited by mtDNA. Most of these mutations are inconsequential



**Fig. 3** The threshold effect illustrating the capacity of cells to maintain function [normal cytochrome *c* oxidase (COX) staining] in the presence of mutated species of mtDNA, until a tissue-specific threshold (70% in this example) is exceeded. Heteroplasmy for mutant species in excess of this threshold results in impaired oxidative phosphorylation and in this case loss of COX activity as demonstrated by blue fibres in the dual stained COX/SDH (succinate dehydrogenase) muscle biopsy section. The precise level of this threshold will vary not only from tissue to tissue, but also with different mutations and between individuals.

but occasionally a stable, replicative mutant mtDNA is produced. However, as there are multiple copies of mtDNA within each mitochondrion, this does not necessarily, nor often, result in clinical pathology. Rather, a dual population of wild type mtDNA and mutant mtDNA flourish within the mitochondrion; a situation known as heteroplasmy. This is a dynamic phenomenon and the level of heteroplasmy (proportion of mutated mtDNA) may vary considerably from tissue to tissue or even from cell to cell [11]. By contrast, homoplasmy describes the state where all copies of mtDNA within a mitochondrion are identical and usually refers to the wild type situation. However, selective pressure such as replicative advantage may favour homoplasmy of the mutant mtDNA. Clinical disease is frequently associated with heteroplasmy; a popular explanation being that the proportion of mutated mtDNA must exceed a predetermined, tissue-specific 'threshold' level, before cellular dysfunction and disease can occur [12] (Fig. 3). Leber's hereditary optic neuropathy (LHON) is a notable exception to this principle, as the mutations responsible are often homoplasmic and present in both symptomatic and asymptomatic individuals [13].

#### *Segregation and tissue variation in threshold*

Mammalian cells contain multiple copies of mtDNA, with oocytes containing greater than 100 000 copies.

Following fertilization, a heteroplasmic mtDNA point mutation present in the oocyte will segregate to either of the two daughter cells. One daughter cell may inherit significantly more mutated mtDNA than the other and as this process recurs during organogenesis, it can lead to significantly higher levels of mutated mtDNA in some tissues compared with others [11]. In addition, some mutations (3243A>G) are lost from tissues such as blood that undergo rapid mitotic division [14], whilst in other postmitotic tissues the proportion of mutated mtDNA is thought to increase with time. Thus, even on a simple random distribution model of segregation there are many factors contributing to the proportion of mutant present in any single tissue. To complicate matters further it has been suggested that the process of mtDNA segregation is not a random event and there is some evidence to support this hypothesis. Battersby *et al.* successfully demonstrated that two distinct polymorphisms specific to two different strains of mice are not randomly segregated to the various internal organs of the crossbred mouse [15]. How this relates to the situation with pathological mutations in humans is not clear, but it is tempting to speculate that some mtDNA mutations in humans are also actively segregated to particular tissues.

Mitochondrial disease commonly presents with a combination of muscle and brain involvement. Both

tissues are postmitotic and have high metabolic requirements, which may influence their involvement in the phenotype. Other organs may be involved depending on the proportion of mutated mtDNA present and their individual threshold for the mutation. However, even accounting for all of these factors the phenotype exhibited is sometimes difficult to explain given the type and severity of clinical features observed in other family members and the proportion of mutated mtDNA present in biopsied muscle.

### Epidemiology

Mitochondrial disease can present at any stage of life from the neonatal period to very old age. The universal and incessant demand for ATP results in a plethora of symptoms that commonly involve more than one tissue [16]. This myriad of symptoms has been less than helpful in collating collective clinical experience of these diseases at individual centres. In many instances clinicians are only alerted to the possibility of mitochondrial disease where there is obvious multi-system involvement of apparently unrelated organs, e.g. diabetes and deafness. This affects not only individual diagnosis, but the phenotypic diversity also impedes epidemiological studies of disease prevalence. Moreover, definitive diagnosis in the proband often requires a muscle biopsy [17]; an invasive procedure that is declined by a small minority of patients. Facilities for obtaining and interpreting muscle biopsies may not be available at some centres. This is particularly true for children, where an open biopsy under general anaesthetic is preferred. These epidemiological problems are further compounded by the ethical complexities of presymptomatic genetic testing in adults and children.

Leber's hereditary optic neuropathy is the commonest mtDNA disorder [18] and is characterized by subacute bilateral visual failure in young adults, predominantly males. Over 95% of LHON patients harbour one of three common mutations in mtDNA genes encoding structural proteins of complex I. Approximately 2% of Australians registered as blind harbour one of these three common LHON mutations [19]. In the North East of England (UK) the minimum point prevalence of visual failure due to LHON is 3.22 per 100 000 (95%

CI 2.47–3.97/100 000), with a minimum point prevalence for mtDNA LHON mutations of 11.82 per 100 000 (95% CI 10.38–13.27/100 000) [20]. This discrepancy between genotype and phenotype illustrates the importance of additional genetic, epigenetic or environmental factors in the expression of mtDNA disease. One such factor is gender: 50% of men with LHON mutations develop visual failure, whereas only 10% of women are clinically affected. This sex bias stimulated the search for an X-linked modifying gene [21].

The m.3243A>G mutation occurs in the *MTTL1* gene (mitochondrial-tRNA<sup>Leu</sup><sup>UUR</sup>) and has a prevalence in Caucasian and Japanese diabetic populations of approximately 1%. Overall, the prevalence of diabetes in Western Europe is between 3% and 6% of the general population, and the prevalence of mitochondrial diabetes due to the m.3243A>G mutation is estimated at 0.06%, or 60/100 000 of the general population [22]. Majamaa *et al.* studied 245 201 adults in Northern Finland [23] and determined the frequency of the m.3243A>G mutation amongst individuals with clinical features and a family history suggestive of mitochondrial disease. Of the 615 patients identified on clinical grounds, 480 were screened for the m.3243A>G mutation and they detected 11 independent maternal pedigrees transmitting the m.3243A>G mutation, giving an overall point prevalence of 16.3/100 000 of the adult population (95% CI 11.3–21.4/100 000). Subgroup analysis revealed a high prevalence of the m.3243A>G mutation in certain subgroups of the Finnish population. Similarly a much higher population prevalence of the m.3243A>G mutation was recorded in a predominantly Caucasian population in Australia, where Manwaring *et al.* established a prevalence of 236/100 000 [24]. Studies in North East England have estimated the prevalence of mtDNA disease to be 9.18/100 000, and identified a further 16.5/100 000 of the adult population who by virtue of their first-degree relation to an affected individual are at increased risk of developing mtDNA disease [25].

The prevalence of mitochondrial disease in the paediatric population has been more difficult to determine, and this is at least in part because of problems encountered in

genetic diagnosis of mitochondrial disease in children, where unidentified nuclear DNA (nDNA) mutations account for the majority of cases. In a paediatric population from Northern Ostrobothnia (Finland), Uusimaa recorded a prevalence for the m.3243A>G of 18.4/100 000, a figure very similar to that seen in the adult population of the same region [26]. This is perhaps not true for the general paediatric population of Finland, and discrepancies in the prevalence of mitochondrial disease due to particular ethnic population subgroups have been noted in previous studies [27]. Overall, it is likely that mitochondrial disease is at least as common in children, although more often than in adults it is caused by mutations of nDNA. Despite this preponderance of (mainly unidentified) nDNA mutations in children with mitochondrial disease, a variety of mtDNA mutations have been described; these are frequently sporadic and often associated with isolated defects of complex I [28, 29]. Early-onset is probably a reflection of disease severity and it remains possible that mtDNA mutations occur more frequently in the paediatric population than is currently recognized, but their prevalence remains low because they are often fatal in infancy.

### Diagnosis of mitochondrial disease

Mitochondrial disease can present with a wide variety of symptoms and signs in one or more organs [30]. Multi-organ involvement, a hallmark of mitochondrial disease, may not be obvious at initial presentation and tissue-specific forms of mitochondrial disease can progress very slowly to involve other systems over a protracted period of time. A number of 'classical' syndromes have been described that are often, but not always, associated with a particular genotype. This association of phenotype with genotype is, in fact, far from concrete in mitochondrial disease, and different mutations in mtDNA or nDNA can result in the same phenotype. Conversely, a single mtDNA mutation may give rise to several different phenotypes; the m.3243A>G mutation is a prime example and results in at least three different phenotypes: mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes (MELAS), maternally inherited diabetes and deafness (MIDD) and chronic progressive external ophthalmoplegia (CPEO). In addition, a substantial

group of patients, particularly children, will not fulfil clinical criteria for a particular syndrome and may have symptoms or signs that overlap one or more clinical syndromes. Common neurological manifestations of mitochondrial disease include seizures, migraine, stroke-like episodes, neuropathy and dystonia. Often though, mitochondrial disease is only considered when such features occur in conjunction with other conditions, such as deafness, diabetes or visual impairment. Nonspecific complaints such as fatigue and myalgia are common in the population and patients who present with these symptoms in isolation are often not referred for specialist neurological advice; yet these symptoms are sometimes the most incapacitating aspects of mitochondrial disease [31]. In contrast, other patients, particularly children, with cardinal signs of respiratory chain dysfunction undergo extensive, but ultimately fruitless investigation, with no biochemical or genetic cause for their mitochondrial disease being identified. Diagnostic criteria exist which allow such children to be classified as probably, possible or unlikely mitochondrial disease [32]. Unfortunately such diagnostic categories are of limited use when counselling parents of an affected child who are considering having further children. In such a situation only basic genetic counselling is possible, and there is no prospect of antenatal testing or preimplantation genetic diagnosis.

In general, childhood presentations of mitochondrial disease tend to be more severe than those with their onset in adult life and frequently involve many different organ systems. Hepatic dysfunction and haemopoietic stem cell failure are uncommon features of adult-onset mitochondrial disease, but are seen more often in children. Renal disease also appears to be a more prominent clinical feature of paediatric mitochondrial disorders, evident in both mitochondrial depletion syndrome [33] and complex III deficiencies (*BCS1L* mutations) [34, 35].

### Investigation

The range of symptoms and signs of mitochondrial disease is extraordinarily diverse and the differential diagnosis consequently wide. In addition, investiga-

tion is made much more difficult by the often poor correlation of phenotype with genotype. A strategy that combines clinical assessment and laboratory evaluation of appropriate tissue is therefore essential.

*Clinical assessment.* The patient with suspected mitochondrial disease may have obvious symptoms or signs of mitochondrial disease at presentation, such as fatigue, ptosis or proximal weakness, but it is important to specifically address issues including maternal health and obstetric history; family history of neonatal or childhood deaths; deafness; diabetes; gastrointestinal complaints; epilepsy; cardiac symptoms; visual impairment and developmental delay. Examination should be directed towards identifying characteristic features of mitochondrial disease such as optic atrophy, ophthalmoparesis, hearing impairment, cardiac enlargement and the neurological signs associated with muscle, brain and peripheral nerve involvement.

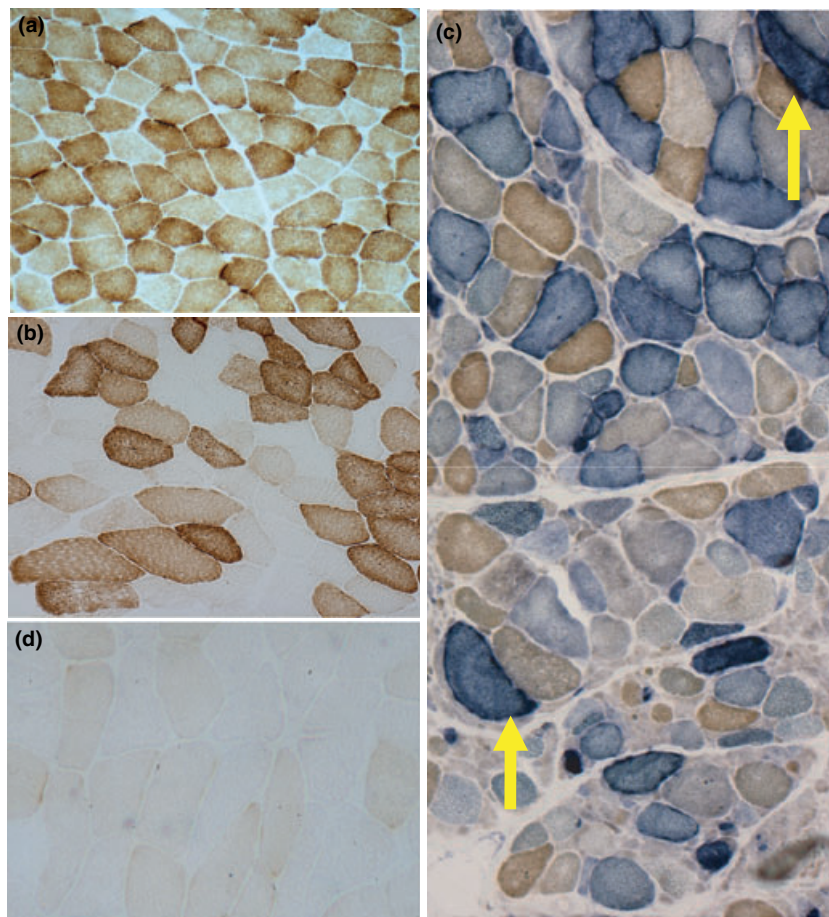
*Clinical investigations.* Urinary dipstick assessment for blood, protein and glucose is a simple yet important investigation that may provide clues to underlying renal disease (renal tubular acidosis) or diabetes mellitus. Routine blood tests can provide supporting evidence for the clinical diagnosis and should include creatine kinase; resting blood lactate; full blood count; urea and electrolytes; liver function tests; bone chemistry; thyroxine and thyroid stimulating hormone; random blood glucose and glycated haemoglobin. Respirometry, in particular forced vital capacity, performed lying and standing is a useful and accurate objective measure of respiratory muscle involvement. An electrocardiogram (ECG) is mandatory as conduction defects or cardiac hypertrophy are common features of mitochondrial disease and amenable to treatment. Chest radiography and echocardiography are appropriate investigations in those individuals with clinical or ECG evidence of cardiorespiratory disease. Lumbar puncture is an important and valuable investigation, as raised cerebrospinal fluid (CSF) lactate and mild protein elevation are consistent with mitochondrial dysfunction [36]. However, an increased CSF lactate following seizure activity or stroke, two common features of mitochondrial disease, should always be interpreted with caution.

Electromyography may be normal, even in the presence of clinical myopathy and nerve conduction studies can demonstrate either an axonal or a mixed axonal-demyelinating peripheral sensorimotor neuropathy. An electroencephalogram (EEG) is often helpful, even in those patients without obvious seizures, and occasionally reveals: (i) a pattern of generalized slow waves indicative of subacute encephalopathy that was clinically unsuspected; or (ii) subclinical seizure activity for which anticonvulsant medication is indicated. Cognitive impairment, central neurological signs or an abnormal EEG all warrant cerebral imaging. A wide range of radiological abnormalities are observed on magnetic resonance imaging (MRI) of brain, and some characteristic patterns involving specific areas of the brain such as the basal ganglia and brainstem alert the radiologist to the possibility of mitochondrial disease. Computed tomography scanning is sometimes a useful adjunct in identifying basal ganglia calcification where this is not obvious on MRI.

*Analysis of skeletal muscle biopsy.* Histological and in particular histochemical analysis of muscle biopsy remains a 'gold standard' for the detection of mitochondrial disease, especially in adult patients. Muscle biopsies are usually obtained from quadriceps femoris, orientated and then frozen in an isopentane bath (cooled to  $-160^{\circ}\text{C}$  in liquid nitrogen). Frozen muscle is then cut into 8–10  $\mu\text{m}$  sections before a variety of enzyme activities can be assayed. The Gomori trichrome stain has traditionally been used to demonstrate abnormal subsarcolemmal accumulations of mitochondria, a unique feature of mitochondrial disease described as the 'Ragged Red Fibre'. The same aggregations are also observed using the succinate dehydrogenase (SDH) assay, a potentially more useful technique as the reaction also identifies disorders involving complex II of the MRC and is completely unaffected by abnormalities of mtDNA. The technique is most powerful when combined with the cytochrome *c* oxidase (COX) reaction. As subunits of COX are encoded on both genomes this reaction is affected by mutations in both mitochondrial and nuclear DNA. Significant variation is observed in COX reactivity between type I (oxidative) and type II (glycolytic) fibres, the former reacting strongly

producing a dark brown fibre (Fig. 4a). Whilst an inherent variation of COX activity between type I and type II fibres is expected, the presence of a mosaic pattern of COX activity is indicative of a heteroplasmic mtDNA mutation (Fig. 4b). The mosaic pattern involves both fibre types and arises from the variation in mutation load between different fibres [17]. In patients where only a very small number of fibres are COX deficient, sequential COX-SDH histochemistry is particularly useful for identifying abnormal fibres

that might otherwise be overlooked; COX-deficient fibres remain dark blue and are easily distinguishable from brown COX-positive fibres (Fig. 4c). A global decrease in COX activity has previously been considered evidence of a nuclear DNA mutation affecting either COX subunits or one of the ancillary proteins involved in COX assembly such as SURF1 [37]. This conclusion is no longer accurate and global COX deficiency can result from pathogenic homoplasmic mt-tRNA mutations [38] (Fig. 4d).



**Fig. 4** Cytochrome *c* oxidase (COX) activity aids diagnosis of mitochondrial disease. (a) COX activity demonstrates a normal variation between type 1 (oxidative) and type 2 (glycolytic) muscle fibres reflecting their relative density of mitochondria and principal metabolic activity. (b) Both fibre types demonstrate a reduction in COX activity, but this is not uniform and results in mosaic pattern of dark and light brown fibres with some fibres barely visible. These extremely pale fibres are more readily observed when stained sequentially for COX then succinate dehydrogenase (SDH) activity (c). The latter produces a blue stain that is lost in the presence of adequate COX activity. Consequently COX-deficient fibres appear vivid blue on the dual COX/SDH stain. This mosaic pattern is typical of heteroplasmic mtDNA mutations affecting complex IV. Nuclear DNA or homoplasmic mtDNA mutations can result in a uniform reduction in COX activity (d) and this can be a useful diagnostic guide for further genetic studies.

Although often informative, mitochondrial enzyme histochemistry should always be interpreted in the clinical context and with regard to other factors such as patient age and the results of biochemical respiratory chain analysis. Patients with defects involving complexes I, III or V will have normal COX and SDH reactions and at present there are no histochemical methods of assessing activity of these enzymes. Consequently, patients with clearly defined mitochondrial diseases can present with normal muscle histochemistry, whilst those with age-related muscle changes can demonstrate low levels of COX-deficient muscle fibres. This COX deficiency is due to clonal expansion of acquired mtDNA deletions within individual fibres. Such focal deficiencies of COX can comprise up to 2% of all fibres in the muscle biopsy from an elderly patient and low levels of COX deficiency must therefore be interpreted with caution in this age group [39].

*Biochemistry.* Although measurement of mitochondrial enzyme activities is a key element in the diagnostic process, unfortunately protocols are often not standardized between different laboratories. The availability of fresh or frozen muscle biopsies and the choice of substrates and/or electron acceptors used in the assays complicate the development of universally accepted 'control ranges' for each of the enzyme activities. Generally though, individual complex activities (I, II, III and IV) are measured in a mitochondrial fraction isolated from frozen skeletal muscle following enzymatic digestion, mechanical disruption and centrifugation. Measurement of each complex activity in isolation avoids some of the difficulties encountered with the linked spectrophotometric assays, where measurement of electron transfer through a section of the respiratory chain can obscure a partial defect, because the linked enzyme exerts a greater influence on electron flux through that section of the respiratory chain. Complex V activity cannot be measured directly in frozen muscle using these techniques, but blue-native polyacrylamide gel electrophoresis (PAGE), an established and powerful technique that allows individual respiratory chain complexes to be isolated intact from the inner mitochondrial membrane, can be used to overcome this

difficulty [40]. Following isolation, the activity of intact complex V can be assayed in-gel. Recent developments including the use of clear native PAGE, a new technique that allows a cleaner, more efficient extraction of complexes from the inner mitochondrial membrane [41, 42], have made this approach feasible even when only small quantities of muscle biopsy material are available.

Often the most profound deficiencies are observed in children with recessive nuclear mutations and these tend to be isolated enzyme defects. In contrast, patients with mtDNA disease have wide-ranging results, from normal enzyme activities, through isolated complex deficiency to multiple enzyme defects involving complexes I, III and IV.

*Molecular genetic analysis.* The investigation of mitochondrial disease at the molecular level can be complex and should not be undertaken without first reviewing the available clinical, histochemical and biochemical evidence. Information gleaned from these various sources will determine a rational approach to molecular investigation, as does an understanding of the genotype-phenotype relationship for specific mutations of both nuclear and mtDNA origin. Mitochondrial disease in the paediatric population is frequently due to autosomal recessive mutations of nuclear genes and children with isolated biochemical deficiencies in muscle should be investigated with this in mind [43]. However, approximately 25% of paediatric presentations are due to mutations in mtDNA, but early-onset disease and atypical (nonclassical) presentation probably result in significant misdiagnosis of this group [28].

Nuclear genetic defects are best and most easily investigated in freshly extracted DNA from peripheral white blood cells. However, blood is less useful for detecting mtDNA mutations. Exceptions are the high levels of heteroplasmy observed for some mt-tRNA point mutations (m.14709T>C and m.8344A>G) and the detection of single deletions or rearrangements in early childhood. Skeletal muscle is the tissue of choice for molecular genetic analysis of mtDNA. This is because skeletal muscle is often an affected tissue,

and for some mutations the levels of heteroplasmy in skeletal muscle parallel those in other affected postmitotic tissues such as the brain [44].

Southern blot is typically used to investigate possible rearrangements of mtDNA including single deletions, duplications and multiple mtDNA deletions, and is considered to be the 'gold standard' assay in this respect. The technique involves linearization of mtDNA using a restriction endonuclease followed by agarose gel separation, denaturation, membrane transfer and hybridization to a radiolabelled D-loop probe. Southern blot is adaptable and can be utilized to detect mtDNA depletion by the addition of a probe targeted to a nuclear gene (commonly 18S rRNA). Used in conjunction with the standard mitochondrial probe this allows a ratio of nuclear to mtDNA to be estimated and compared with an age-dependent control range [45]. Southern blotting is however far from perfect: interpretation of individual bands is not easy and the technique will occasionally fail to detect low levels of multiple mtDNA deletions. For this reason nonquantitative PCR-based techniques such as long-range PCR (LRPCR) are often employed where there is a strong clinical suspicion of multiple deletions (e.g. autosomal dominant CPEO phenotype) [46]. Being PCR based, this technique preferentially amplifies smaller templates (deleted mtDNA molecules) over full-length wild type mtDNA. One caution in interpreting results from LRPCR is that deleted mtDNA molecules can be found at low levels in otherwise healthy elderly individuals.

Restriction fragment length polymorphism (RFLP) analysis is the most commonly employed method for detecting 'common' point mutations in mtDNA. In particular, m.3243A>G, m.8993T>G/C, m.13513G>A and m.14709T>C are frequently detected and quantified using radioactive (or fluorescent) 'last cycle' PCR-based RFLP. These assays are reliant on the mutation creating or removing a restriction site for a particular enzyme. Patient and control mtDNA are amplified (with label incorporated in the last cycle), digested with the restriction enzyme and separated on polyacrylamide gel. The resulting pattern of bands can be viewed and analysed to determine the proportion of mutant heteroplasmy.

Sequencing of mtDNA is an effective means of identifying novel mutations in specific well-characterized mitochondrial genes such as *MTATP6*. In some cases, such as detection of the m.8344A>G mutation in *MTTK* is more time-efficient and reliable than performing an RFLP. Similarly, the ease with which sequencing of the entire mitochondrial genome can be undertaken has revealed the true extent of mtDNA variation [47], and proved useful in excluding mtDNA involvement prior to investigating candidate nuclear genes. However, this abundance of mitochondrial genetic data is not without its problems and particular difficulties arise in deciding which variants are polymorphisms and which are pathogenic mutations. Again, molecular investigations should not be viewed in isolation and the pathogenicity of possible mtDNA mutations should be considered in the light of clinical, biochemical and molecular genetic information. DiMauro and Schon have proposed a concise canon of criteria that should be met by pathogenic mtDNA mutations [12]. However, these are not without exception and some well-recognized heteroplasmic mutations fail to satisfy a number of these criteria, particularly with regard to evolutionary conservation. Homoplasmic mutations are an extreme example and cannot fulfil any criteria that specify segregation of mutated mtDNA, irrespective of whether this is at the level of the cell, tissue, individual or family. Additional 'functional' evidence is necessary to demonstrate pathogenicity of these homoplasmic mutations [48]. The contribution that homoplasmic mutations make to the overall morbidity and mortality associated with mitochondrial disease is difficult to estimate. Their frequency is likely to be underestimated by routinely employed whole genome screening methods such as denaturing high performance liquid chromatography. This technique relies on the formation of heteroduplexes that only occur in the presence of heteroplasmy [49, 50].

A large and increasing number of nuclear genes have been described in association with mitochondrial disease. These genes encode a variety of proteins relevant to normal functioning of mitochondria including structural elements (e.g. *NDUFS* and *NDUFV* genes), complex assembly (e.g. *SURF1*, *SCO2*, *BCS1L*),

import machinery (*DDP*), synthesis and repair of mtDNA (*POLG*, *PEO1*), maintenance of intra-mitochondrial nucleotide pools (*ANT1*, *DGOUK*, *TK2*), mitochondrial protein synthesis (*EFG1*) and mitochondrial integrity (e.g. *OPA1*, *MFN2*). Of these genes, *POLG* has the largest number of mutations and is responsible for the greatest proportion of human disease. Phenotypes associated with these mutations in *POLG* range from a fatal, infantile-onset epileptic encephalopathy with liver failure (Alpers) through a variety of ataxia-neuropathy syndromes and premature ovarian failure to a relatively benign CPEO. Interestingly, the phenotype appears to be related to the site of the mutation within the *POLG* gene, and in particular whether one or other of the mutations occur within the linker, DNA polymerase or exonuclease domains.

### Managing specific complications of mitochondrial disease

#### *Mitochondrial myopathy*

Skeletal muscle weakness is a prominent clinical feature of several 'classical' mitochondrial syndromes, and rarely, it is the only clinical feature of mitochondrial disease. Typically affecting the proximal muscles of the hip and shoulder girdle, mitochondrial myopathy is usually associated with a gradual deterioration in power and a waddling gait. The hip girdle musculature is often more obviously affected than that of the shoulders and patients often have difficulty rising from the floor and climbing stairs. As the weakness progresses patients have increasing difficulty with mobility and activities of daily living such as getting in and out of a bath. In some patients the myopathy extends to involve respiratory muscles to such an extent that patients require nocturnal ventilatory support and life expectancy is severely limited. Rhabdomyolysis and muscle pain are common features of mitochondrial myopathy and whilst stiffness is described it is less often a predominant symptom [51–53]. Patients with mitochondrial myopathy should be advised to avoid physical overexertion and maintain adequate hydration so that the risk of renal complications is reduced.

#### *Cardiomyopathy and conduction defects*

Cardiac involvement, particularly as part of a syndrome such as MELAS or myoclonic epilepsy with ragged red fibres, is a common complication of mitochondrial disease. Maternal, autosomal recessive and X-linked inheritance (Barth syndrome) have been described in mitochondrial cardiomyopathy, which is usually hypertrophic, involving both ventricles and the septal wall. Significant progression of cardiomyopathy may take many years, but in some genotypes, e.g. m.3243A>G, it may progress more rapidly to end-stage failure despite treatment with  $\beta$ -blockers and/or angiotensin-converting enzyme inhibitors. Mitochondrial disease should certainly be considered in the differential diagnosis of patients who present with biventricular hypertrophy and appropriate investigations undertaken to exclude this diagnosis. Conduction defects also occur with alarming regularity in patients with large-scale single deletions of mtDNA, m.3243A>G and LHON. This is often a Wolff-Parkinson-White preexcitation pattern particularly in patients with m.3243A>G [54], but various forms of conduction block are also described and may require permanent cardiac pacing [55–57].

#### *Eye disease – Leber's hereditary optic neuropathy*

Leber's hereditary optic neuropathy is a historically important condition in that it was the first mitochondrial disease ascribed to an mtDNA point mutation. It is an acute or subacute, bilateral (though may be asymmetric initially), painless, central visual loss and the commonest cause of blindness in young men [58]. Theodor Leber first reported the clinical condition in 1871, when he described it as a 'familial neuro-ophthalmic disease'. It was over 117 years before Wallace *et al.* [13] could demonstrate that the majority of LHON families harbour the same mtDNA mutation (m.11778G>A). Numerous mtDNA mutations have subsequently been described in association with LHON, but three mutations (m.11778G>A, m.3460G>A and m.14484T>C) are present in at least 95% of families [20]. These mutations in complex I (NADH: ubiquinone-oxidoreductase) encoding genes *MTND4*, *MTND1* and *MTND6*, respectively, are

considered to be primary LHON mutations on the basis of their frequency, penetrance and clinical severity. The excess of affected males suffering from this condition has been attributed to an X-linked visual-loss susceptibility locus although the precise genetic details of this remain unclear [21].

Clinical examination reveals peripapillary telangiectasia, microangiopathy, disc pseudo-oedema and tortuous retinal vessels [59]. Onset of disease is commonest after 20 and before 40 years, with initial unilateral involvement being typical. The other eye is commonly involved within a short period (typically months), but the subsequent decline in visual acuity may be rather slowly progressive over a period of several years. Both the disease penetrance and the clinical course appear to be determined by the mutation responsible, with measurements of final visual acuity ranging from 20/60 to difficulty in perceiving light. Similarly, the extent of visual recovery also varies in relation to the mutation, with only 4% of m.11778G>A patients showing recovery [60]; whilst there are reports of up to 71% of m.14484T>C patients recovering [61]. Extra-ocular clinical features such as cardiac conduction defects (Wolff-Parkinson-White and Lown-Ganong-Levine) are evident occasionally and minor neurological problems are also not uncommon. Specific mutations (m.14459G>A) can be associated with a severe, predominantly neurological, phenotype involving early-onset dystonia accompanied by bilateral basal ganglial degeneration [62].

### *Sensorineural deafness*

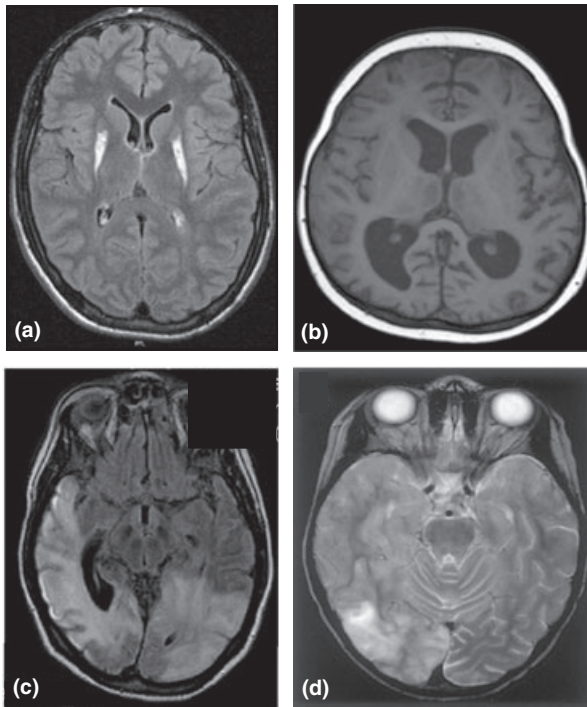
Bilateral sensorineural deafness is a common disabling feature of mitochondrial disease with variable age of onset from birth. The deafness frequently occurs in conjunction with diabetes, and features of neurological disease may also be present, particularly in families harbouring the m.3243A>G mutation [63] or other *MTT* gene mutations {m.14709T>C (*MTTE*) [64], m.7511T>C(*MTTS1*) [65], m.7445A>G(*MTTS1/COI*) [66] and m.7472Cins (*MTTS1*) [67]}. The genetic aetiology is heterogeneous and patients with sporadic large-scale single deletions, both Kearns-Sayre and CPEO phenotypes, also experience sensori-

neural hearing loss [68, 69], as do patients with nuclear gene mutations in *OPA1* and *DDP1*, where the phenotypes also include optic atrophy and dystonia (Mohr-Tranebjaerg syndrome) respectively [5, 70, 71]. One particular genotype associated with deafness, m.1555A>G in *MTRNR1*, demonstrates the complex interaction of nuclear and mitochondrial genomes, and the environment. Deafness is the only symptom of the m.1555A>G mutation, but its expression is extremely variable [72] and appears to be under direct nuclear genetic control with at least two (*MTO1* and *TFB1M*), and possibly a third, putative nuclear genetic modifiers [73, 74]. In addition, the deafness phenotype is very clearly related to environmental exposure to aminoglycoside antibiotics, which are thought to bind more readily to the ribosome in the presence of the m.1555A>G mutation [75, 76]. There is some evidence to suggest that the m.1555A>G mutation decreases the accuracy of protein translation and it is postulated that binding of aminoglycoside to the ribosome, or the action of nuclear modifiers, then further impairs translation efficiency beyond a threshold for disease expression [77]. However, the remarkable organ specificity of this disease remains unexplained.

### **Classical syndromes of mitochondrial disease**

#### *Mitochondrial disease with onset in infancy or early childhood*

*Leigh syndrome.* Leigh syndrome is a progressive neurodegenerative condition of infancy and childhood, although rare adult-onset forms have been described [78]. Leigh first described the characteristic symmetric necrotic lesions distributed along the brainstem, diencephalon and basal ganglia on postmortem tissue [79], but these can now be identified *in vivo* using MRI techniques (Fig. 5a). There is considerable variation in the onset and progress of Leigh syndrome, but signs of brainstem or basal ganglia dysfunction such as respiratory abnormalities, nystagmus, ataxia, dystonia, hypotonia and optic atrophy are common to many patients. Developmental delay and regression are prominent but nonspecific clinical features of this disorder: their diagnostic usefulness is improved when they occur in conjunction with raised CSF lactate.



**Fig. 5** Brain MRI in three different mitochondrial diseases. (a) An MRI FLAIR image demonstrating bilateral symmetrical putaminal necrosis in a patient with marked dystonia caused by the m.11778G>A point mutation in mtDNA. Cerebral involvement is a common feature of mitochondrial disease and the parieto-occipital lobes are most frequently involved. In Alpers syndrome (b) this takes the form of an occipital atrophy, particularly evident on the right in this T1-weighted image. In MELAS (c, d), areas of apparent infarction extend across vascular territories and may result in significant swelling (c). The point mutation m.12147G>A is responsible for the bilateral infarcts observed on the FLAIR image (c), whereas the more common m.3243A>G mutation has caused the right parieto-occipital infarct observed on the T2-weighted image (d).

Stepwise developmental deterioration with some recovery of skills between episodes of regression is usual, but some patients experience an aggressive unrelenting neurocognitive decline. A severe failure of oxidative metabolism due to a variety of biochemical and molecular defects, including nuclear and mtDNA mutations have been described in Leigh syndrome [80]. MtDNA mutations in both protein encoding (e.g. m.8993T>G; m.9176T>C/G and m.13513G>A) and mitochondrial tRNA genes (e.g. m.1624C>T and m.5537insT) are responsible for maternally inherited Leigh syndrome (MILS), but

other forms of inheritance (X-linked recessive; autosomal recessive) are possible depending on the genetic defect. A particular variant of Leigh syndrome [variously known as Leigh syndrome French-Canadian (OMIM 220111) type; or Saguenay-Lac-Saint-Jean (SLSJ) cytochrome oxidase deficiency] has an extremely high incidence (1 in 2178 live births) in north-eastern Quebec, Canada [81]. An integrative genomic approach has shown that the condition is caused by mutations in the leucine-rich pentatricopeptide repeat cassette gene [82].

*Depletion syndromes.* These are severe disorders often presenting in early infancy or childhood with a variety of features including profound weakness, encephalopathy, seizures and liver failure. In one form of 'hepatocerebral' depletion known as Alpers-Huttenlocher's disease or progressive neuronal degeneration of childhood, explosive onset of seizures, developmental delay, cortical blindness and spasticity are followed by catastrophic liver failure and parieto-occipital cerebral atrophy [83] (Fig. 5b). In the 'myopathic' form of depletion profound weakness impairs mobility and eventually involving the diaphragm causing respiratory failure. A number of genes have been associated with specific variations of the depletion syndromes: myopathic (*TK2*, *RRM2B*) [33, 84], hepatocerebral (*DGOUK*, *POLG1*, *MPV17*) [85–87], encephalomyopathy with methylmalonic acidemia (*SUCLA2*) [88] and fatal infantile lactic acidosis (*SUCLG1*) [89]. Although this currently has little bearing on treatment options, it does provide useful genetic information for prenatal diagnosis in future pregnancies.

*Pearson syndrome.* Pearson syndrome is an extremely rare disorder that results from large-scale rearrangements of mtDNA. Onset is usually in the first year of life and severe congenital pancytopenia and profound lactic acidosis may bring the infant to medical attention at birth. Refractory (transfusion-dependent) macrocytic sideroblastic anaemia together with exocrine pancreatic dysfunction are the major clinical features of this disorder and frequently result in death during infancy [90]. Survival through childhood leads to an improvement in anaemia consistent with an

active selection process in the rapidly dividing haematopoietic tissue. Unfortunately the same is not true of postmitotic tissue and patients eventually develop features of Kearns-Sayre syndrome (KSS) with short stature, ophthalmoparesis and multi-organ failure. For Pearson syndrome, KSS and CPEO the clinical severity appears to correlate with the tissue localization of deleted mtDNA. In Pearson syndrome (and to a lesser extent KSS), deleted mtDNA can be demonstrated in a wide variety of tissues, whereas in CPEO the defective mtDNA is confined to muscle.

*Kearns-Sayre syndrome.* The onset of ophthalmoparesis and pigmentary retinopathy before the age of 20 years is characteristic of KSS. This sporadic condition is usually the result of either a large-scale single deletion or complex rearrangements of mtDNA [91, 92]. Other clinical features include cerebellar ataxia, proximal myopathy, complete heart block, cardiomyopathy, endocrinopathies, short stature, deafness and an elevated CSF protein. As might be predicted from the early onset of this multisystem disorder, life expectancy is often considerably reduced, but with supportive care and early treatment of cardiac complications, long-term survival is still possible.

#### *Mitochondrial disease with onset in late childhood or adult life*

*Mitochondrial encephalopathy lactic acidosis and stroke-like episodes.* The hallmark of this clinical syndrome is the stroke-like episode, an event that often has a stuttering onset with a migraine-like prodrome lasting several hours. This may progress from a simple aura to impairment of consciousness with focal seizures and hemiparesis or monoparesis. Vision is also frequently affected with visual field loss consistent with cortical involvement: typically homonymous hemianopia. Stroke-like episodes are usually paroxysmal events with no clear precipitant, but the role of recurrent focal seizures may be important and it remains unclear whether (subclinical) seizure activity invokes, or results from, a stroke-like episode. Certainly controlling seizure activity is a primary focus of the acute management strategy for stroke-like episodes, together with fluid

resuscitation and correction of acid-base balance with bicarbonate. The latter is often unnecessary with prompt administration of adequate fluids. Investigations include determination of blood pH, bicarbonate, glucose, lactate and electrolytes as well as EEG and MRI of brain. Stroke-like episodes visible on MRI are usually found in the parieto-occipital region and generally do not conform to recognized vascular territories (Fig. 5c,d), making them distinguishable from strokes of vascular origin to which some of these diabetic, hypertensive MELAS patients are also prone. There may be significant brain swelling associated with this infarcts, sufficient to cause significant mass effect (Fig). Other features of MELAS include intermittent encephalopathic episodes associated with elevated plasma and CSF lactate, but without evidence of stroke-like episodes or seizure activity. Recurrent stroke-like episodes and encephalopathy eventually lead to significant cognitive impairment and in some individuals to a premature dementia with global brain atrophy.

Muscle biopsy most commonly reveals a complex I deficiency, but histochemistry may reveal COX-negative fibres and COX-positive ragged red fibres. The m.3243A>G mutation in the *MTTL1* gene was the first and most frequently described mtDNA mutation associated with this clinical phenotype [93–95]. However, mutations in other *MTT* genes [96, 97], as well as *MTND1* [29] and *MTND5* [98] genes have been described in association with this presentation and the m.3243A>G mutation also causes other distinct clinical phenotypes such as diabetes and deafness (see below) [63]. Extraordinarily, such variations in phenotype can occur between individuals in the same family harbouring identical mutations [99, 100].

*Maternally inherited diabetes and deafness.* The association between MIDD and mtDNA mutations is strong and well recognized [101, 102]. Several mutations have been described in association with this phenotype, including the m.3243A>G *MTTL1* [103] and m.14709T>C (*MTTE*) point mutations [104]. The association of mtDNA mutation with MIDD is so robust that patients presenting to diabetes clinics with this combination of features should be screened first

for the m.3243A>G mutation in urinary sediment and then referred to a specialist neuromuscular clinic for further advice should this prove negative [105]. Interestingly, although some patients with diabetes due to the m.3243A>G mutation manage for a variable period of time without insulin, all show a gradual progression towards insulin requirement irrespective of the level of heteroplasmy identified in urine or muscle at diagnosis [106].

*Neuropathy, ataxia and retinitis pigmentosa (NARP).* The clinical combination of developmental delay, retinitis pigmentosa, dementia, seizures, ataxia, proximal neurogenic muscle weakness and sensory neuropathy was first described in four members of a single family who harboured a heteroplasmic m.8993T>G transversion [107]. Since then, the phenotype associated with this mutation has broadened to include cardiomyopathy and MILS [108]. A T>C mutation at m.8993 has also subsequently been described with a generally milder clinical phenotype, but higher frequency of ataxia [109]. The MILS phenotype and impaired ATP synthesis is also observed with other mutations in the ATP6 gene including m.9176T>C [110] and m.9176T>G [111]. It is important to note from a diagnostic perspective that mutations in mitochondrial ATPase subunits do not affect cytochrome oxidase activity and therefore routine COX/SDH histochemistry will appear normal in patients with NARP.

*Mitochondrial neuro-gastrointestinal encephalopathy.* This multisystem disorder is characterized by onset of progressive external ophthalmoparesis, ptosis, gastrointestinal dysmotility (pseudo-obstruction), diffuse leucoencephalopathy, peripheral neuropathy and myopathy [112]. This is often clinically evident before the age of 20 years but the disorder is clinically heterogeneous and patients may present much later. The inheritance is autosomal recessive and causative mutations have been identified in *ECGFI*, the gene encoding thymidine phosphorylase [113, 114] and *POLG* [115]. Elevated levels of deoxyuridine in urine (and blood) are helpful in confirming clinical diagnosis prior to genetic studies. This is one of the few areas of mitochondrial disease where progress has been

made towards cure. Peritoneal dialysis had no effect on blood nucleoside levels, but did improve gastrointestinal symptoms [116] and allogeneic stem cell transplantation has been shown to successfully correct the biochemical alterations associated with defective thymidine phosphorylase [117].

*Chronic progressive external ophthalmoplegia.* This condition is one of the commonest presentations of mitochondrial disease in adults and is characterized by a slowly progressive paresis of eye musculature, bilateral ptosis, mild proximal weakness, fatigue and as the disease progresses, cardiac conduction defects. Many patients first notice onset of an initially asymmetric ptosis often in their late thirties. A sporadic, single deletion of mtDNA (4977 bp) is a common cause of CPEO, but other single deletions of variable length and some *MTT* mutations result in an identical phenotype. Recessive and dominant families with CPEO are well recognized and are associated with multiple species of deleted mtDNA [118, 119]. In these patients the ophthalmoparesis and ptosis demonstrate a slowly progressive course with a wide range in the age of onset. Other features can include fatigue, optic atrophy, cataracts, ataxia, peripheral neuropathy, deafness, cardiomyopathy and depression. A number of causative nuclear gene mutations occurring in *ANTI*, *PEO1* and *POLG* and *POLG2* have subsequently been described in these families [120, 121].

## Conclusions

The last two decades have seen an intense interest in mitochondrial disease and genetics that has been fuelled by ingenious technological advances. Nevertheless, despite 20 years of clinical, biochemical and genetic research, the mitochondrion and mitochondrial disease remain an enormous challenge. The interaction between nuclear and mitochondrial genomes is an area of flourishing research and holds the prospect of defining the pathophysiological mechanisms underlying many forms of mitochondrial disease. The development of a mouse model will permit a clearer understanding of the role that mitochondria play in the ageing process and increasingly

sophisticated imaging techniques such as magnetic resonance spectroscopy should improve diagnosis and monitoring of patients. Mitochondrial medicine is clearly coming of age and with progress being made on a number of fronts this is an exciting, stimulating time for mitochondrial research and one that will hopefully bring benefit to patients and their families.

### Conflict of interest statement

The authors declare that no conflicts of interest were incurred in the design, preparation or publication of this manuscript.

### References

- Margulis L. Genetic and evolutionary consequences of symbiosis. *Exp Parasitol* 1976; **39**: 277–349.
- Lister R, Hulett JM, Lithgow T, Whelan J. Protein import into mitochondria: origins and functions today. *Mol Membr Biol* 2005; **22**: 87–100 (Review).
- Gabalton T, Huynen MA. Shaping the mitochondrial proteome. *Biochim Biophys Acta* 2004; **1659**: 212–20.
- Margineantu DH, Gregory Cox W, Sundell L, Sherwood SW, Beechem JM, Capaldi RA. Cell cycle dependent morphology changes and associated mitochondrial DNA redistribution in mitochondria of human cell lines. *Mitochondrion* 2002; **1**: 425–35.
- Hudson G, Amati-Bonneau P, Blakely EL *et al.* Mutation of OPA1 causes dominant optic atrophy with external ophthalmoplegia, ataxia, deafness and multiple mitochondrial DNA deletions: a novel disorder of mtDNA maintenance. *Brain* 2008; **131**: 329–37.
- Anderson S, Bankier AT, Barrell BG *et al.* Sequence and organization of the human mitochondrial genome. *Nature* 1981; **290**: 457–65.
- Andrews RM, Kubacka I, Chinnery PF, Turnbull DM, Lightowlers RN, Howell N. Reanalysis and revision of the Cambridge Reference Sequence. *Nat Genet* 1999; **23**: 147.
- Schwartz M, Vissing J. Paternal inheritance of mitochondrial DNA. *N Engl J Med* 2002; **347**: 576–80.
- Taylor RW, Giordano C, Davidson MM *et al.* A homoplasmic mitochondrial transfer ribonucleic acid mutation as a cause of maternally inherited hypertrophic cardiomyopathy. *J Am Coll Cardiol* 2003; **41**: 1786–96.
- Filosto M, Mancuso M, Vives-Bauza C *et al.* Lack of paternal inheritance of muscle mitochondrial DNA in sporadic mitochondrial myopathies. *Ann Neurol* 2003; **54**: 524–6.
- Lightowlers RN, Chinnery PF, Turnbull DM, Howell N. Mammalian mitochondrial genetics: heredity, heteroplasmy and disease. *Trends Genet* 1997; **13**: 450–5.
- DiMauro S, Schon EA. Mitochondrial DNA mutations in human disease. *Am J Med Genet* 2001; **106**: 18–26.
- Wallace DC, Singh G, Lott MT *et al.* Mitochondrial DNA mutation associated with Leber's hereditary optic neuropathy. *Science* 1988; **242**: 1427–30.
- Rahman S, Poulton J, Marchington D, Suomalainen A. Decrease of 3243 A→G mtDNA mutation from blood in MELAS syndrome: a longitudinal study. *Am J Hum Genet* 2001; **68**: 238–40.
- Battersby BJ, Loredó-Osti JC, Shoubridge EA. Nuclear genetic control of mitochondrial DNA segregation. *Nat Genet* 2003; **33**: 183–6.
- McFarland R, Taylor RW, Turnbull DM. The neurology of mitochondrial DNA disease. *Lancet Neurol* 2002; **1**: 343–51.
- Taylor RW, Schaefer AM, Barron MJ, McFarland R, Turnbull DM. The diagnosis of mitochondrial muscle disease. *Neuromuscul Disord* 2004; **14**: 237–45.
- Chinnery PF, Johnson MA, Wardell TM *et al.* The epidemiology of pathogenic mitochondrial DNA mutations. *Ann Neurol* 2000; **48**: 188–93.
- Mackey DA, Buttery RG. Leber hereditary optic neuropathy in Australia. *Aust N Z J Ophthalmol* 1992; **20**: 177–84.
- Man PY, Griffiths PG, Brown DT, Howell N, Turnbull DM, Chinnery PF. The epidemiology of Leber hereditary optic neuropathy in the North East of England. *Am J Hum Genet* 2003; **72**: 333–9.
- Hudson G, Keers S, Yu Wai Man P *et al.* Identification of an X-chromosomal locus and haplotype modulating the phenotype of a mitochondrial DNA disorder. *Am J Hum Genet* 2005; **77**: 1086–91.
- Gerbitz K-D, van den Ouweland JMW, Maassen JA, Jaksch M. Mitochondrial diabetes: a review. *Biochim Biophys Acta* 1995; **1271**: 253–60.
- Majamaa K, Moilanen JS, Uimonen S *et al.* Epidemiology of A3243G, the mutation for mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes: prevalence of the mutation in an adult population. *Am J Hum Genet* 1998; **63**: 447–54.
- Manwaring N, Jones MM, Wang JJ *et al.* Population prevalence of the MELAS A3243G mutation. *Mitochondrion* 2007; **7**: 230–3.
- Schaefer AM, McFarland R, Blakely EL *et al.* Prevalence of mitochondrial DNA disease in adults. *Ann Neurol* 2008; **63**: 35–9.
- Uusimaa J, Remes AM, Rantala H *et al.* Childhood encephalopathies and myopathies: a prospective study in a defined population to assess the frequency of mitochondrial diseases. *Pediatrics* 2000; **105**: 598–603.
- Skladal D, Halliday J, Thorburn DR. Minimum birth prevalence of mitochondrial respiratory chain disorders in children. *Brain* 2003; **126**: 1905–12.
- McFarland R, Kirby DM, Fowler KJ *et al.* De novo mutations in the mitochondrial ND3 gene as a cause of infantile mitochondrial encephalopathy and complex I deficiency. *Ann Neurol* 2004; **55**: 58–64.
- Kirby DM, McFarland R, Ohtake A *et al.* Mutations of the mitochondrial ND1 gene as a cause of MELAS. *J Med Genet* 2004; **41**: 784–9.

- 30 Taylor RW, Turnbull DM. Mitochondrial DNA mutations in human disease. *Nat Rev Genet* 2005; **6**: 389–402.
- 31 Griggs RC, Karpati G. Muscle pain, fatigue, and mitochondrialopathies. *N Engl J Med* 1999; **341**: 1077–8.
- 32 Bernier FP, Boneh A, Dennett X, Chow CW, Cleary MA, Thorburn DR. Diagnostic criteria for respiratory chain disorders in adults and children. *Neurology* 2002; **59**: 1406–11.
- 33 Bourdon A, Minai L, Serre V *et al*. Mutation of RRM2B, encoding p53-controlled ribonucleotide reductase (p53R2), causes severe mitochondrial DNA depletion. *Nat Genet* 2007; **39**: 776–80.
- 34 de Lonlay P, Valnot I, Barrientos A *et al*. A mutant mitochondrial respiratory chain assembly protein causes complex III deficiency in patients with tubulopathy, encephalopathy and liver failure. *Nat Genet* 2001; **29**: 57–60.
- 35 De Meirleir L, Seneca S, Damis E *et al*. Clinical and diagnostic characteristics of complex III deficiency due to mutations in the BCS1L gene. *Am J Med Genet A* 2003; **121**: 126–31.
- 36 Jackson MJ, Schaefer JA, Johnson MA, Morris AAM, Turnbull DM, Bindoff LA. Presentation and clinical investigation of mitochondrial respiratory chain disease. *Brain* 1995; **118**: 339–57.
- 37 Zhu Z, Yao J, Johns T *et al*. SURF1, encoding a factor involved in the biogenesis of cytochrome *c* oxidase, is mutated in Leigh syndrome. *Nat Genet* 1998; **20**: 337–43.
- 38 McFarland R, Clark KM, Morris AA *et al*. Multiple neonatal deaths due to a homoplasmic mitochondrial DNA mutation. *Nat Genet* 2002; **30**: 145–6.
- 39 Brierley EJ, Johnson MA, Lightowers RN, James OF, Turnbull DM. Role of mitochondrial DNA mutations in human aging: implications for the central nervous system and muscle. *Ann Neurol* 1998; **43**: 217–23.
- 40 Schagger H, von Jagow G. Blue native electrophoresis for isolation of membrane protein complexes in enzymatically active form. *Anal Biochem* 1991; **199**: 223–31.
- 41 Wittig I, Karas M, Schagger H. High resolution clear native electrophoresis for in-gel functional assays and fluorescence studies of membrane protein complexes. *Mol Cell Proteomics* 2007; **6**: 1215–25.
- 42 Wittig I, Carozzo R, Santorelli FM, Schagger H. Functional assays in high-resolution clear native gels to quantify mitochondrial complexes in human biopsies and cell lines. *Electrophoresis* 2007; **28**: 3811–20.
- 43 Loeffen JL, Smeitink JA, Trijbels JM *et al*. Isolated complex I deficiency in children: clinical, biochemical and genetic aspects. *Hum Mutat* 2000; **15**: 123–34.
- 44 Oldfors A, Holme E, Tulinius M, Larsson NG. Tissue distribution and disease manifestations of the tRNA(Lys) A→G(8344) mitochondrial DNA mutation in a case of myoclonus epilepsy and ragged red fibres. *Acta Neuropathol* 1995; **90**: 328–33.
- 45 Morten KJ, Ashley N, Wijburg F *et al*. Liver mtDNA content increases during development: a comparison of methods and the importance of age- and tissue-specific controls for the diagnosis of mtDNA depletion. *Mitochondrion* 2007; **7**: 386–95.
- 46 He L, Chinnery PF, Durham SE *et al*. Detection and quantification of mitochondrial DNA deletions in individual cells by real-time PCR. *Nucleic Acids Res* 2002; **30**: e68.
- 47 MITOMAP. 2008. A Human Mitochondrial Genome Database. <http://www.mitomap.org>, accessed on 27 October 2008.
- 48 McFarland R, Elson JL, Taylor RW, Howell N, Turnbull DM. Assigning pathogenicity to mitochondrial tRNA mutations: when “definitely maybe” is not good enough. *Trends Genet* 2004; **20**: 591–6.
- 49 van Den Bosch BJ, de Coo RF, Scholte HR *et al*. Mutation analysis of the entire mitochondrial genome using denaturing high performance liquid chromatography. *Nucleic Acids Res* 2000; **28**: E89.
- 50 Biggin A, Henke R, Bennetts B, Thorburn DR, Christodoulou J. Mutation screening of the mitochondrial genome using denaturing high-performance liquid chromatography. *Mol Genet Metab* 2005; **84**: 61–74.
- 51 McFarland R, Taylor RW, Chinnery PF, Howell N, Turnbull DM. A novel sporadic mutation in cytochrome *c* oxidase subunit II as a cause of rhabdomyolysis. *Neuromuscul Disord* 2004; **14**: 162–6.
- 52 Saunier P, Chretien D, Wood C *et al*. Cytochrome *c* oxidase deficiency presenting as recurrent neonatal myoglobinuria. *Neuromuscul Disord* 1995; **5**: 285–9.
- 53 Deschauer M, Wieser T, Neudecker S, Lindner A, Zierz S. Mitochondrial 3243 A→G mutation (MELAS mutation) associated with painful muscle stiffness. *Neuromuscul Disord* 1999; **9**: 305–7.
- 54 Sproule DM, Kaufmann P, Engelstad K, Starc TJ, Hordof AJ, De Vivo DC. Wolff-Parkinson-White syndrome in patients with MELAS. *Arch Neurol* 2007; **64**: 1625–7.
- 55 Karanikis P, Korantzopoulos P, Kountouris E *et al*. Kearns-Sayre syndrome associated with trifascicular block and QT prolongation. *Int J Cardiol* 2005; **101**: 147–50.
- 56 Subbiah RN, Kuchar D, Baron D. Torsades de pointes in a patient with Kearns-Sayre syndrome: a fortunate finding. *Pacing Clin Electrophysiol* 2007; **30**: 137–9.
- 57 Welzing L, von Kleist-Retzow JC, Kribs A, Eifinger F, Huenseler C, Sreeram N. Rapid development of life-threatening complete atrioventricular block in Kearns-Sayre syndrome. *Eur J Pediatr* 2008; Sep 24 [Epub ahead of print].
- 58 Kleiner L, Sherman J. Leber’s hereditary optic neuropathy: historical and contemporary considerations. *Optom Clin* 1996; **5**: 77–112.
- 59 Huoponen K. Leber hereditary optic neuropathy: clinical and molecular genetic findings. *Neurogenetics* 2001; **3**: 119–25.
- 60 Stone EM, Newman NJ, Miller NR, Johns DR, Lott MT, Wallace DC. Visual recovery in patients with Leber’s hereditary optic neuropathy and the 11778 mutation. *J Clin Neuroophthalmol* 1992; **12**: 10–4.
- 61 Harding AE, Riordan-Eva P, Govan GG. Mitochondrial DNA diseases: genotype and phenotype in Leber’s hereditary optic neuropathy. *Muscle Nerve* 1995; **3**: S82–4.
- 62 Jun AS, Brown MD, Wallace DC. A mitochondrial DNA mutation at nucleotide pair 14459 of the NADH dehydrogenase subunit 6 gene associated with maternally inherited Leber

- hereditary optic neuropathy and dystonia. *Proc Natl Acad Sci USA* 1994; **91**: 6206–10.
- 63 Hammans SR, Sweeney MG, Hanna MG, Brockington M, Morgan-Hughes JA, Harding AE. The mitochondrial DNA transfer RNA<sup>Leu</sup>(UUR) A→G(3243) mutation. A clinical and genetic study. *Brain* 1995; **118**: 721–34.
- 64 Rigoli L, Prisco F, Caruso RA *et al.* Association of the T14709C mutation of mitochondrial DNA with maternally inherited diabetes mellitus and/or deafness in an Italian family. *Diabet Med* 2001; **18**: 334–6.
- 65 Sue CM, Tanji K, Hadjigeorgiou G *et al.* Maternally inherited hearing loss in a large kindred with a novel T7511C mutation in the mitochondrial DNA tRNA(Ser(UCN)) gene. *Neurology* 1999; **52**: 1905–8.
- 66 Reid FM, Vernham GA, Jacobs HT. A novel mitochondrial point mutation in a maternal pedigree with sensorineural deafness. *Hum Mutat* 1994; **3**: 243–7.
- 67 Tiranti V, Chariot P, Carella F *et al.* Maternally inherited hearing loss, ataxia and myoclonus associated with a novel point mutation in mitochondrial tRNA<sup>Ser</sup>(UCN) gene. *Hum Mol Genet* 1995; **4**: 1421–7.
- 68 Ballinger SW, Shoffner JM, Hedaya EV *et al.* Maternally transmitted diabetes and deafness associated with a 10.4 kb mitochondrial DNA deletion. *Nat Genet* 1992; **1**: 11–5.
- 69 Zupanc ML, Moraes CT, Shanske S, Langman CB, Ciafaloni E, DiMauro S. Deletion of mitochondrial DNA in patients with combined features of Kearns-Sayre and MELAS syndromes. *Ann Neurol* 1991; **29**: 680–3.
- 70 Amati-Bonneau P, Valentino ML, Reynier P *et al.* OPA1 mutations induce mitochondrial DNA instability and optic atrophy 'plus' phenotypes. *Brain* 2008; **131**: 338–51.
- 71 Jin H, May M, Tranebjaerg L *et al.* A novel X-linked gene, DDP, shows mutations in families with deafness (DFN-1), dystonia, mental deficiency and blindness. *Nat Genet* 1996; **14**: 177–80.
- 72 Prezant TR, Agapian JV, Bohlman MC *et al.* Mitochondrial ribosomal RNA mutation associated with both antibiotic-induced and non-syndromic deafness. *Nat Genet* 1993; **4**: 289–94.
- 73 Guan MX, Fischel-Ghodsian N, Attardi G. Nuclear background determines biochemical phenotype in the deafness-associated mitochondrial 12S rRNA mutation. *Hum Mol Genet* 2001; **10**: 573–80.
- 74 Bykhovskaya Y, Mengesha E, Wang D *et al.* Phenotype of non-syndromic deafness associated with the mitochondrial A1555G mutation is modulated by mitochondrial RNA modifying enzymes MTO1 and GTPBP3. *Mol Genet Metab* 2004; **83**: 199–206.
- 75 Hutchin T, Haworth I, Higashi K *et al.* A molecular basis for human hypersensitivity to aminoglycoside antibiotics. *Nucleic Acids Res* 1993; **21**: 4174–9.
- 76 Estivill X, Govea N, Barcelo A *et al.* Familial progressive sensorineural deafness is mainly due to the mtDNA A1555G mutation and is enhanced by treatment with aminoglycosides. *Am J Hum Genet* 1998; **62**: 27–35.
- 77 Hobbie SN, Bruell CM, Akshay S, Kalapala SK, Shcherbakov D, Bottger EC. Mitochondrial deafness alleles confer misreading of the genetic code. *Proc Natl Acad Sci USA* 2008; **105**: 3244–9.
- 78 Nagashima T, Mori M, Katayama K *et al.* Adult Leigh syndrome with mitochondrial DNA mutation at 8993. *Acta Neuropathol* 1999; **97**: 416–22.
- 79 Leigh D. Subacute necrotizing encephalomyelopathy in an infant. *J Neurol Neurosurg Psychiatr* 1951; **14**: 216–21.
- 80 Rahman S, Blok RB, Dahl HH *et al.* Leigh syndrome: clinical features and biochemical and DNA abnormalities. *Ann Neurol* 1996; **39**: 343–51.
- 81 De Braekeleer M. Genetic epidemiology of autosomal recessive spastic ataxia of Charlevoix-Saguenay in northeastern Quebec. *Genet Epidemiol* 1993; **10**: 17–25.
- 82 Mootha VK, Lepage P, Miller K *et al.* Identification of a gene causing human cytochrome *c* oxidase deficiency by integrative genomics. *Proc Natl Acad Sci USA* 2003; **100**: 605–10.
- 83 Huttenlocher PR, Solitare GB, Adams G. Infantile diffuse cerebral degeneration with hepatic cirrhosis. *Arch Neurol* 1976; **33**: 186–92.
- 84 Saada A, Shaag A, Mandel H, Nevo Y, Eriksson S, Elpeleg O. Mutant mitochondrial thymidine kinase in mitochondrial DNA depletion myopathy. *Nat Genet* 2001; **29**: 342.
- 85 Mandel H, Szargel R, Labay V *et al.* The deoxyguanosine kinase gene is mutated in individuals with depleted hepatocerebral mitochondrial DNA. *Nat Genet* 2001; **29**: 337–41.
- 86 Naviaux RK, Nguyen KV. POLG mutations associated with Alpers' syndrome and mitochondrial DNA depletion. *Ann Neurol* 2004; **55**: 706–12.
- 87 Spinazzola A, Viscomi C, Fernandez-Vizarra E *et al.* MPV17 encodes an inner mitochondrial membrane protein and is mutated in infantile hepatic mitochondrial DNA depletion. *Nat Genet* 2006; **38**: 570–5.
- 88 Elpeleg O, Miller C, Hershkovitz E *et al.* Deficiency of the ADP-forming succinyl-CoA synthase activity is associated with encephalomyopathy and mitochondrial DNA depletion. *Am J Hum Genet* 2005; **76**: 1081–6.
- 89 Ostergaard E, Christensen E, Kristensen E *et al.* Deficiency of the alpha subunit of succinate-coenzyme A ligase causes fatal infantile lactic acidosis with mitochondrial DNA depletion. *Am J Hum Genet* 2007; **81**: 383–7.
- 90 Morikawa Y, Matsuura N, Kakudo K, Higuchi R, Koike M, Kobayashi Y. Pearson's marrow/pancreas syndrome: a histological and genetic study. *Virchows Arch A Pathol Anat Histochem* 1993; **423**: 227–31.
- 91 Zeviani M, Moraes CT, DiMauro S *et al.* Deletions of mitochondrial DNA in Kearns-Sayre syndrome. *Neurology* 1988; **38**: 1339–46.
- 92 Poulton J, Morten KJ, Marchington D *et al.* Duplications of mitochondrial DNA in Kearns-Sayre syndrome. *Muscle Nerve* 1995; **3**: S154–8.
- 93 Goto Y, Nonaka I, Horai S. A mutation in the tRNA<sup>Leu</sup>(UUR) gene associated with the MELAS subgroup of mitochondrial encephalomyopathies. *Nature* 1990; **348**: 651–3.
- 94 Morten KJ, Poulton J, Sykes B. Multiple independent occurrence of the 3243 mutation in mitochondrial tRNA<sup>Leu</sup>(UUR) in

- patients with the MELAS phenotype. *Hum Mol Genet* 1995; **4**: 1689–91.
- 95 Sternberg D, Chatzoglou E, Laforet P *et al*. Mitochondrial DNA transfer RNA gene sequence variations in patients with mitochondrial disorders. *Brain* 2001; **124**: 984–94.
- 96 Bataillard M, Chatzoglou E, Rumbach L *et al*. Atypical MELAS syndrome associated with a new mitochondrial tRNA glutamine point mutation. *Neurology* 2001; **56**: 405–7.
- 97 de Coo IF, Sistermans EA, de Wijs IJ *et al*. A mitochondrial tRNA(Val) gene mutation (G1642A) in a patient with mitochondrial myopathy, lactic acidosis, and stroke-like episodes. *Neurology* 1998; **50**: 293–5.
- 98 Liolitsa D, Rahman S, Benton S, Carr LJ, Hanna MG. Is the mitochondrial complex I ND5 gene a hot-spot for MELAS causing mutations? *Ann Neurol* 2003; **53**: 128–32.
- 99 Martinuzzi A, Bartolomei L, Carozzo R *et al*. Correlation between clinical and molecular features in two MELAS families. *J Neurol Sci* 1992; **113**: 222–9.
- 100 Damian MS, Seibel P, Reichmann H *et al*. Clinical spectrum of the MELAS mutation in a large pedigree. *Acta Neurol Scand* 1995; **92**: 409–15.
- 101 van den Ouweland JWM, Lemkes HHPJ, Ruitenbeek K. Mutation in mitochondrial tRNA<sup>Leu(UUR)</sup> gene in a large pedigree with maternally transmitted type II diabetes mellitus and deafness. *Nat Genet* 1992; **1**: 368–71.
- 102 Walker M, Taylor RW, Turnbull DM. Mitochondrial diabetes. *Diabet Med* 2005; **22**(Suppl. 4): 18–20.
- 103 Maassen JA, LM TH, Van Essen E *et al*. Mitochondrial diabetes: molecular mechanisms and clinical presentation. *Diabetes* 2004; **53**(Suppl. 1): S103–9.
- 104 Vialettes BH, Paquis-Flucklinger V, Pelissier JF *et al*. Phenotypic expression of diabetes secondary to a T14709C mutation of mitochondrial DNA. Comparison with MIDD syndrome (A3243G mutation): a case report. *Diabetes Care* 1997; **20**: 1731–7.
- 105 Whittaker RG, Schaefer AM, McFarland R, Taylor RW, Walker M, Turnbull DM. Diabetes and deafness: is it sufficient to screen for the mitochondrial 3243A>G mutation alone? *Diabetes Care* 2007; **30**: 2238–9.
- 106 Whittaker RG, Schaefer AM, McFarland R, Taylor RW, Walker M, Turnbull DM. Prevalence and progression of diabetes in mitochondrial disease. *Diabetologia* 2007; **50**: 2085–9.
- 107 Holt IJ, Harding AE, Petty RK, Morgan-Hughes JA. A new mitochondrial disease associated with mitochondrial DNA heteroplasmy. *Am J Hum Genet* 1990; **46**: 428–33.
- 108 Santorelli FM, Shanske S, Jain KD, Tick D, Schon EA, DiMauro S. A T→C mutation at nt 8993 of mitochondrial DNA in a child with Leigh syndrome. *Neurology* 1994; **44**: 972–4.
- 109 de Vries DD, van Engelen BGM, Gabreels FJM, Ruitenbeek W, van Oost BA. A second missense mutation in the mitochondrial ATPase 6 gene mutation in Leigh's syndrome. *Ann Neurol* 1993; **34**: 410–2.
- 110 Thyagarajan D, Shanske S, Vazquez-Memije M, De Vivo D, DiMauro S. A novel mitochondrial ATPase 6 point mutation in familial bilateral striatal necrosis. *Ann Neurol* 1995; **38**: 468–72.
- 111 Carozzo R, Tessa A, Vazquez-Memije ME *et al*. The T9176G mtDNA mutation severely affects ATP production and results in Leigh syndrome. *Neurology* 2001; **56**: 687–90.
- 112 Hirano M, Silvestri G, Blake DM *et al*. Mitochondrial neurogastrointestinal encephalomyopathy (MNGIE): clinical, biochemical, and genetic features of an autosomal recessive mitochondrial disorder. *Neurology* 1994; **44**: 721–7.
- 113 Nishino I, Spinazzola A, Hirano M. Thymidine phosphorylase gene mutations in MNGIE, a human mitochondrial disorder. *Science* 1999; **283**: 689–92.
- 114 Spinazzola A, Marti R, Nishino I *et al*. Altered thymidine metabolism due to defects of thymidine phosphorylase. *J Biol Chem* 2002; **277**: 4128–33.
- 115 Van Goethem G, Schwartz M, Lofgren A, Dermaut B, Van Broeckhoven C, Vissing J. Novel POLG mutations in progressive external ophthalmoplegia mimicking mitochondrial neurogastrointestinal encephalomyopathy. *Eur J Hum Genet* 2003; **11**: 547–9.
- 116 Yavuz H, Ozel A, Christensen M *et al*. Treatment of mitochondrial neurogastrointestinal encephalomyopathy with dialysis. *Arch Neurol* 2007; **64**: 435–8.
- 117 Hirano M, Marti R, Casali C *et al*. Allogeneic stem cell transplantation corrects biochemical derangements in MNGIE. *Neurology* 2006; **67**: 1458–60.
- 118 Zeviani M, Sevidi S, Gallera C *et al*. An autosomal dominant disorder with multiple deletions of mitochondrial DNA starting in the D-loop region. *Nature* 1989; **339**: 309–11.
- 119 Bohlega S, Tanji K, Santorelli FM, Hirano M, al-Jishi A, DiMauro S. Multiple mitochondrial DNA deletions associated with autosomal recessive ophthalmoplegia and severe cardiomyopathy. *Neurology* 1996; **46**: 1329–34.
- 120 Agostino A, Valletta L, Chinnery PF *et al*. Mutations of ANT1, Twinkle, and POLG1 in sporadic progressive external ophthalmoplegia (PEO). *Neurology* 2003; **60**: 1354–6.
- 121 Longley MJ, Clark S, Yu Wai Man C *et al*. Mutant POLG2 disrupts DNA polymerase gamma subunits and causes progressive external ophthalmoplegia. *Am J Hum Genet* 2006; **78**: 1026–34.

*Correspondence:* Dr Robert McFarland, Mitochondrial Research Group, School of Neurology, Neurobiology and Psychiatry, 4th Floor, The Medical School, Framlington Place, Newcastle University, Newcastle-upon-Tyne NE2 4HH, UK.  
(fax: +44 (0) 191 222 8553; e-mail: robert.mcfarland@ncl.ac.uk) ■