Mitochondrial diseases

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Abstract | Mitochondrial diseases are a group of genetic disorders that are characterized by defects in oxidative phosphorylation and caused by mutations in genes in the nuclear DNA (nDNA) and mitochondrial DNA (mtDNA) that encode structural mitochondrial proteins or proteins involved in mitochondrial function. Mitochondrial diseases are the most common group of inherited metabolic disorders and are among the most common forms of inherited neurological disorders. One of the challenges of mitochondrial diseases is the marked clinical variation seen in patients, which can delay diagnosis. However, advances in next-generation sequencing techniques have substantially improved diagnosis, particularly in children. Establishing a genetic diagnosis allows patients with mitochondrial diseases to have reproductive options, but this is more challenging for women with pathogenetic mtDNA mutations that are strictly maternally inherited. Recent advances in *in vitro* fertilization techniques, including mitochondrial donation, will offer a better reproductive choice for these women in the future. The treatment of patients with mitochondrial diseases remains a challenge, but guidelines are available to manage the complications of disease. Moreover, an increasing number of therapeutic options are being considered, and with the development of large cohorts of patients and biomarkers, several clinical trials are in progress.

Mitochondrial diseases are a group of genetic disorders that are characterized by dysfunctional mitochondria. Mitochondria are ubiquitous cellular organelles, except in erythrocytes, and are crucial integrators of intermediary metabolism in various cellular metabolic pathways, including oxidative phosphorylation, fatty acid oxidation, Krebs cycle, urea cycle, gluconeogenesis and ketogenesis[1]. Mitochondria also have an important role in other important cellular processes, including (non-shivering) thermogenesis, amino acid metabolism, lipid metabolism, biosynthesis of haem and iron–sulfur clusters, calcium homeostasis and apoptosis[2–5]. The pathophysiology of mitochondrial diseases is complex and involves genetic mutations in mitochondrial DNA (mtDNA) and nuclear DNA (nDNA). This complex genetics means that mitochondrial diseases can have any pattern of inheritance, including autosomal and X-linked inheritance for nDNA mutations and maternal inheritance for mtDNA mutations. Rare sporadic cases due to *de novo* mutations have also been noted. In patients with mtDNA mutations, inheritance and clinical presentation are further complicated by the presence of multiple mtDNA genomes in an individual cell, which can often lead to a mixture of mutated and wild-type genomes, called heteroplasmy (BOX 1). The level of heteroplasmy is crucial in determining the extent of cellular dysfunction. Conventionally, mitochondrial diseases lead to a primary defect in oxidative phosphorylation, the process by which cells produce ATP, but other factors, such as dysfunction of the Krebs cycle and folate cycle, lack of enzyme intermediates and accumulation of toxic substances, among others, might also have a role in disease.

Mitochondrial diseases are clinically heterogeneous, can occur at any age and can manifest with a wide range of clinical symptoms. Mitochondrial diseases can involve any organ or tissue, characteristically involve multiple systems, typically affecting organs that are highly dependent on aerobic metabolism, and are often relentlessly progressive with high morbidity and mortality[6]. Some of the clinical features that are associated with mitochondrial diseases can be grouped into specific syndromes, for example, Leigh syndrome (also known as subacute necrotizing encephalomyelopathy) and Alpers–Huttenlocher syndrome. The heterogeneity in the clinical manifestation of mitochondrial diseases means that both diagnosis and management of these disorders are extremely difficult. Diagnosis often relies on genetic testing, in addition to histochemical and biochemical analysis of tissue biopsies. The management of patients with mitochondrial diseases includes strategies to reduce morbidity and mortality, the early treatment of organ-specific complications and interventional strategies where possible.

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Considerable advances in our understanding of the molecular basis of mitochondrial diseases and their genetic aetiology have been made. Moreover, next-generation sequencing technologies have already transformed the diagnosis of mitochondrial diseases that are caused by mutations in nDNA and, for some conditions, has increased the diagnostic success from <20% to >60%. However, this has been possible only after meticulous clinical and biochemical characterization of patients. Many challenges remain, not least of which is elaborating the mechanisms involved in the phenotypic expression of the genetic defect. Other important challenges include improving the care of patients with mitochondrial diseases, the development of curative treatments and strategies to prevent inheritance. The advent of mitochondrial donation for women with mtDNA mutations might be a novel way to prevent disease transmission to their offspring. This Primer provides an insight into the understanding and current management of patients with mitochondrial diseases and also provides information about the epidemiology and diagnosis of these disorders.

Epidemiology

The prevalence of all forms of childhood-onset (<16 years of age) mitochondrial diseases has been estimated to range from 5 to 15 cases per 100,000 individuals (TABLE 1). Substantial contributors to this variation are the inclusion criteria being used, particularly in the diagnostic algorithms, and the age ranges being studied. In addition, variation in prevalence occurs owing to the presence of genetic founder mutations and high consanguinity. Genetic founder mutations have been shown to make specific mitochondrial diseases more common in certain populations. For example, recessive mutations in *POLG* (encoding DNA polymerase subunit γ1, which is the catalytic subunit of mtDNA polymerase) are one of the most common causes of childhood-onset mitochondrial diseases in many countries, due to the spread of two ancient European founder mutations[7]. The most common childhood presentation of a mitochondrial disease is Leigh syndrome, which comprises >75 monogenic disorders[8]. The prevalence of Leigh syndrome is ~2.5 cases per 100,000 births, but is up to tenfold higher in Saguenay-Lac-St-Jean, Canada, due to a genetic founder mutation in *LRPPRC*[8]. Populations with high consanguinity often show an increased prevalence of inherited diseases, for example, autosomal recessive childhood-onset mitochondrial diseases in the Australian Lebanese and Irish travelling communities[9,10]. Moreover, the large number of genes associated with recessive mitochondrial diseases makes it likely that a single consanguineous community will have an increased prevalence of several different recessive mitochondrial diseases.

The most detailed estimates of the prevalence of adults with mitochondrial diseases are from a cohort study in the North East of England, a largely white population with little evidence of consanguinity (TABLE 2). This study determined that, in adults, the prevalence of mitochondrial diseases caused by mutations in mtDNA is estimated at 9.6 cases per 100,000 individuals and the prevalence of mitochondrial diseases caused by mutations in nDNA is 2.9 cases per 100,000 individuals[11]. In addition, 10.8 per 100,000 adults were identified as being at risk of developing mitochondrial diseases caused by mutations in mtDNA, owing to having an affected first-degree relative with mtDNA mutations[11]. Interestingly, pathogenetic mutations in mtDNA have been shown to be much more prevalent in population-based studies than in cohort studies of affected individuals[12,13]. For example, the population prevalence of the m.3243A>G mutation (in *MT-TL1*), which is associated with mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes (MELAS) syndrome, has been estimated at up to ~236 cases per 100,000 individuals in an Australian Caucasian-based population study; all individuals with this mutation had mild-to-moderate hearing loss, which is one of the clinical manifestations of the m.3243A>G mutation[12]. Thus, a substantial number of patients with mitochondrial diseases caused by mtDNA mutations might be eluding diagnosis. Currently available data imply that most global regions will have a minimum birth prevalence for mitochondrial diseases of 20 cases per 100,000 individuals, but this might be much higher in regions with high consanguinity or specific genetic founder effects. Moreover, mtDNA mutations seem to

Box 1 | **Mitochondrial DNA heteroplasmy**

The majority of pathogenetic mitochondrial DNA (mtDNA) mutations are heteroplasmic, with a mixture of mutated and wild-type mtDNA inside an individual cell. High levels of heteroplasmy refer to cells with high levels of mutant mtDNA and low levels of wild-type mtDNA, whereas low levels of heteroplasmy refer to cells with low levels of mutant mtDNA but high levels of wild-type mtDNA. Studies in single cells from patients with mitochondrial diseases have shown that the level of mutated and wild-type mtDNA is very important for determining the cellular phenotype. For example, cells become respiratory deficient if they contain high levels of mutated mtDNA and low levels of wild-type mtDNA (that is, high levels of heteroplasmy). The threshold at which this deficiency occurs depends on the precise mutation and the cell type. Typically, high percentage levels of mutated mtDNA (>50%) are required to result in cellular defects, but some mtDNA mutations only generate a deficiency if present at very high levels (typically mt-tRNA mutations) and others (such as single, large-scale mtDNA deletions) produce a deficiency when there is ~60% deleted mtDNA.

Table 1 | **Prevalence of adult-onset and childhood-onset mitochondrial diseases**

Age range (years)	Prevalence per 100,000 individuals (95% CI)	Region	Refs
0–6	8.9 (5.3–14)	Western Sweden	189
0–10	15 (9.8–21)	Central region of Portugal	190
0–16	4.7 (2.8–7.6)	Western Sweden	189
0–16	6.2 (4.5–8.4)*	South eastern Australia	9
0–16	71 (32–136)*	South eastern Australia (families of Lebanese ancestry)	9
0–18	7.5 (5–10)	North West Spain	191
0–18	~12	Northern Finland	192
Childhood	~11	Ireland	10
Childhood	~10	Japan	193
Adults (>16)	9.6 (8.3–11)	North East of England (mtDNA)	11
Adults (>16)	2.9 (2.2–3.7)	North East of England (nDNA)	11

mtDNA, mitochondrial DNA; nDNA, nuclear DNA. *Time interval: 1991–1994.

be responsible for ~80% of mitochondrial diseases in adults. Conversely, mtDNA mutations have been found in only 20–25% of cases of childhood-onset mitochondrial diseases[14,15] and most childhood-onset mitochondrial diseases are caused by autosomal recessive mutations in nDNA[9].

Mechanisms/pathophysiology

Mitochondrial biogenesis and biochemistry

Over ~1,500 proteins are required for healthy mitochondrial function[16,17]. Thirteen proteins are encoded by mtDNA and the rest are encoded by nDNA, which are translated in the cytoplasm and the proteins are imported across the mitochondrial membrane through an intricate import machinery. Only ~100 proteins are directly involved in oxidative phosphorylation and the production of ATP (FIG. 1). Oxidative phosphorylation requires the transport of electrons to molecular oxygen by the mitochondrial respiratory chain (also known as the electron transport chain), which involves four multisubunit complexes (known as complex I–complex IV) and two mobile electron carriers, ubiquinone (also known as coenzyme Q10, coenzyme Q or CoQ) and cytochrome *c*. The respiratory chain generates a transmembrane proton gradient that is harnessed by complex V (also known as ATP synthase) to synthesize ATP. Some studies have shown that the respiratory chain complexes often form larger structures called respiratory supercomplexes (also known as respirasomes). Defects in one complex in a respirasome can have a knock-on effect on the other complexes[18].

The mitochondrial respiratory chain is a substantial source of reactive oxygen species (ROS), particularly by complex I and complex III. Although several enzymes, including superoxide dismutase, in mitochondria are involved in ROS disposal and a role is emerging for ROS in the physiological regulation of mitochondrial biogenesis, excessive ROS might damage lipid membranes, proteins and nucleic acids and have a role in the pathogenesis of mitochondrial diseases.

mtDNA

Human mtDNA is a molecule of double-stranded DNA that encodes 13 structural peptide subunits of the oxidative phosphorylation system and 24 RNA molecules that are required for intra-mitochondrial protein synthesis[19,20] (FIG. 2). As opposed to nDNA, mtDNA has a circular structure and lacks an intron–exon structure. In addition, replication, transcription and translation of mtDNA are all controlled by a single non-coding region, known as the displacement loop (D loop). In addition, multiple copies of mtDNA exist within each cell and the total amount can vary between a few hundred and many tens of thousands of copies, depending on the cell type. Many patients with a mitochondrial disease have a mixture of mutated and wild-type mtDNA (known as heteroplasmy); the proportion of mutated and wild-type mtDNA is a key factor that determines whether a cell expresses a biochemical defect (BOX 1). Inheritance of mtDNA is strictly maternal and thus undergoes negligible intra-molecular recombination at the population level. Finally, subtle but important differences exist in the mtDNA genetic code compared with nDNA. For example, in mtDNA, the codons AUA and AUG code for methionine, the codon UGA codes for tryptophan (not a stop codon, as in the nDNA) and the codons UAA and UAG are the only stop codons used by mitochondria[21].

Pathogenetic mutations in mtDNA can affect the structural subunits of the respiratory chain or the mitochondrial protein synthesis machinery. Hundreds of different point mutations and large-scale mtDNA rearrangements have been shown to cause disease. Mutations of mtDNA causing mitochondrial diseases can be classified into three types: mtDNA rearrangements (for example, sporadic, single, large-scale mtDNA deletions), mutations in genes involved in protein synthesis (for example, mutations in mitochondrial tRNA or rRNA) or mutations in genes encoding structural proteins (for example, mitochondrial mRNA mutations) (BOX 2; FIG. 3).

nDNA

Over ~1,500 different nuclear genes encode mitochondrial proteins[17], which are not only involved in oxidative phosphorylation but also in the many other functions of the mitochondria. An increasing number of mutations in these genes have been shown to cause mitochondrial diseases (BOX 2; FIG. 3) and can have autosomal dominant, autosomal recessive or X-linked inheritance patterns[22]. Although complex, classification of these Mendelian disorders can be simplified based on the impairment of mitochondrial and cellular function. The pathological consequences of mutations in nDNA affecting the mitochondria are varied and include defects in mtDNA maintenance, mtDNA translation and mitochondrial homeostasis, among others. Defects in mtDNA maintenance are due to mutations in genes encoding proteins involved in mtDNA replication and in proteins controlling replication or deoxynucleotide triphosphate (dNTP) synthesis and/or salvage, both of which result in secondary mtDNA abnormalities that manifest as either

mtDNA copy number loss or multiple mtDNA deletions. Defective mitochondrial function can also be due to altered mtDNA translation, which can be caused by mutations in genes encoding translation and releasing factor proteins, mitochondrial tRNA-modifying proteins, mitochondrial mRNA-processing enzymes, mitochondrial aminoacyl-tRNA synthetases and mitoribosomal proteins. Other causes of defective mitochondrial function caused by nDNA mutations include apoptosis, mitochondrial chaperones and mitochondrial metabolism, among others[23] (FIG. 3). Inextricably, mtDNA is dependent on numerous nuclear-encoded proteins for replication and repair[24–26] (BOX 2). Severance in mtDNA replication or repair due to mutations in these genes can manifest qualitatively in the generation of multiple point mutations or large-scale mtDNA deletions that might appear over the lifespan of a patient[27], or quantitatively by the near-complete loss of the mitochondrial genome (known as mtDNA depletion)[28], or both[29]. These mutations in the nDNA, which encodes proteins with a role in essential mitochondrial processes, are recognized to manifest with a wide variety of clinical syndromes similar to mtDNA-related mitochondrial disorders[30].

Diagnosis, screening and prevention

Clinical features and syndromes

The onset of mitochondrial diseases has been shown to have a bimodal distribution with a peak in the first 3 years of life followed by a second broader peak beginning towards the end of teenage years and into the fourth decade of life (adult-onset diseases), although mitochondrial diseases can present much later, particularly with chronic progressive external ophthalmoplegia (CPEO). The clinical diagnosis of mitochondrial diseases is complicated by a diverse phenotype that encompasses dysfunction of almost any organ system[6] (FIG. 4). Single-organ involvement might occur in a relatively small number of cases, for example, isolated eye involvement in Leber hereditary optic neuropathy (LHON). However, most patients present with multi-system involvement. Individuals might present with a constellation of clinical features that are compatible with a discrete clinical syndrome (TABLE 3). However, categorizing patients into syndromes is of limited value, as the majority of patients do not fit into discrete categories.

The diagnosis of mitochondrial diseases is further complicated by an often tenuous relationship between the genotype and the observed clinical phenotype; some mtDNA mutations can give rise to several different clinical syndromes, for example, the m.3243A>G mutation (in *MT-TL1*) can cause CPEO, MELAS syndrome and MIDD (maternally inherited diabetes and deafness)[31]. The reverse is also true in that specific syndromes can have a diverse genetic aetiology, for example, Leigh syndrome can be caused by an array of mtDNA and nDNA mutations in several genes[8]. Often, the variable clinical manifestation of mtDNA mutations is a direct consequence of the level of heteroplasmy or, perhaps more physiologically relevant, the absolute copy number of wild-type mtDNA in the tissue. Moreover, other, as yet unidentified, genetic, environmental and epigenetic factors also seem to be important in determining the clinical phenotype of many mtDNA mutations[32]. No definitive evidence is available regarding the factors that affect the penetrance of disease, apart from the clear male prevalence in LHON (see below). The penetrance of LHON might be influenced by efficient mitochondrial biogenesis[33,34], although the factors influencing this increase in mitochondrial copy number are unknown. For patients with single, large-scale mtDNA deletions, the phenotype is influenced by the size of the deletion and the level of heteroplasmy[35].

Childhood-onset mitochondrial diseases. Childhood-onset mitochondrial diseases are generally severe, although not always fatal[36–38], and are most often a consequence of recessive nDNA mutations or mtDNA mutations that are present at very high levels of mtDNA heteroplasmy. Common, nonspecific clinical features can present in childhood-onset mitochondrial diseases, including hypotonia, generalized weakness, failure to thrive, dysautonomia, fatigue, exercise intolerance, vomiting, seizures and encephalopathy. Limb spasticity with axial hypotonia is typical of central nervous system involvement, but is not specific for mitochondrial diseases. The pattern of hypomyelination and leukodystrophy observed on cranial MRI scans can help to distinguish, for example, mitochondrial diseases (caused by mutations in *NDUFS1*, *DARS2* and *ISCA2*) from other causes of white matter disease[39–41]. Renal involvement in young children with mitochondrial diseases can occur due to several different genetic defects and can be a cystic disorder (for example, associated with mutations in *RMND1*) or a proximal tubulopathy (for example, associated with mutations in *RRM2B*), although in adolescent and adult patients, focal segmental glomerulosclerosis is more common (for example, associated with a mutation in *MT-TL1* (m.3243A>G)[42–44]. Hypertrophic cardiomyopathy is much more frequently observed than dilated cardiomyopathy in patients with

Table 2 | **Prevalence estimates for mitochondrial diseases**

Mutation	Number of adult cases per 100,000 individuals (95% CI)	Number of adults and children at risk per 100,000 individuals (95% CI)
In genes associated with LHON	3.7 (2.9–4.6)	4.4 (3.7–5.3)
MT-TL1 (m.3243A>G)	3.5 (2.7–4.4)	4.4 (3.7–5.3)
Single, large-scale mtDNA deletion	1.5 (1–2.1)	0
MT-TK (m.8344A>G)	0.2 (0.1–0.5)	0.5 (0.2–0.8)
SPG7, ar	0.8 (0.5–1.3)	1.3 (0.9–1.8)
PEO1, ad	0.7 (0.4–1.2)	2.3 (1.7–2.9)
OPA1, ad	0.4 (0.2–0.7)	0.7 (0.4–1.1)
POLG, ar	0.3 (0.1–0.6)	0.3 (0.1–0.6)
RRM2B, ad	0.2 (0.1–0.5)	0.7 (0.4–1)
nDNA (GD)	0.2 (0.1–0.5)	0.3 (0.1–0.6)

ad, autosomal dominant inheritance; ar, autosomal recessive inheritance; GD, adults with chronic progressive external opthalmoplegia and multiple mtDNA deletions in muscle that have not been genetically determined; LHON, Leber hereditary optic neuropathy; mtDNA, mitochondrial DNA; nDNA, nuclear DNA. Data from REF. 11.

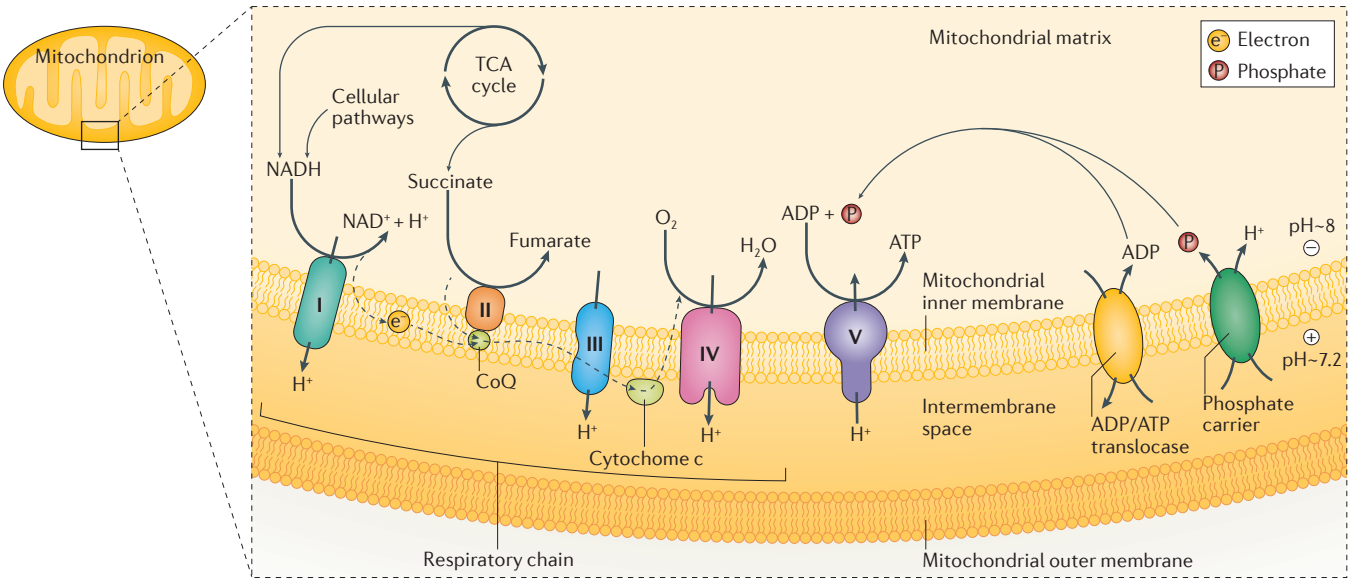

Figure 1 | **Schematic representation of oxidative phosphorylation.** Oxidative phosphorylation is a metabolic pathway that cells use to oxidize nutrients, thereby releasing energy in the form of ATP. The respiratory pathway includes complexes I–IV of the respiratory chain and complex V, an ATP synthase. Complex I (NADH:coenzyme Q oxidoreductase) oxidizes NADH with the reduction of coenzyme Q10 (also known as CoQ) from its ubiquinone (CoQ; Q) form to ubiquinol (QH2), generating an electrochemical gradient across the inner mitochondrial membrane. Complex II (succinate-CoQ oxidoreductase) intricately links the Krebs cycle (also known as the tricarboxylic acid (TCA) cycle) to the respiratory chain. Complex II oxidizes succinate with the reduction of CoQ from its ubiquinone (CoQ; Q) form to ubiquinol (QH2). Complex III (ubiquinol-cytochrome *c* oxidoreductase) catalyses the reduction of cytochrome *c* by oxidation of ubiquinol with the generation of an electrochemical gradient. Complex IV (cytochrome *c* oxidase) is responsible for the terminal enzymatic reaction of the respiratory chain that transfers electrons (e^-) to molecular oxygen and generates an electrochemical gradient. Complex V converts transmembrane electrochemical proton (H^+) gradient energy into mechanical energy, which catalyses the chemical bond energy between ADP and phosphate (P) to form ATP.

mitochondrial diseases (although dilated cardiomyopathy can be observed as the myocardium fails), and can occur in isolation of other clinical features (for example, in individuals with mutations in *AARS2* or *MTO1*) or in association with multi-system disease (for example, in individuals with mutations in *MT-TK* (m.8344A>G), *MT-TL1* (m.3243A>G) or *AGK*)[31,45–48]. Hypertrichosis (excessive hair growth) is a common finding in Leigh syndrome caused by *SURF1* mutations, and dysmorphic features can be observed in several mitochondrial diseases caused by mutations in nDNA, including in *FBXL4* (REFS 49,50). Sensorineural hearing loss, both syndromic and non-syndromic, can occur in children and adults with mitochondrial diseases and has a diverse genetic aetiology that includes mutations in *MT-TL1* (m.3243A>G), *MT-RNR1* (m.1555A>G), *MT-TS1*, *SUCLA2*, *SUCLG1*, *RMND1* and *RRM2B*, and single, large-scale deletions of mtDNA[51,52].

Several syndromes of mitochondrial diseases that arise during childhood have been identified, such as Leigh syndrome, Alpers–Huttenlocher syndrome and Pearson syndrome (TABLE 3). The most common syndrome associated with childhood-onset mitochondrial diseases is Leigh syndrome, which usually begins between 3 months and 2 years of age and can be caused by mutations in at least 75 different genes[8]. Most cases of Leigh syndrome have their onset in infancy, although some cases have a rapidly progressive course from birth and, rarely, others have a much later onset in adolescence or early adult life. Previously, Leigh syndrome used to be a neuropathological diagnosis with typical findings that included symmetric spongiform degeneration of the corpus striatum and brainstem with demyelination and vascular proliferation. Now, cranial MRI is used, which shows symmetric T2 hyperintensities in the striatum and brainstem[53,54] (FIG. 5a–c).

Alpers–Huttenlocher syndrome is also a childhood-onset mitochondrial disease that is characterized by intractable epilepsy, psychomotor regression and liver disease[55–57] (TABLE 3). The majority of cases are a consequence of mutations in *POLG*, whereas an Alpers-like syndrome has also been reported in individuals with mutations in genes encoding mitochondrial tRNA synthetase (*FARS2, NARS2* and *PARS2*)[58–60].

Other clinical syndromes that are observed in infancy include Pearson syndrome and congenital lactic acidosis[61–63] (TABLE 3). Other syndromes include a progressive pure myopathy or spinal muscular atrophy-like phenotype, which is associated with mtDNA depletion in skeletal muscle and mutations in *TK2* (REF. 64), MEGDEL syndrome (also known as 3-methylglutaconic aciduria with deafness, encephalopathy and Leigh-like syndrome), which is associated with mutations in *SERAC1* (REF. 65), and Sengers syndrome of congenital cataracts, proximal myopathy and hypertrophic cardiomyopathy, which is associated with mutations in *AGK*[45,66].

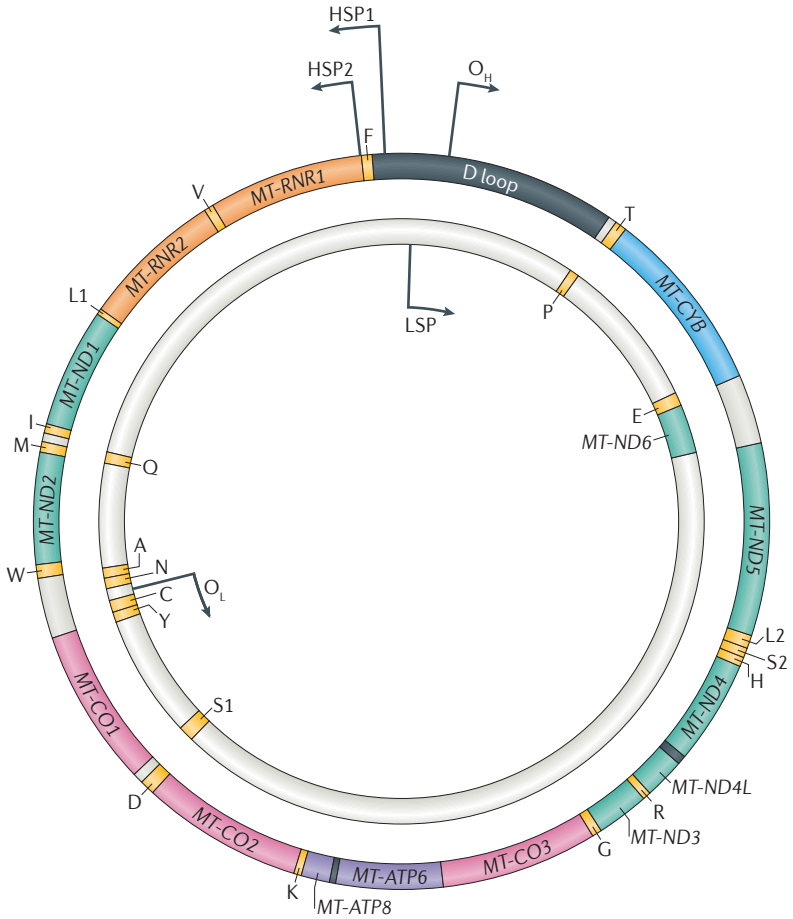

Figure 2 | **Human mitochondrial genome.** The human mitochondrial genome is a ~16.6-kb circular, double-stranded DNA molecule. It includes a 1.1-kb non-coding region, called the displacement loop (D loop), which is involved in the regulation of transcription and replication of DNA. Seven (out of 44) complex I, one (out of 11) complex III, three (out of 14) complex IV and two (out of 16) complex V subunits are encoded by mitochondrial DNA (mtDNA). *MT-RNR1* and *MT-RNR2* are rRNA genes and encode 12S and 16S rRNA, respectively. Single letters, such as 'Q' and 'L', indicate the tRNA genes, which provide RNA components for intra-mitochondrial protein synthesis. O_H and O_L indicate the origins of heavy-strand and light-strand mtDNA replication. HSP, heavy-strand promoter; LSP, light-strand promoter. Adapted from REF. 187, Nature Publishing Group.

Mitochondrial diseases in adolescents and adults. Similar to childhood-onset mitochondrial diseases, adult-onset mitochondrial diseases can present and progress in a myriad of ways, making diagnosis and management challenging. Increasingly, the identification of one family member with mitochondrial disease might alert physicians to other individuals with disease in the family. Although many adult patients do not neatly fit into specific syndromes, the description of the syndromes is helpful in highlighting the multiple systems involved in disease.

LHON is a maternally inherited disorder that is characterized by degeneration of retinal ganglion cells and their axons, which culminates in acute or subacute central visual loss[67]. In the North East of England, three primary LHON mtDNA mutations account for almost 50% of all adult-onset mitochondrial diseases, but in the French-Canadian population, there is a large genetic founder effect for mutations in *MT-ND6* (m.14484T>C)[68,69]. Disease expression is variable even in individuals with homoplasmic levels of the mutation and can depend on sex and lifestyle factors. For example, male sex is associated with an increased risk of developing LHON compared with female sex, and cigarette smokers and heavy alcohol drinkers have a significantly greater risk of developing LHON, with evidence of a dose–response relationship. Women with LHON have a greater risk of developing clinical and/or radiological features that resemble multiple sclerosis than men with LHON. Together, these data indicate that nuclear genetic and environmental factors exert a strong modulating effect on disease expression[70–72].

Kearns–Sayre syndrome[73,74] is a progressive cardioencephalomyopathy caused by a single, large-scale deletion or rearrangement of mtDNA, which also gives rise to Pearson syndrome[61,62] in infancy and CPEO in middle age, and was the first genetic defect in mtDNA noted to be associated with human disease. The condition is characterized by the triad of cardinal clinical features described by Kearns and Sayre (retinitis pigmentosa, ophthalmoplegia and cardiac conduction defects) and typically has its onset before 20 years of age. Cardiac pacing is frequently required and multiple endocrinopathies (such as in the adrenal gland, pancreas, thyroid and parathyroid gland) frequently develop in patients with the most severe disease.

MELAS syndrome is characterized by mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes[75] (FIG. 5d–f). Nearly 80% of all cases of MELAS syndrome harbour an m.3243A>G mutation in *MT-TL1* (REF. 76). The genetic aetiology of the remaining ~20% of cases is very heterogeneous; various mutations in *MT-TL1* and other mtDNA genes encoding tRNAs have been associated with this syndrome[77]. The m.3243A>G mutation in *MT-TL1* can also give rise to other conditions, including MIDD, Leigh syndrome, CPEO or a cardioencephalomyopathy syndrome[31] (TABLE 3).

Myoclonic epilepsy with ragged red fibres is a well-characterized but rare form of mitochondrial diseases[11,78]. Mutations in *MT-TK* (particularly m.8344A>G)[79] account for the genetic aetiology of the vast majority of cases (>90%).

Neurogenic muscle weakness, ataxia and retinitis pigmentosa (NARP) is a progressive neurodegenerative disorder that often presents in early childhood, but might remain quiescent or stable into adult life, and forms a clinical continuum with maternally inherited Leigh syndrome[80]. The genetic aetiology of NARP seems to be confined to pathogenetic variants in *MT-ATP6* (m.8993T>G or m.8993T>C)[80,81]. Both of these mutations exhibit the strongest genotype–phenotype correlation of any mtDNA pathogenetic variants, with a strong relationship between the level of mtDNA mutations and the disease severity[82,83]. In general, individuals with heteroplasmy levels of <70% of the m.8993T>G mutation are often asymptomatic, those with heteroplasmy levels of 70–90% manifest clinically with a NARP phenotype, whereas those with heteroplasmy levels of >90% manifest clinically as Leigh syndrome. Individuals with the

m.8993T>C mutation characteristically only manifest with symptoms at heteroplasmy levels of >90%[83].

CPEO is a common manifestation of adult-onset mitochondrial diseases that is commonly associated with either primary mtDNA mutations or secondary mtDNA defects due to a nuclear genetic disorder of mtDNA maintenance. Mutations in ~75% of the known maintenance genes have been shown to cause adult-onset CPEO. Despite the identification of a large number of genes associated with CPEO, a substantial proportion of patients do not have a genetic diagnosis, but these patients have evidence of muscle-restricted multiple mtDNA deletions[84].

Another group of disorders, resulting from impaired mitochondrial dynamics (fission and fusion events), have emerged, including mutations in *OPA1* (which can manifest clinically as optic atrophy, ataxia and deafness)[85] and *MFN2* (which causes Charcot–Marie–Tooth neuropathy type 2A)[86]. These disorders indirectly result in acquired mtDNA defects[86,87].

Box 2 | **Nuclear gene defects in mitochondrial diseases and their function**

Phospholipid metabolism
AGK, *SERAC1* and *TAZ*

Metabolism of toxic compounds
HIBCH, *ECHS1*, *ETHE1* and *MPV17*

Disulfide relay system
GFER

Iron–sulfur protein assembly
ISCU, *BOLA3*, *NFU1* and *IBA57*

tRNA modification
MTO1, *GTP3BP*, *TRMU*, *PUS1*, *MTFMT*, *TRIT1*, *TRNT1* and *TRMT5*

Aminoacyl-tRNA synthetases
AARS2, *DARS2*, *EARS2*, *RARS2*, *YARS2*, *FARS2*, *HARS2*, *LARS2*, *VARS2*, *TARS2*, *IARS2*, *CARS2*, *PARS2*, *NARS2*, *KARS*, *GARS*, *SARS2* and *MARS2*

Release factors
C12orf65

Elongation factors
TUFM, *TSFM* and *GFM1*

Mitoribosomal proteins
MRPS16, *MRPS22*, *MRPL3*, *MRP12* and *MRPL44*

mRNA processing
LRPPRC, *TACO1*, *ELAC2*, *PNPT1*, *HSD17B10*, *MTPAP* and *PTCD1*

Mitochondrial fusion and fission
OPA1 and *MFN2*

Deoxynucleotide triphosphate synthesis
DGUOK, *TK2*, *TYMP*, *MGME1*, *SUCLG1*, *SUCLA2*, *RNASEH1*, *C10orf2*, *POLG*, *POLG2*, *DNA2* and *RRM2B*

Solute carriers of thiamine and phosphate
SLC19A3, *SLC25A3* and *SLC25A19*

Respiratory chain subunits

- Complex I: *NDUFS1*, *NDUFS2*, *NDUFS3*, *NDUFS4*, *NDUFS6*, *NDUFS7*, *NDUFS8*, *NDUFV1*, *NDUFV2*, *NDUFA1*, *NDUFA2*, *NDUFA9*, *NDUFA10*, *NDUFA11*, *NDUFA12*, *NDUFA13*, *NDUFAF2*, *NDUFAF6* and *NDUFB11*
- Complex II: *SDHA*, *SDHB*, *SDHC*, *SDHD* and *SDHAF1*
- Complex III: *UQCRB*, BCS1L, *UQCRQ*, *UQCRC2*, *CYC1*, *TTC19*, *LYRM7*, *UQCC2* and *UQCC3*
- Complex IV: *COA5*, *SURF1*, *COX10*, *COX14*, *COX15*, *COX20*, *COX6B1*, *FASTKD2*, *SCO1*, *SCO2*, *LRPPRC*, *TACO1* and *PET100*
- Complex V: *ATPAF2*, *TMEM70*, *ATP5E* and *ATP5A1*
- Coenzyme Q10 deficiency: *PDSS1*, *PDSS2*, *COQ2*, *COQ4*, *COQ6*, *COQ8A*, *COQ8B* and *COQ9* (secondary defects: *ETFDH* and *APTX*)

Protein quality control and degradation
FBXL4, *AFG3L2* and *SPG7*

ATP and ADP transport
ANT1

Diagnostic algorithm

Devising a diagnostic algorithm that encompasses all mitochondrial diseases is fraught with difficulties, not least of which are the diverse clinical manifestations of disease and heterogeneous genetics (FIG. 6). The key to any successful diagnostic algorithm for mitochondrial diseases is astute clinical observation and awareness. The recognition of mitochondrial disease syndromes or specific clinical features can permit targeted genetic analysis, which enables the rapid diagnosis of patients and family members. For example, cardiomyopathy and cataracts are frequently associated with *AGK* mutations (Sengers syndrome)[45,66]. Equally, detailed clinical and laboratory tests (BOX 3) are vital for the accurate interpretation of new genetic mutations that are identified through next-generation sequencing technologies. The diagnostic algorithm is heavily influenced by the age of the patient, and the presence of consanguinity and common genetic founder mutations in certain parts of the world.

In young children with common phenotypes, targeted gene sequencing for the commonly affected genes in both nDNA and mtDNA that cause the phenotype is both sensible and efficient. In adults, the high incidence of mtDNA mutations highlights the importance of investigating the mitochondrial genome. Most mutations in mtDNA, apart from *de novo* mutations (for example, single, large-scale deletions), can be detected using sequencing of mtDNA that is isolated from blood or urinary sediment[88].

If no diagnosis is made, then either further genetic studies, including exome sequencing to detect nDNA mutations, or the testing of clinically relevant or affected tissue should be performed. Performing exome sequencing might avoid the need for an invasive skeletal muscle biopsy, but some cases will still require biopsy for biochemical confirmation of the consequences of mutations of unknown importance. Skeletal muscle biopsy can be extremely helpful and can be used for histochemical and biochemical analysis (BOX 3). Skeletal muscle histochemistry and the sequential assessment of cytochrome *c* oxidase (complex IV) and succinate dehydrogenase (complex II) activities[89] have been helpful in the diagnosis of adult-onset mitochondrial diseases. Indeed, the qualitative assessment of complex IV-deficient muscle fibres, together with the clinical history of the patient, can often suggest the underlying genetic problem. A new technique, quadruple immunofluorescence, which uses fluorescently labelled antibodies raised against subunits of complexes I–IV, has been shown to provide accurate data on the relative abundance of complex I and complex IV[90] and could contribute to the diagnosis of mitochondrial diseases.

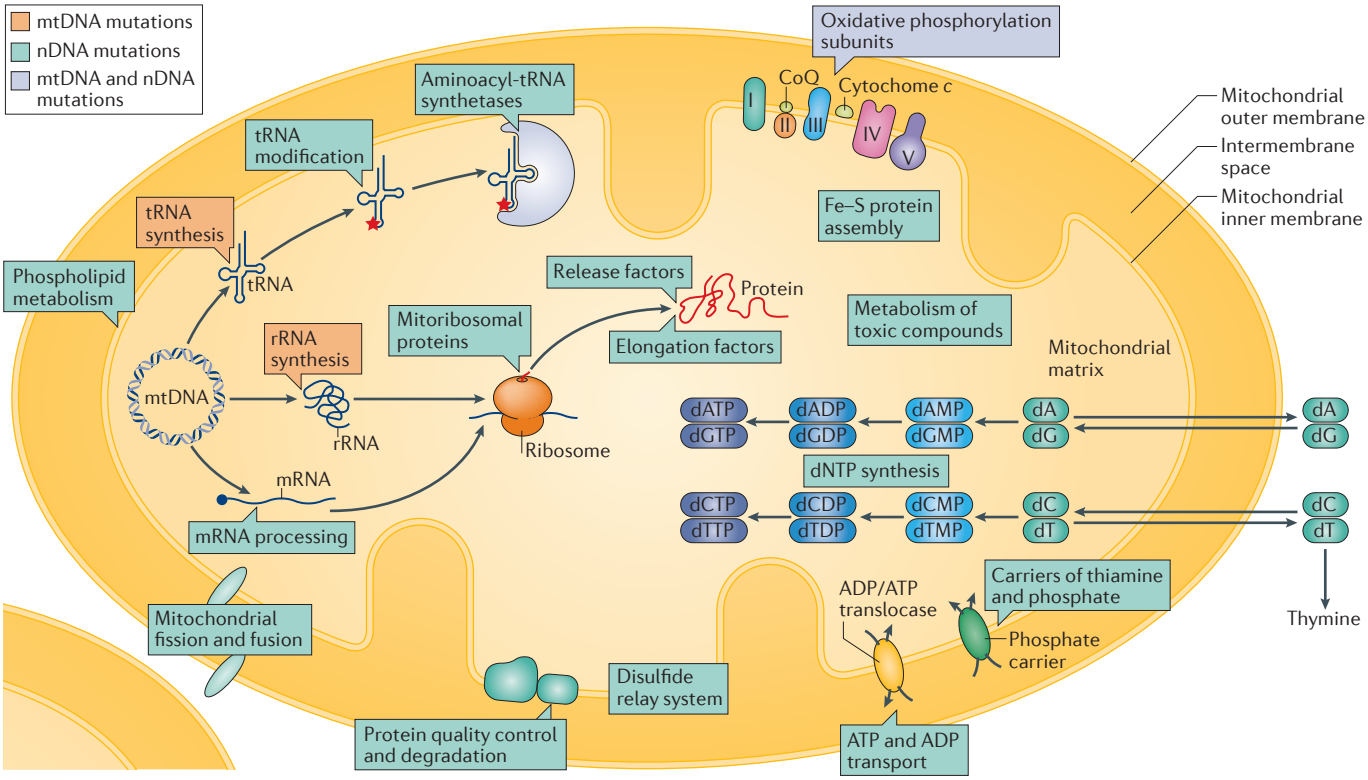

Figure 3 | **Genes associated with human mitochondrial diseases and their role in mitochondrial function.** Defects in nuclear DNA (nDNA) can cause abnormalities in oxidative phosphorylation, mitochondrial DNA (mtDNA) maintenance, solute carriers of thiamine and phosphate, and respiratory chain deficiency, among others. Mutations in mtDNA can cause defects in structural proteins, defective mitochondrial RNA synthesis and respiratory chain deficiency[30,84]. CoQ, coenzyme Q10; dNTP, deoxynucleotide triphosphate; Fe–S, iron–sulfur.

Depending on the results of biochemical and histochemical analysis, further genetic investigations will involve sequencing of specific genes, analysis of a panel of genes (for example, complex I gene panel), exome sequencing or whole-genome sequencing. A skin biopsy to isolate fibroblasts should be considered when taking a muscle sample. Cultured skin fibroblasts can sometimes recapitulate the deficiency in oxidative phosphorylation identified in skeletal muscle and can be a vital resource in assigning pathogenicity to new mutations. Once a diagnosis has been established in an index case, testing of relatives might proceed (with consent) in a much less invasive manner using buccal, urine and blood DNA samples.

Biomarkers

The conventional biomarkers used to support a diagnosis of a mitochondrial disease in clinical practice are mostly metabolic intermediates, specific enzymes or the end products of anaerobic glucose metabolism, resulting from impairment of oxidative phosphorylation[91] (BOX 3). These biomarkers include lactate, pyruvate, creatine kinase, alanine, thymidine and deoxyuridine, acylcarnitines and organic acids. In practice, these biomarkers are often not mitochondrial disease-specific, exhibit poor sensitivity and specificity, and the interpretation is not always straightforward[92]. Some biomarkers are only useful for the diagnosis of specific mitochondrial disease syndromes, for example, measurements of plasma thymidine and deoxyuridine levels in mitochondrial neurogastrointestinal encephalopathy (MNGIE) syndrome[93] (TABLE 3).

The identification of molecular signals or metabolic fingerprints of a deficiency in oxidative phosphorylation have the potential to be more-useful biomarkers for mitochondrial diseases. For example, the levels of fibroblast growth factor 21 (FGF21) have been shown to be increased in the muscle and serum of mice with mitochondrial diseases and have been reported as a useful biomarker for muscle-manifesting mitochondrial diseases[94]. Although the levels of FGF21 are also increased in a wide range of metabolic disorders, such as diabetes mellitus, obesity and metabolic syndrome, its sensitivity and specificity for mitochondrial diseases (both 92%) seems to exceed conventional biomarkers to date[94,95]. The levels of growth/differentiation factor 15 (GDF15), a member of the transforming growth factor-β superfamily, have been shown to be increased in the serum of patients with mitochondrial diseases and have also been proposed as another biomarker for these diseases[96]. The GDF15 biomarker has been shown to have a sensitivity of 98% and a specificity of 86% for mitochondrial diseases[96], but the levels of GDF15 are also increased in cardiac failure, renal insufficiency and prostate cancer. The factors that

modulate the concentrations of both GDF15 and FGF21 remain to be fully elucidated, but these biomarkers might provide important information for the diagnosis and potentially the management of mitochondrial diseases.

Other biomarkers for the detection of childhood-onset mitochondrial diseases include the detection of abnormal quantities of organic acids in urine. This can be particularly helpful for the diagnosis of numerous mitochondrial disorders, for example, 3-methylglutaconic aciduria can be caused by mutations in *TAZ*, *TMEM70* and *SERAC1*, and methylmalonic aciduria can be caused by mutations in *SUCLA2* and *SUCLG1*, which can also cause mtDNA depletion[97–99]. Hyperammonaemia is a nonspecific finding in a range of metabolic disorders, but can indicate mitochondrial disease due to mutations in *TMEM70* when associated with concomitant hypertrophic cardiomyopathy[97].

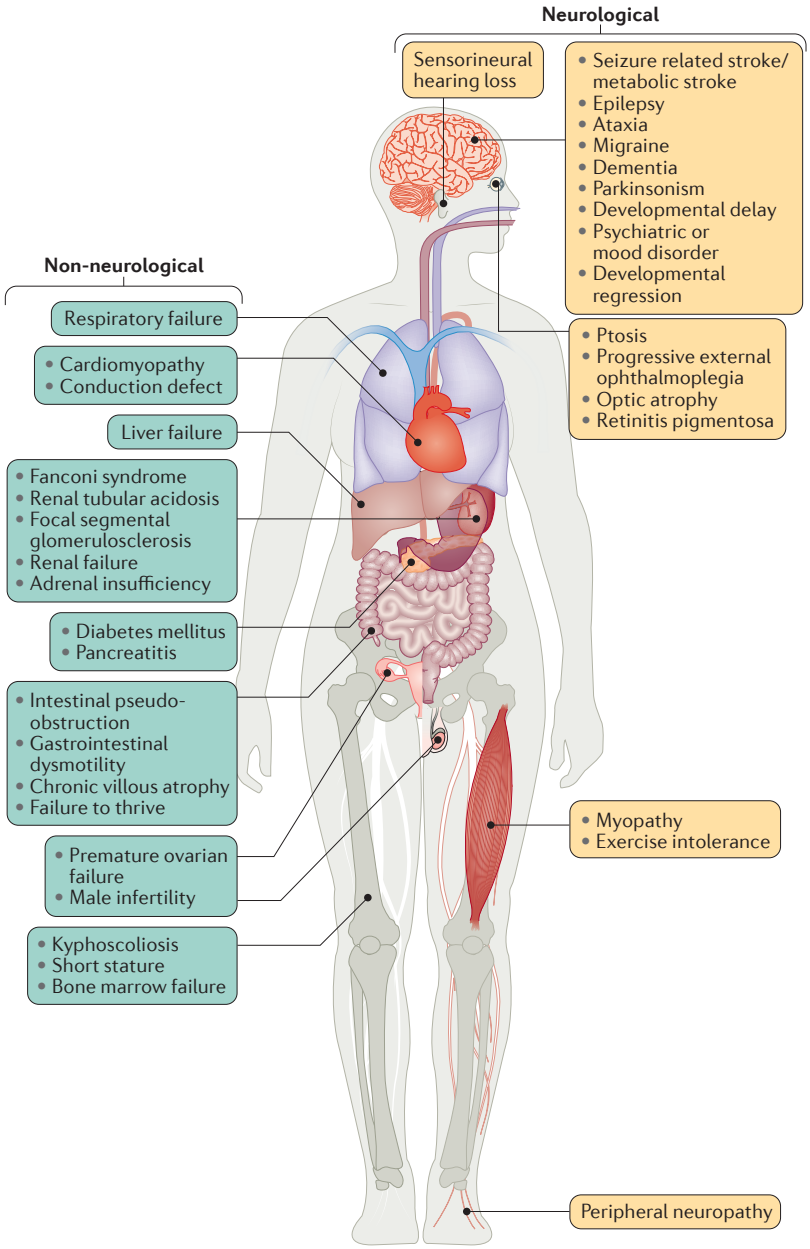

Figure 4 | **Clinical presentations of mitochondrial diseases.** The clinical presentation of mitochondrial diseases is variable between patients and can encompass dysfunction of any organ or tissue. Broadly speaking, the clinical presentations have non-neurological or neurological characteristics and usually involve multiple organ systems.

Prevention

Although it remains crucial that we focus on the development of new treatments for mitochondrial diseases — as for the vast majority of patients the condition is relentlessly progressive leading to considerable morbidity and, in those most severely affected, death — preventive strategies are also required. One of the major advantages of the vast improvement in the genetic diagnosis of mitochondrial diseases has been the opportunity to provide genetic counselling (for reproductive purposes) to families with a history of mitochondrial diseases. Patients with mitochondrial diseases caused by mutations in nDNA can consider prenatal or, in some cases, preimplantation genetic diagnosis, similar to patients with other nuclear genetic diseases. However, genetic advice is more complicated for families with mtDNA mutations. Heteroplasmy causes a genetic bottleneck during development, which can lead to offspring that can have markedly different levels of heteroplasmy than their mother (FIG. 7). Even with homoplasmic mtDNA mutations, which are transmitted to all offspring, the risk of the offspring having a mitochondrial disease can change owing to the different penetrance of the mutations. A classic example is the difference in the penetrance of mutations that cause LHON between men and women. Women with mtDNA mutations have various reproductive options. Some women might choose not to have children, not to have their own children but adopt, or use oocyte donation. Other women might opt to reproduce naturally and hope for a successful outcome, whereas others will consider prenatal diagnosis with the option of a termination. In addition, women with heteroplasmic mtDNA mutations can use preimplantation genetic diagnosis to determine the embryos with only low (or the lowest possible) level of heteroplasmy for implantation[100] (FIG. 8a). Finally, techniques that are broadly called mitochondrial donation, including pronuclear transfer, metaphase II spindle transfer and polar body transfer (FIG. 8b,c), have been developed that could potentially prevent the transmission of mtDNA mutations from mother to offspring[101,102]. These techniques have undergone extensive review and were permitted by the UK parliament in 2015, subject to individual centres obtaining a licence from the statutory regulator, the Human Fertilisation and Embryology Authority. However, no children have yet been born using these techniques and, in other countries, such as the United States, there is still debate as to the use of mitochondrial donation. Importantly, reproductive options are now available for many families with mitochondrial diseases and it is essential that these families have access to expert advice on which to make their decisions.

Mitochondrial diseases, particularly the use of mitochondrial donation to prevent disease transmission to the offspring, has raised numerous ethical issues, which have been discussed in the UK Nuffield Council on Bioethics report[103] and in another report by the US Institute

Table 3 | **Clinical syndromes, inheritance pattern and phenotypic features of mitochondrial diseases**

Syndrome	Genetics	Clinical features	Refs
Childhood-onset mitochondrial diseases			
Leigh syndrome	>75 genes in nDNA and mtDNA	Acute periods of neurodevelopmental regression followed by partial recovery, hypotonia, dystonia, hypopnoea, dysphagia, epilepsy, failure to thrive, encephalopathy, and basal ganglia and brainstem lesions	8,53, 54
Alpers–Huttenlocher syndrome (AHS)	nDNA (*POLG*-related)	Intractable epilepsy, psychomotor regression and liver disease; might also include the clinical features of MCHS and MEMSA	55–57
Childhood myocerebrohepatopathy spectrum (MCHS)		Neuropathy, ataxia, hypotonia, myoclonus (spontaneous muscle contractions), choreoathetosis (the occurrence of involuntary jerky, writhing movements of muscles or muscle groups) and Parkinsonism, in addition to renal tubulopathy	
Ataxia neuropathy spectrum (ANS; previously referred to as mitochondrial recessive ataxia syndrome (MIRAS) and sensory ataxia neuropathy dysarthria and ophthalmoplegia (SANDO))		Sensory axonal neuropathy with variable sensory and cerebellar ataxia	
Myoclonic epilepsy myopathy sensory ataxia (MEMSA; previously referred to as spinocerebellar ataxia with epilepsy (SCAE))		Epilepsy, PEO, seizures, dysarthria, dementia, spasticity and myopathy	
Sengers syndrome	nDNA (*AGK* mutations)	Congenital cataracts, hypertrophic cardiomyopathy, skeletal myopathy, exercise intolerance and lactic acidosis. Patients can present with or without mtDNA depletion in muscle	45,66
MEGDEL syndrome (also known as 3-methylglutaconic aciduria with deafness, encephalopathy and Leigh-like syndrome)	nDNA (*SERAC1* mutations)	Sensorineural hearing loss, encephalopathy, failure to thrive, hypotonia, psychomotor delay, dystonia, spasticity, hypoglycaemia, hepatopathy and lactic acidosis	65
Pearson syndrome	Single, large-scale mtDNA deletion or rearrangements of mtDNA (often sporadic)	Sideroblastic anaemia of childhood associated with exocrine and/or endocrine pancreatic dysfunction, pancytopaenia and renal tubulopathy	61,62
Congenital lactic acidosis (CLA)	nDNA, *de novo* mtDNA point mutations or inherited mtDNA point mutations	Childhood onset, often fatal, progressive neuromuscular weakness, accumulation of lactate in the blood (acidosis), urine and/or CSF	63
Adult-onset mitochondrial diseases			
Leber hereditary optic neuropathy (LHON)	Mutations in mtDNA, for example, m.11778G>A (*MT-ND4*), m.14484T>C (*MT-ND6*) and m.3460G>A (*MT-ND1*)	Subacute painless bilateral visual loss. Might also include dystonia, cardiac pre-excitation syndromes and LHON associated with multiple sclerosis-like symptoms (Harding syndrome)	69,194
Kearns–Sayre syndrome (KSS)	Single, large-scale mtDNA deletion (often sporadic)	PEO, pigmentary retinopathy (progressive vision impairment due to degeneration of the rod photoreceptors), CSF protein levels of >1 g per l, cerebellar ataxia, cardiac conduction abnormalities (age of onset <20 years), myopathy, diabetes mellitus, deafness, bulbar weakness and dementia	73,74
Mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes (MELAS) syndrome	Mutations in mtDNA, such as m.3243A>G in *MT-TL1*, and 23 other pathogenetic variants in *MT-TL1* and in other mtDNA genes (for example, *MT-TF*, *MT-TV* and *MT-TQ*)	Stroke-like episodes. Can also include deafness, diabetes mellitus, pigmented retinopathy, cardiomyopathy, cerebellar ataxia, seizures, encephalopathy, lactic acidosis and mitochondrial myopathy	31,75, 76
Myoclonic epilepsy with ragged red fibres (MERRF)	Mutations in mtDNA, such as m.8344A>G in *MT-TK*, and other pathogenetic variants in *MT-TF*, *MT-TL1*, *MT-TI* and *MT-TP*	Progressive myoclonic epilepsy, ataxia, weakness. Can also include pigmented retinopathy, sensorineural hearing loss, lactic acidosis, lipomata, spasticity and cardiac conduction defects (Wolff–Parkinson–White syndrome)	78,79
Neurogenic muscle weakness, ataxia and retinitis pigmentosa (NARP)	Mutations in mtDNA, such as *MT-ATP6* m.8993T>G, which is the most common variant, and m.8993T>C, a less severe variant	Early childhood onset, can remain quiescent or stable into adulthood, sensorimotor neuropathy, ataxia and pigmentary retinopathy. Can also include seizures, learning disability, dementia, proximal neurogenic muscle weakness, basal ganglia abnormalities, sensorineural hearing loss, short stature, CPEO, cardiac conduction defects, obstructive sleep apnoea and neuropsychiatric disorders[8]. These syndromes form a clinical continuum with Leigh syndrome	80–83

Table 3 (cont.) | **Clinical syndromes, inheritance pattern and phenotypic features of mitochondrial diseases**

Syndrome	Genetics	Clinical features	Refs
Adult-onset mitochondrial diseases (cont.)			
Chronic progressive external opthalmoplegia (CPEO)[84]	*TYMP*, which encodes thymidine phosphorylase	Gastrointestinal dysmotility, muscle weakness and atrophy, neuropathy, retinopathy, hearing loss, leukoencephalopathy and depleted TYMP activity	93
	POLG1, which encodes α-DNA polymerase subunit γ1	Ataxia, peripheral sensory neuronopathy, Parkinsonism, premature ovarian failure, psychiatric symptoms, MELAS syndrome and epilepsy	195
	POLG2, which encodes DNA polymerase subunit γ2	Ptosis and proximal myopathy, dystrophy, cerebellar ataxia and gastrointestinal symptoms	196
	MPV17, which encodes protein MPV17	Distal limb weakness, neuropathy, exercise intolerance, diabetes mellitus, ptosis, hearing loss, gastrointestinal dysmotility, depression and Parkinsonism	197
	DGUOK, which encodes deoxyguanosine kinase	Exercise intolerance, limb girdle weakness, muscle cramps and occasional dysphagia	198
	TK2, which encodes thymidine kinase 2	Ptosis, scapular winging, facial and proximal muscle weakness and wasting, dysphagia and respiratory deficiency	199
	RRM2B, which encodes ribonucleotide-diphosphate reductase subunit M2B	Hearing loss, bulbar dysfunction, renal disease and gastrointestinal disturbance	200
	C10orf2, which encodes Twinkle protein	Isolated PEO with or without mild proximal myopathy and exercise intolerance, fatigue, hearing loss, Parkinsonism psychiatric symptoms and cardiac involvement	201
	SLC25A4, which encodes ADP/ATP translocase 1	Indolent or mild PEO with or without ptosis, facial weakness, mild hearing loss, psychiatric symptoms and neuropathy	202
	SPG7, which encodes paraplegin	Ataxia, optic atrophy, myopathy, spastic paraparesis and cerebellar atrophy	203
	AFG3L2, which encodes AFG3-like protein 2	Ataxia, spastic paraparesis, myopathy and cerebellar atrophy	204
	OPA1, which encodes dynamin-like 120 kDa protein	Optic atrophy, early-onset visual loss, ataxia and deafness, spasticity, axonal neuropathy, diabetes mellitus, epilepsy and dementia	85,87
	C20orf7, which encodes mitochondrial genome maintenance exonuclease 1	Proximal muscle weakness, generalized muscle wasting, respiratory deficiency, chronic renal failure, cardiac arrhythmias and depression	24
	DNA2, which encodes DNA replication ATP-dependent helicase/nuclease DNA2	Progressive myopathy	205
	RNASEH1, which encodes ribonuclease H1	Myopathy, dysphagia and spinocerebellar signs	206
	DNM2, which encodes dynamin 2	Facial and proximal weakness, axonal neuropathy and cardiomyopathy	207
Mitochondrial neurogastro-intestinal encephalopathy (MNGIE) syndrome	*TYMP*, *RRM2B* and *POLG*	Gastrointestinal dysmotility, muscle weakness and atrophy, PEO, neuropathy, retinopathy, hearing loss, leukoencephalopathy* and depleted TYMP activity*	93, 122–124

CSF, cerebrospinal fluid; mtDNA, mitochondrial DNA; nDNA, nuclear DNA; PEO, progressive external ophthalmoplegia. *Not affected in *RRM2B* and *POLG* variants.

of Medicine[104]. Considerable challenges exist with any new *in vitro* fertilization technique. In addition, concerns have been raised that if this technique resulted in a female pregnancy, the change would result in germline transmission. However, evidence supports the safety profile of mitochondrial donation and the potential to reduce the risk of offspring developing mtDNA disease, but this technique does not guarantee the prevention of disease transmission[105]. Many individuals have argued against the legalisation of mitochondrial donation, but others argue that, if a technique is available that could prevent the transmission of mitochondrial diseases, what is the justification for not giving families the choice. As with many ethical debates in this area, the use of mitochondrial donation is likely to come down to personal choice. In the United Kingdom, the whole debate was focused on providing women with mtDNA mutations with more reproductive options.

Management

Guidance for patient management

The management of patients with mitochondrial diseases is challenging. The natural history of many subtypes of mitochondrial diseases is not known and some conditions (for example, Leigh syndrome) have a fluctuating clinical course. The frequent involvement of many

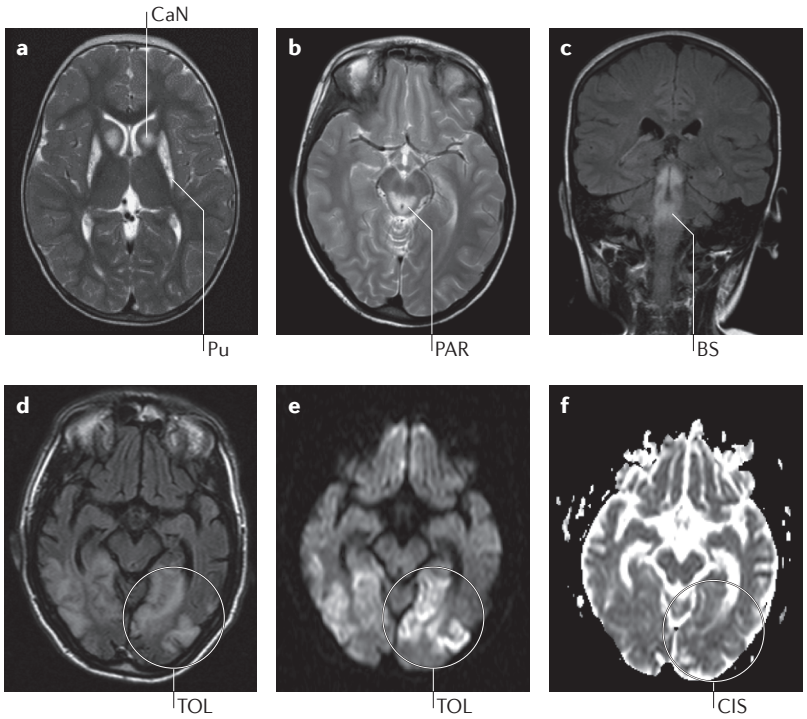

Figure 5 | **Brain MRI of patients with Leigh syndrome or mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes syndrome.** **a–c** | Axial T2-weighted and oblique coronal fluid attenuation inversion recovery (FLAIR) imaging demonstrates symmetrical hyperintensity in regions of the striatum, such as the caudate nucleus (CaN) and putamen (Pu) (part **a**), and periaqueductal (PAR; part **b**); the hyperintensity extends throughout the brainstem (BS), which is consistent with Leigh syndrome (part **c**). **d** | Axial FLAIR reveals hyperintensity in both temporo-occipital lobes (TOLs) with loss of cortico-subcortical differentiation and generalized cerebral atrophy in a patient with mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes (MELAS) syndrome. **e** | Axial diffusion weighted imaging demonstrates hyperintense lesions in both TOLs. **f** | Relatively increased and cortical iso-signal (CIS) intensity can be seen on apparent diffusion coefficient maps.

different organ systems can mean that the effective management of disease in one organ (for example, by organ transplantation) still allows for the progression of disease in other organ systems. For very severe mitochondrial diseases in childhood, management involves predominantly supportive care and the early treatment of organ-specific complications. For both children and adults with less-severe mitochondrial diseases, the overarching aim of clinical care is to reduce morbidity and mortality, by first identifying patients who are most likely to develop severe complications of the disorder and, second, the timely instigation of effective therapeutic strategies to off-set these complications.

Dietary supplements

Dietary supplements are increasingly used for the symptomatic management of patients with mitochondrial diseases, as they are readily available and their use negates the need for pharmaceutical prescription and dispensing. However, efficacy and safety evidence for the use of most dietary supplements are lacking[106]. Current dosing practices often greatly exceed the recommended dietary reference intakes and, although manufacturers are obligated to adhere to good manufacturing standards, no requirements exist to neither confirm efficacy nor undertake stringent post-marketing reviews. Moreover, dietary supplements are often consumed as multiple or compounded products, further heightening the difficulties in confirming efficacy or discerning potential compound–compound or compound–drug interactions[107]. Dietary supplements that are frequently used by patients with mitochondrial diseases include antioxidants[108] (such as CoQ[109–112], α-lipoic acid, vitamin C and vitamin E), agents to modulate mitochondrial electron transfer flux (such as vitamin B2 (also known as riboflavin)[113] and CoQ), nitric acid precursors (such as L-arginine and L-citrulline), energy buffers (such as creatine)[109,114], drugs involved in fatty acid uptake (such as L-carnitine) and mitochondrial biogenesis (such as vitamin B3). L-Arginine might provide a clinical benefit in MELAS syndrome by a reduction in the frequency and severity of stroke-like episodes, but, thus far, only an open-label study has been published[115–117]. The Office of Dietary Supplements (US NIH) in collaboration with leading mitochondrial specialists are striving to address these shortcomings of dietary supplements and are endeavouring to provide evidence for their use in patients with mitochondrial diseases[107].

Organ-specific interventions

Owing to the often multi-system nature of mitochondrial diseases, organ-specific guidelines are important in the daily care of patients, including advice on the management of diabetes, ptosis, stroke-like episodes and cardiac, respiratory, gastrointestinal and renal involvement. Other advice to guide clinical decision making in patients with mitochondrial diseases and other health issues that can affect their underlying mitochondrial disease (such as pregnancy, anaesthesia, surgery and vaccination) are now increasingly recognized as paramount. The identification of the underlying genetic defect allows specific advice to be given to individual patients, which can have major implications for clinical care. For example, patients with certain mitochondrial diseases are at risk of cardiac arrhythmias leading to sudden death[118], but early therapeutic intervention with cardiac pacemakers or ablation of secondary conduction pathways will greatly reduce the incidence of such complications. Detailed expert opinion guidelines that cover many of these pertinent issues are beyond the remit of this Primer, but can be found in REF. 119.

Targeted treatments

Scavenging toxic compounds. Pharmacological strategies have been implemented to modify the course of certain mitochondrial diseases that are caused by metabolic blocks, leading to toxic substance accumulation. *N*-acetylcysteine and metronidazole treatment can be used to reduce the high levels of hydrogen sulfide that occur in ethylmalonic encephalopathy[120]. Ethylmalonic encephalopathy is a devastating, autosomal recessive childhood-onset mitochondrial disease due to mutations in *ETHE1* (REF. 121). ETHE1 is a mitochondrial sulfur dioxygenase, which detoxifies hydrogen sulfide. Hydrogen sulfide can inhibit the terminal segment of fatty acid β-oxidation as well as complex IV, and directly damages

the endothelial lining of small vessels. *N*-acetylcysteine acts as an intracellular buffer for hydrogen sulfide, and metronidazole is an antibiotic that specifically targets hydrogen sulfide-producing anaerobic bacteria and protozoa in the microbiota — an important source of hydrogen sulfide. Co-administration of *N*-acetylcysteine and metronidazole has been shown to significantly prolong the lifespan of *Ethe1*$^{-/-}$ mice and mitigate some of the characteristic clinical disease features of ethylmalonic encephalopathy, including chronic diarrhoea and diffuse microvasculopathy with acrocyanosis in patients[120,121]. Treatment also improved alertness and wakefulness and decreased the frequency and duration of epileptic seizures[120]. This therapy has now become standard care in the palliative regimen of ethylmalonic encephalopathy.

Enzyme replacement therapy. Enzyme replacement therapy has been successfully applied to multiple metabolic diseases, including MNGIE syndrome, the first mitochondrial disease to be treated by this approach. MNGIE syndrome is caused by loss-of-function mutations in *TYMP*, which encodes thymidine phosphorylase[93,122] (TABLE 3). Erythrocyte-encapsulated thymidine phosphorylase has shown preliminary biochemical and clinical success in one patient under a research protocol[123].

Allogenic haematopoietic stem cell transplantation. Allogeneic haematopoietic stem cell transplantation to restore thymidine phosphorylase activity has effectively decreased the levels of thymidine and deoxyuridine in the blood and led to symptomatic improvements in patients with MNGIE syndrome. However, this is burdened by a >50% post-graft mortality due to the required immunosuppression, which can further damage the mitochondria, in addition to the poor clinical conditions of the recipient patients[124].

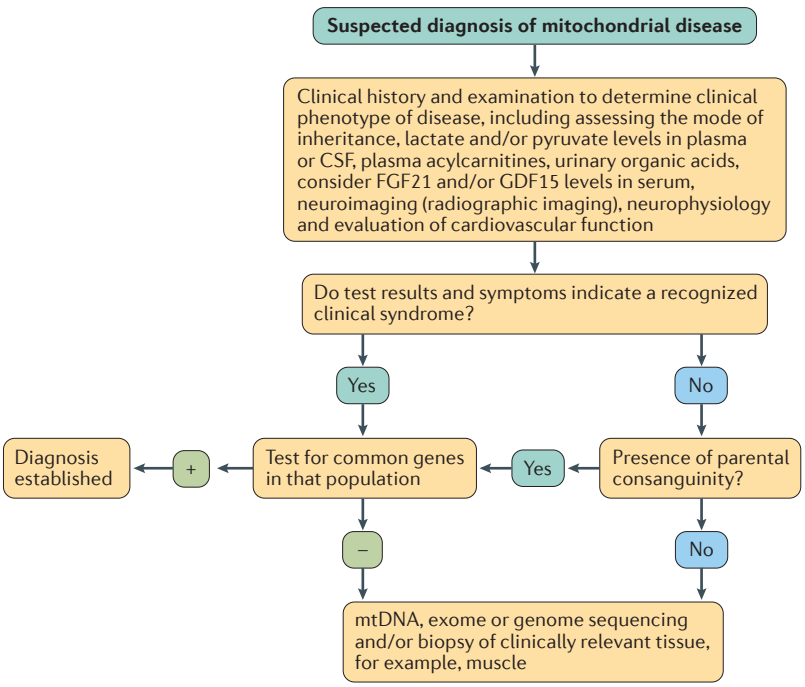

Figure 6 | **Diagnostic algorithm for suspected mitochondrial diseases.** Diagnosis of mitochondrial diseases depends on patient history, clinical symptoms, and laboratory and genetic tests. –, negative; +, positive; CSF, cerebrospinal fluid; FGF21, fibroblast growth factor 21; GDF15, growth/differentiation factor 15; mtDNA, mitochondrial DNA.

Molecular bypass therapy. In some mitochondrial diseases caused by enzyme deficiency, biochemical homeostasis can be restored by administration of the deficient enzyme product, for example, the use of CoQ supplementation in primary CoQ deficiency. Primary CoQ deficiency encompasses a group of genetic defects of the CoQ biosynthetic pathway, which manifest with variable phenotypes, including childhood-onset mitochondrial diseases, encephalonephropathies with nephrotic syndrome and isolated cerebellar ataxia[125,126]. Although improvement of symptoms to CoQ supplementation has been variable, perhaps owing to poor bioavailability of this highly lipophilic molecule, CoQ supplementation is routinely used by patients with mitochondrial diseases[125,126].

However, promising newer agents are under investigation. In a randomized, placebo-controlled trial, idebenone, a CoQ analogue and cofactor for NADPH dehydrogenase, was shown to preserve vision in patients with LHON and discordant vision at baseline and, in the open-label follow-up study, preservation of vision persisted for >2 years[127,128]. Based on these findings, the European Commission has granted marketing authorization for the use of idebenone in patients with LHON. Other novel antioxidants that are being tested in clinical trials include EPI-743 (which is hypothesized to augment glutathione levels) and cysteamine bitartrate (also known as RP103; which converts intralysosomal cystine into cysteine–cysteamine disulfide) for Leigh syndrome[129,130] (TABLE 4).

Cofactor therapy. Vitamin B2 is the central component of cofactors flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN). Supplementation with high doses of vitamin B2 has been shown to improve symptoms in mitochondrial diseases caused by genes encoding FMN-dependent or FAD-dependent proteins, such as NADH dehydrogenase flavoprotein 1 (NDUFV1; the FMN-binding subunit of complex I), apoptosis-inducing factor 1, mitochondrial (AIFM1), acyl-CoA dehydrogenase family member 9, mitochondrial (ACAD9) and succinate dehydrogenase flavoprotein subunit, mitochondrial (SDHA)[131–133], presumably by improving the function of residual enzyme activity.

Quality of life

Health-related quality of life (HRQOL) is a multifaceted index of well-being, exemplifying the subjective effect of disease on all aspects of an individual's daily living, including physical and mental well-being[134]. Studies evaluating the effect of mitochondrial diseases on patient HRQOL are, to date, surprisingly limited. Those few studies that have been performed speculate that disease burden affects HRQOL indices to varying degrees, suggesting that other factors also have a role[135]. Chronic diseases do not only affect the well-being of the patient but also affect their relatives and wider social support network.

The effect on caregivers, who include the mother and siblings who might also have mitochondrial diseases, is perhaps one of the least studied aspects of mitochondrial diseases. Moreover, studies have almost exclusively focused on parental caregivers of children with mitochondrial diseases rather than on those providing care for adults with mitochondrial diseases[136–142]. Higher rates of psychopathology[136], comorbid anxiety and depressive symptoms have been reported in the caregivers of children with mitochondrial diseases[137–139]. In addition, a significantly greater caregiver burden[139] and socioeconomic and psycho-affective strain have been noted for caregivers of those with mitochondrial diseases than caregivers of children with other chronic neurological and metabolic disorders[136]. Yet, the effect of a chronic illness does not always have to be negative, with clarity about life's meaning and personal growth cited as potential positive strengths to help devise coping strategies[143].

Little is known about the economic effect of mitochondrial diseases on the patient, their family and society in general. The use of mitochondrial donation has the potential to reduce some of the disease burden and might also have an economic effect. A pilot study has estimated the net benefit of the implementation of mitochondrial donation in the first year of policy, scaled to 20 individuals per year, as GBP£32 million[144]. Although further cost–benefit analysis needs to be conducted on a larger scale, these preliminary findings hold substantial implications for the planning of future services, informing financial support schemes for patients and their families and evaluating mitochondrial donation and other therapeutic intervention strategies.

Box 3 | **Overview of the diagnostic tests used for mitochondrial diseases**

Common clinical symptoms are, for example, optic atrophy, chronic progressive external ophthalmoplegia and neurodevelopmental regression. Diagnosis of mitochondrial diseases depends on the age of the patient and the clinical manifestation of disease. As such, patients will require different diagnostic work-ups, which can include combinations of the following laboratory tests:

- Standard blood tests: blood cell count, blood urea nitrogen and liver blood tests
- Metabolites: glucose, lactate, pyruvate, creatine kinase, alanine, thymidine and deoxyuridine, acylcarnitines and organic acids
- Muscle biopsies
  - General morphology
  - Histochemical analysis, for example, for cytochrome *c* oxidase and succinate dehydrogenase (COX/SDH) assessment to detect respiratory chain deficiency and for ragged red fibres (clumps of affected mitochondria that accumulate in the subsarcolemmal region of the muscle fibre and stained red following modified Gömöri trichrome staining)
  - Specific immununohistochemistry to detect the abundance of complexes of the respiratory chain, including quadruple immunofluorescence
  - Biochemical quantification of coenzyme Q10 levels
  - Photometric analysis of respiratory chain activity
  - Measurement of oxygen uptake to determine oxidative phosphorylation activity
- Skin biopsy: fibroblast culture to confirm changes in oxidative phosphorylation activity
- Molecular genetics
  - Nuclear DNA (nDNA): whole-genome sequencing and exome sequencing on nDNA and targeted gene sequencing for common phenotypes, such as Leigh syndrome
  - Mitochondrial DNA (mtDNA): real-time PCR to detect mtDNA depletion, long-range PCR to detect mtDNA deletions and mtDNA sequencing or specific point mutation assays to detect point mutations; mtDNA can be isolated from clinically relevant tissue

Outlook

Remarkable progress in mitochondrial diseases has been achieved over the past decade with several scientific advances directly affecting patient care. However, several interesting and challenging aspects of mitochondrial diseases remain that will continue to be a major focus of research.

New mechanisms of disease

The often-quoted dictum, 'mitochondrial diseases manifest at any age and in any tissue system' is undoubtedly true considering the spectrum of mitochondrial diseases, but specific entities of disease are likely to manifest at specific ages and tissues in a manner that cannot be explained entirely on the basis of ATP deficiency. Establishing the molecular mechanisms that are responsible for the exceptional variability and tissue specificity of disease manifestations remains challenging.

Next-generation sequencing[145] has enabled the identification of the underlying genetic defects in an increasing number of patients with mitochondrial diseases. For the first time, true comparison of patient genotypes and phenotypes in large international collaborative data repositories has become possible (for example, Mitochondrial Disease Sequence Data Resource)[146]. These data repositories are a prerequisite for generating understanding of the natural history of different disorders. Furthermore, these data are invaluable for international collection of patients for clinical trials, as the individual disease entities are limited in patient number. However, it has become apparent that genetic defects causing mitochondrial diseases do not explain why defects of proteins with very similar tasks, or the same mutation in a single gene, can lead to a multitude of non-overlapping clinical phenotypes.

Interestingly, detailed knowledge of the pathophysiology of primary mitochondrial diseases might be relevant for common disorders with secondary mitochondrial dysfunction. This includes disorders such as Parkinson disease[147], inclusion body myositis[148] and even obesity[149].

Tissue specificity. Mitochondrial diseases that affect mtDNA replication or nucleotide metabolism have provided important insights into why tissue-specific phenotypes arise. These disorders show three main phenotypes: onset in early childhood with focal epilepsy, encephalopathy and liver failure; juvenile disorders that manifest with severe acute-onset epilepsy; and adult-onset disorders that most often affect muscle[150]. These clinical manifestations indicate that the type of tissue determines the susceptibility for mtDNA maintenance defects and the susceptibility of a given tissue for specific defects changes with the age of the patient. As mtDNA maintenance is required in all tissues, the finding that defects in mtDNA maintenance typically present in post-mitotic tissues and not in cell types that undergo constant cell division and DNA synthesis (such as the haematopoietic system, gut or skin) is surprising. The intracellular dNTP levels are highly asymmetric and levels are up to 1,000-fold lower in post-mitotic tissues than in proliferating cells, suggesting that high levels of dNTP in dividing cells could

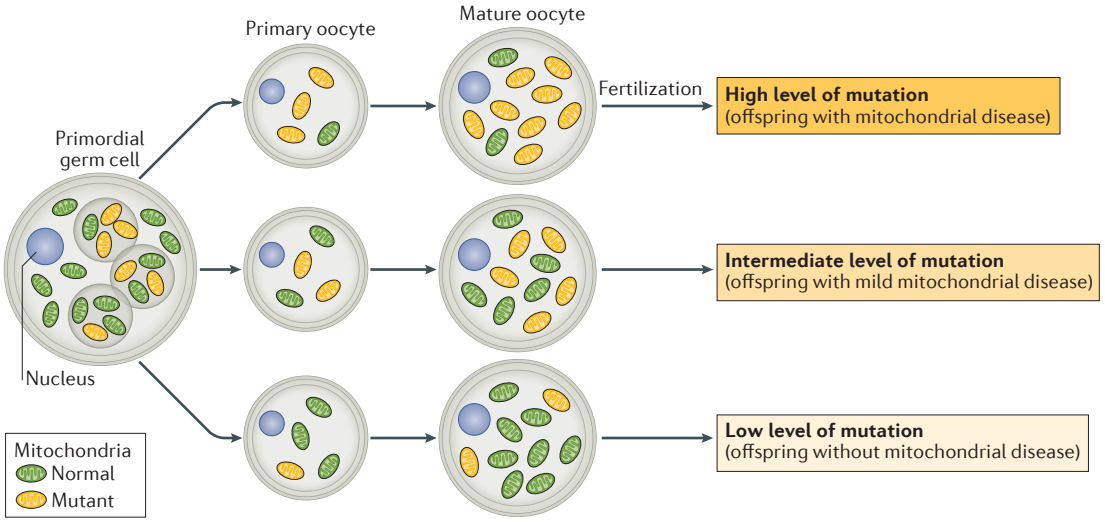

Figure 7 | **Heteroplasmy causes mitochondrial bottleneck during oogenesis.** The transmission of heteroplasmic mitochondrial DNA (mtDNA) mutations from mother to offspring is complicated by the genetic bottleneck during development. This bottleneck occurs owing to a profound dilution of mtDNA during the formation of the primordial germ cell, followed potentially by selective replication of mtDNA genomes. This can lead to profoundly different levels of heteroplasmy in different mature oocytes of women with heteroplasmic mtDNA mutations and is an important consideration when counselling mothers with heteroplasmic mtDNA mutations about the risks of having offspring with mitochondrial diseases. Adapted from REF. 188, Nature Publishing Group.

compensate for the functional defects of mtDNA replication enzymes. Cell-type-specific availability of dNTPs — or single cofactors or metabolites — could contribute to tissue-specific manifestations of disease[151–153]. Indeed, the first promising results have already been obtained by nucleotide supplementation for mtDNA depletion[153].

The importance of mitochondrial translation defects in the pathogenesis of mitochondrial diseases has become increasingly clear, with next-generation sequencing methodology revealing mutations in nuclear-encoded mitoribosomal proteins and translation factors, in addition to mtDNA-encoded tRNAs and rRNAs[145]. This group of disorders shows the largest variability of phenotypes, especially in mitochondrial diseases caused by mutations in genes encoding aminoacyl-tRNA synthetases and mitochondrial tRNA[154]. The reasons for the variability in clinical phenotype are unknown, as the only established function of these proteins is to support the synthesis of proteins encoded by the 13 mtDNA-encoded oxidative phosphorylation subunits. What has become clear is that severe defects of mtDNA translation manifest as perinatal or early-infantile rapidly progressive, hypertrophic cardiomyopathies, indicating the absolute requirement of functional mitochondrial translation machinery immediately before and after birth. Few patients survive this severe heart disease, but of those who do, most show spontaneous stabilization of the heart condition around 6 years of age, but can later develop brain disorders. These observations indicate that cardiac metabolism undergoes postnatal metabolic maturation and that compensatory mechanisms exist to support heart function even with poorly functioning mitochondrial translation machinery. Understanding the compensatory mechanisms that enable complementation of the metabolic defect could reveal clues for new treatments.

View beyond the organelle. The mitochondrial disease field still mostly focuses on studying the consequences of specific mitochondrial defects for the respiratory chain and ATP synthesis, for example, nutrient catabolism, inside the organelle. However, mitochondria have major anabolic functions and fine-tune nutrient signalling in the whole cell through cofactors (such as NAD^+/NADH, NADP/NADPH, oxygen radicals, AMP/ATP and calcium)[155]. The Krebs cycle and mitochondrial folate cycle provide metabolites and cofactors for the main cellular anabolic biosynthetic pathways, contributing to, for example, cellular dNTP metabolism, glutathione biosynthesis and methylation reactions (such as creatine and phospholipid synthesis and genome methylation)[156]. The biosynthetic pathways are highly cell-type dependent, so might affect specific cells in different ways. For example, γ-aminobutyric acid (GABA)ergic neurons and erythrocyte precursors both use Krebs cycle metabolites for biosynthetic reactions, that is, for synthesis of GABA and haem, respectively. These Krebs cycle metabolites are not limiting for fibroblasts, as similar large-scale production of GABA or haem does not occur in these cells, making fibroblasts less vulnerable to deficiencies in these metabolites. Whether mitochondrial dysfunction modifies the balance between anabolic biosynthesis and catabolic energy metabolism is an intriguing possibility and offers potential mechanisms for the tissue specificity of disease and paves the way for new supplementation strategies.

Normal tissue metabolism generates inhibitors of respiration, which introduces a potential metabolic feedback mechanism to balance mitochondrial respiration and biosynthetic reactions. Decreased flux or breakdown of these substances can cause mitochondrial dysfunction and disease[121]. Such feedback systems orchestrated directly by metabolite flux might be a parallel regulatory

mechanism to transcriptional and post-translational regulation, but are still relatively uncharacterized. For example, hydrogen sulfide produced by cysteine metabolism is a potent inhibitor of complex IV[121]. In addition, formate, the product of the mitochondrial folate cycle, is a strong inhibitor of mitochondrial respiration[157].

Dietary interventions and supplements. Metabolic insults, for example, nutritional status and exercise, have been shown to initiate not only local pathology but also variable cell non-autonomous responses. These responses are mediated by metabolites, metabolic cytokines and hormone-like molecules, which have the

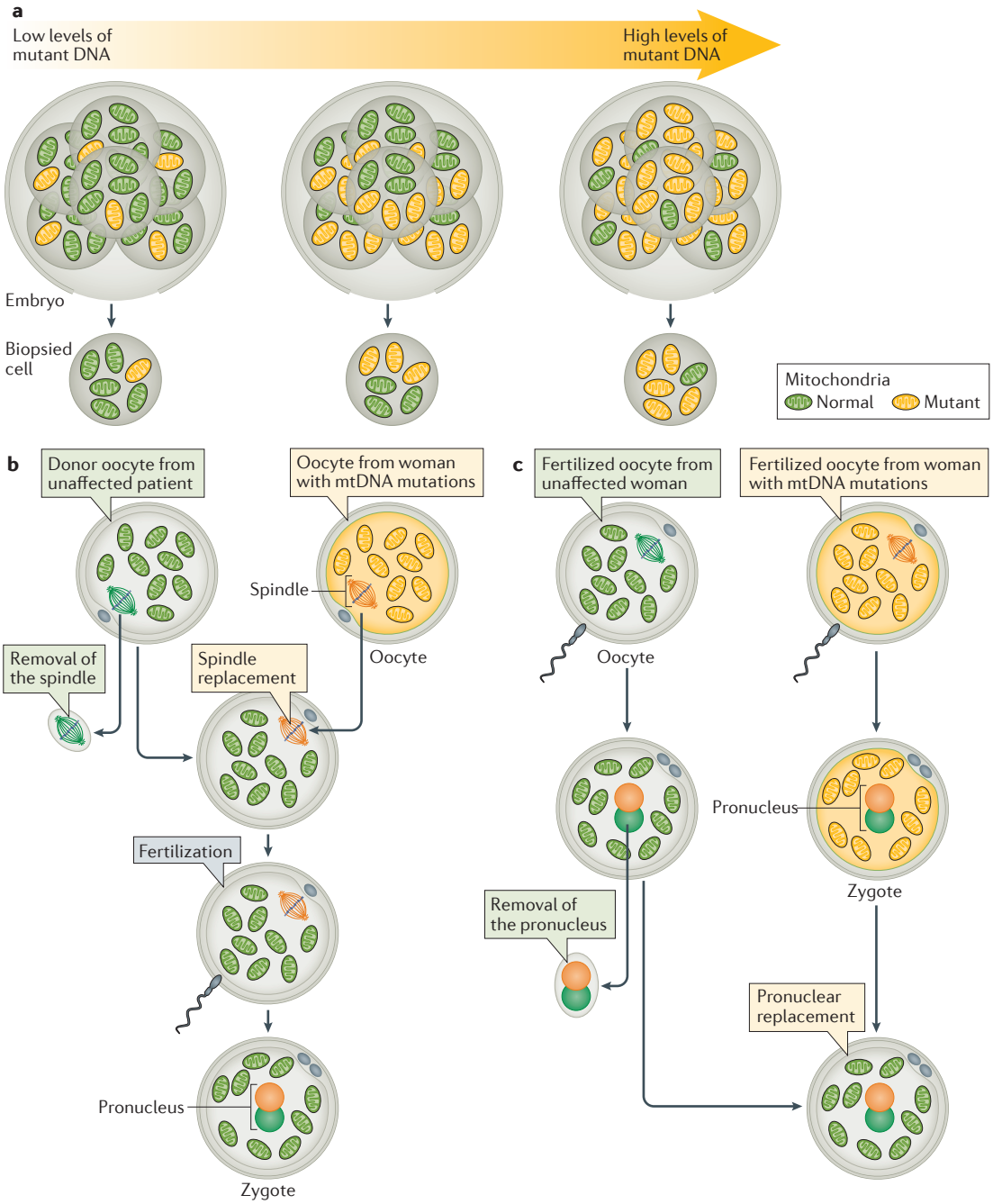

Figure 8 | **Reproductive options for women with mitochondrial DNA mutations. a** | Preimplantation genetic diagnosis involves patients undergoing *in vitro* fertilization and all oocytes being fertilized. Developing embryos are then biopsied (either individual blastomeres or at the blastocyst stage). The high-quality embryos with the lowest level of mutated mitochondrial DNA (mtDNA) are then used for implantation. **b**,**c** | Mitochondrial donation involves the transfer of the nuclear DNA (nDNA) from an oocyte or zygote from a woman with a pathogenetic mtDNA mutation into an enucleated, recipient donor oocyte or zygote. In these techniques, mtDNA is from the donor. Metaphase II spindle transfer involves the removal of the spindle from the donor egg and transfer of the patient spindle into the donor oocyte followed by fertilization and development (part **b**). Pronuclear transfer occurs after fertilization and the pronuclei are transferred from the patient zygote to the donor zygote (part **c**). Adapted with permission from REF. 30, AAAS.

potential to modify disease progression and, as such, are potentially relevant for therapy[158]. To date, the use of high-fat or ketogenic diets in children with complex I defects (to attempt to bypass the defect by channelling more electrons into the respiratory chain via electron-transferring flavoprotein dehydrogenase-CoQ reductase) has shown promise. The use of the ketogenic diet has shown efficacy in improving symptoms in mouse models[159,160] and in children with mitochondrial diseases that manifest with epilepsy[161], but further work is still needed. Existing knowledge from primary vitamin deficiencies, such as folate deficiency, which closely mimic infantile mitochondrial epileptic encephalopathies, indicates the need for early intervention to prevent permanent tissue damage[162]. Thus, first-line genomic analyses will be increasingly important in routine evaluation of patients with mitochondrial diseases, to enable tailored early-stage therapeutic interventions.

Technological future: prevention of disease

The high conservation of mitochondrial proteins between species offers a wide choice of different disease models (ranging from mice to worms to yeast) to study the consequences of human mutations. New gene editing technologies[163] allow fast development of disease models, even in large animals such as pigs, whose physiology is closer to humans. Traditional knockout animal models are not always optimal because most disease-associated mutant proteins show residual activity, and a complete excision of an essential mitochondrial protein will target the selected cell type for death. Thus, models with mutations that have been identified in patients (for example, point mutations) and the use of their tissue materials for various 'hypothesis-generating' analyses — namely, genomic, transcriptomic, proteomic and metabolomic analyses — are essential to uncover pathogenetic routes. Furthermore, protein modelling, including the use of

Table 4 | **Current clinical trials in mitochondrial diseases**

Intervention	Mechanism of action	Syndrome	Status	Clinical trials registry identifier
MTP-131	Small peptide that stabilizes cardiolipin	Mitochondrial myopathy	Phase I/II randomized, double-blind, placebo-controlled, multiple ascending-dose clinical study	NCT02367014
Cysteamine bitartrate delayed-release	Cystine-depleting agent	Childhood mitochondrial diseases	Phase II/III open-label, dose-escalation and long-term extension	NCT02023866 and NCT02473445
EPI-743	NADP dehydrogenase modulator	Leigh syndrome	Phase IIb randomized, placebo-controlled	NCT01721733
EPI-743	NADP dehydrogenase modulator	Children (2–11 years of age) with mitochondrial or metabolic diseases	Phase II randomized, placebo-controlled cross-over	NCT01642056
5 ALA and SFC	NADP dehydrogenase modulator	Mitochondrial diseases, mainly to cranial nerve symptoms	Phase II randomized, placebo-controlled	JMA-IIA00200
RTA 408	Activation of nuclear factor erythroid 2-related factor 2 and inhibitor of nuclear factor-κB	Mitochondrial myopathy	Phase II randomized, placebo-controlled, double-blind, dose-escalation, and safety, efficacy and pharmacodynamics	NCT02255422
Bezafibrate	Peroxisome proliferator-activated receptor agonist to enhance mitochondrial biogenesis	Mitochondrial diseases	Phase II, open-label, non-randomized feasibility study	NCT02398201
Allogenic haematopoietic stem cell transplantation	Enhance thymidine phosphorylase activity	Mitochondrial neurogastrointestinal encephalomyopathy	Phase I	NCT02427178
GS010	AAV–*MT-ND4* gene therapy	Leber hereditary optic neuropathy	Phase I	NCT02064569
L-Arginine (intravenous)	Nitric oxide donor for endothelial dysfunction	MELAS acute phase of stroke-like episodes	Phase II/III open-label, 2-year study	JMA-IIA00023
L-Arginine (oral)	Nitric oxide donor for endothelial dysfunction	MELAS interictal phase to prevent stroke-like episodes	Phase II/III open-label, 2-year study	JMA-IIA00025
Arginine and citrulline	Nitric oxide donor	MELAS syndrome	Phase II open-label	NCT01339494
Taurine	Taurine modification	MELAS syndrome	Phase II/III open-label	UMIN000011908
Pyruvate	NAD donor	MELAS and MELA syndromes	Phase II randomized, placebo-controlled	JMA-IIA00093
Idebenone	Synthetic analogue of coenzyme Q10, antioxidant	MELAS syndromes	Phase IIa double-blind, randomized, placebo-controlled	NCT00887562

Data sourced from NCT (ClinicalTrials.gov), JMA (Japan Medical Association Center for Clinical Trials) and UMIN (University Hospital Medical Information Network) (accessed 22 May 2016). AAV, adeno-associated virus; MELA, mitochondrial encephalomyopathy, lactic acidosis; MELAS, mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes.

crystal structures, nuclear magnetic resonance as well as atomic-level molecular dynamics (computer simulations of protein functions), for wild-type and mutated proteins might reveal molecular clues of disease mechanisms. New mitochondrial transfer methodologies provide a possibility to shift heteroplasmy levels in the oocytes of women carrying mtDNA mutations. These early treatments will complement preimplantation genetic diagnosis and enable women with high levels of mutant mtDNA to have offspring. How quickly these techniques of early zygote or oocyte manipulation spread into practice of specialized clinics remains to be seen and depends on approval.

New treatment approaches

Although therapies for specific mitochondrial diseases, such as MNGIE syndrome and ethylmalonic encephalopathy, are emerging, treatment for the vast majority of mitochondrial disorders is limited. Cocktails of nutritional supplements and vitamins have been widely used to treat mitochondrial diseases, but the available sparse evidence suggests that these treatments have modest beneficial effects[107,109,164]. More encouraging data are from studies of aerobic exercise training, which have demonstrated improvements in exercise capacity and quality of life in patients with mtDNA mutations due to the reversal of the effects of sedentary behaviour[165,166]. Nevertheless, given the paucity of effective therapies, new treatment approaches are clearly needed[164] and fortunately are being developed.

Mitochondrial proliferation. Although mitochondrial proliferation is a compensatory attempt to enhance mitochondrial biogenesis[167], it might be possible to improve on this strategy through activation of peroxisome proliferator-activated receptor (PPAR), AMP-activated protein kinase (AMPK) or PPARγ co-activator 1α (PGC1α)-dependent pathways. Although mitochondrial disease-induced mitochondrial proliferation favours mutated mtDNAs (which can be observed as ragged red fibres in skeletal muscle), pharmacological upregulation of mitochondrial biosynthesis increases the number of both mutated and wild-type mtDNAs, which allows wild-type genomes to compensate for mutant genomes. Improved phenotypes have been obtained in mice with complex IV-deficient myopathy and mtDNA maintenance myopathy using a PPAR agonist (bezafibrate)[168,169] or an AMPK agonist (5-aminoimidazole-4-carboxamide ribonucleotide (AICAR)), although in some models, bezafibrate has proven to be ineffective[170]. Nicotinamide riboside, a vitamin B3 precursor, and poly(ADP-ribose) polymerases have been shown to be beneficial in mouse models of mitochondrial diseases by boosting the levels of NAD^+, which activates NAD-dependent protein deacetylase sirtuin 1-mediated mitochondrial biogenesis[171–173].

Mitophagy. Targeting the autophagic elimination of defective mitochondria (known as mitophagy) offers yet another approach to shifting the levels of heteroplasmic mtDNA mutations[174]. Increased mitophagy via the mechanistic target of rapamycin complex (mTORC) inhibitor rapamycin is a promising approach, although in the *Ndufs4*$^{-/-}$ (encoding NADH dehydrogenase iron–sulfur protein 4, mitochondrial) mouse (which lacks the NDUFS4 subunit of complex I and can be used to model Leigh syndrome), rapamycin treatment ameliorated neurodegeneration and enhanced survival, without enhancing complex I activity, indicating unknown alternative mechanisms of action[175].

Hypoxia. Another interesting and surprising, promising therapeutic approach has come from a study testing a large-scale CRISPR–Cas9-mediated whole-genome knockout screen[176]. The most effective genetic strategy to ameliorate mitochondrial diseases was the inhibition of the von Hippel–Lindau disease tumour suppressor, a key regulator in the hypoxia response pathway. Subsequent experiments have shown that a chronic mildly hypoxic environment (consisting of 11% oxygen) improved the lifespan of the *Ndufs4*$^{-/-}$ mouse. Moreover, several indicators of disease progression (such as maintenance of body weight and temperature) were improved, along with a dramatic restoration of locomotor function. Whether these experiments will lead to new therapeutic approaches remains uncertain but highlights the different experimental approaches that are being used to investigate new therapies for the treatment of mitochondrial diseases.

Molecular bypass. For mtDNA depletion due to thymidine kinase 2, mitochondrial (TK2) deficiency, which, as previously stated, causes an imbalance in the dNTP pool, promising results have been obtained in *Tk2*-mutant mice (*Tk2* p.H122N knock-in mice) treated with a pharmacological molecular bypass therapy using deoxynucleotide thymidine and cytidine monophosphates (dTMP and dCMP)[177]. Clear dose-dependent improvements of the biochemical and clinical phenotypes of these mice and a twofold to threefold extension of lifespan were observed following treatment with dNTPs compared with untreated mutant mice. Extremely promising results of dTMP and dCMP therapy have emerged from the treatment of severely affected TK2-deficient children, who have shown amelioration of their devastating neuromuscular disorder (M.H. *et al.*, unpublished observations).

Gene therapy approaches. Besides the growing number of pharmacological agents that are being lauded as antioxidants and respiratory chain boosters, several gene therapies, including tissue-specific adeno-associated virus (AAV)-mediated gene therapy, are being assessed for the treatment of mitochondrial diseases in animals and patients. Mitochondrially targeted zinc-finger nucleases and transcription activator-like effector nucleases (mito-TALENS) that digest specific mtDNA mutations have been shown to reduce the levels of mutated mtDNA *in vitro*[178,179] and have prevented the transmission of mtDNA haplotypes in mice[180]. Perhaps even more promising, mito-TALENS have been effective in reducing heteroplasmy levels in mammalian oocytes[180]. This approach to germline heteroplasmy shift might be an effective alternative to mitochondrial replacement techniques to prevent the maternal transmission of mitochondrial diseases caused by mtDNA defects.

The correction of a mutation by expressing the wild-type gene in crucial organs has long been envisaged as the definitive cure for numerous genetic diseases. The introduction of AAV vectors has provided new potency to gene therapy. AAVs belong to the *Parvoviridae* family, are not associated with any disease in humans or animals and remain episomal in the cells for a prolonged time, which reduces the risk of insertional mutagenesis. Several serotypes of AAV, with different cellular specificities, have been selected, allowing specific targeting of several organs and tissues.

In the context of mitochondrial disease models, a recombinant construct expressing human *ETHE1*^{wt} was targeted to the liver using a hepatotropic AAV2/8 serotype[181]. When injected into 3-week-old *Ethe1*$^{-/-}$ mice, ETHE1-associated sulfur dioxygenase activity was completely recovered in the liver, leading to efficient clearance of hydrogen sulfide from the bloodstream. In addition, this treatment was associated with significant rescue of the profound complex IV deficiency, correction of the main ethylmalonic encephalopathy biomarkers, remarkable clinical improvement and prolongation of the lifespan, from a few weeks in untreated animals to >8 months in AAV-treated littermates. The liver-specific AAV2/8 vector, which contains the *TYMP* transgene, has been injected into the thymidine phosphorylase and uridine phosphorylase double knockout (*Tymp*$^{-/-}$/*Upp*$^{-/-}$) mouse model of MNGIE syndrome[182]. Although *Tymp*$^{-/-}$/*Upp*$^{-/-}$ mice show minimal clinical signs of disease, they have markedly abnormal dNTP pools, similar to patients with MNGIE syndrome[183]. Intravenous injection of AAV2/8 normalized the levels of deoxycytidine triphosphate and deoxythymidine triphosphate in the plasma and tissues (such as the liver, skeletal muscle and brain) of these mice for up to 8 months of age. This encouraging proof-of-principle result supports the application of the AAV2/8 treatment to treat patients with MNGIE syndrome. Both these observations in animal models suggest that similar therapeutic effects might be obtained by liver transplantation, a procedure that is already approved for the treatment of numerous metabolic disorders. In one patient with ethylmalonic encephalopathy, liver transplantation (in which the liver was donated by the mother) has successfully been performed, with dramatic amelioration of the biomarker indexes of disease and a clear improvement in neurological as well as non-neurological symptoms over a >8-month period. However, longer-term follow-up is required. Two patients with MNGIE syndrome[184] have also undergone cadaveric liver transplantation, again with normalization of plasma biomarkers, dramatic amelioration of the gastrointestinal symptoms of disease and a slight improvement of the body mass index and peripheral neuropathy, over a follow-up period of a few months (M.H., unpublished observations). Summary of the advantages and disadvantages of liver transplantation for mtDNA depletion syndromes can be found elsewhere[185].

Clinical trials. Although many of the novel therapeutic approaches for mitochondrial diseases are still being developed preclinically, several therapies are already under investigation in active clinical trials[186] (TABLE 4). Of practical importance, pharmaceutical companies have initiated clinical trials in partnership with academic institutions, thereby accelerating the development of new therapies for mitochondrial diseases. Indeed, the future of mitochondrial therapy looks brighter than ever.

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Competing interests
The authors declare no competing interests.