Mitochondrial disease in adults: recent advances and future promise

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Mitochondrial diseases are some of the most common inherited neurometabolic disorders, and major progress has been made in our understanding, diagnosis, and treatment of these conditions in the past 5 years. Development of national mitochondrial disease cohorts and international collaborations has changed our knowledge of the spectrum of clinical phenotypes and natural history of mitochondrial diseases. Advances in high-throughput sequencing technologies have altered the diagnostic algorithm for mitochondrial diseases by increasingly using a genetics-first approach, with more than 350 disease-causing genes identified to date. While the current management strategy for mitochondrial disease focuses on surveillance for multisystem involvement and effective symptomatic treatment, new endeavours are underway to find better treatments, including repurposing current drugs, use of novel small molecules, and gene therapies. Developments made in reproductive technology offer women the opportunity to prevent transmission of DNA-related mitochondrial disease to their children.

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Introduction

Inherited mitochondrial diseases are complex neurogenetic conditions that present clinical, diagnostic, and treatment challenges for neurologists. The substantial clinical variability and frequent involvement of other tissues means that recognition of potential mitochondrial disease might be delayed. Establishing the genetic diagnosis of mitochondrial disease can be difficult because mitochondria are under dual genetic control by the nuclear and mitochondrial genomes. For individuals who present with a recognised clinical syndrome or a positive family history, genetic diagnosis is straightforward, but for many people, mitochondrial disease is just part of the differential diagnosis of complex neurogenetic disorders. Nevertheless, establishing the genetic cause is crucial for both prognosis and genetic counselling, particularly in the case of mitochondrial DNA (mtDNA)-related disease, which presents unique challenges for affected women in terms of reproductive options. In past years, even with a diagnosis of mitochondrial disease, few treatment options were available and there were no care guidelines.

The aim of this Review is to highlight the substantial progress made in mitochondrial disease research in the past few years, showcasing the important aspects for neurologists to recognise, diagnose, and manage adult patients with mitochondrial disease. Our understanding of the clinical spectrum of mitochondrial disease has progressed with development of large patient cohorts and recognition of genotype-specific symptoms. Advances in genetic sequencing technology have led to recognition that defects in more than 350 genes of both mitochondrial and nuclear origin might cause mitochondrial disease, thereby transforming diagnosis. Although no cure for mitochondrial disease currently exists, consensus-based clinical guidelines are available to help manage patients and, for some mitochondrial diseases, potential treatments are emerging. An increasing number of clinical trials are investigating new or repurposed small molecules and gene therapy. Finally, the development of reproductive techniques, such as mitochondrial replacement therapy, has provided hope for mothers with mtDNA-related disease.

Clinical aspects of mitochondrial disease

Since mitochondria are essential organelles in all human cells, mitochondrial disease can affect all organs; involvement of the nervous system, however, can be substantial. Initially, many mitochondrial diseases were defined as a cluster of clinical syndromic features—eg, mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS) syndrome and Kearns-Sayre syndrome.[1] However, only a few individuals show the full spectrum of signs at presentation. Key to recognising patients with mitochondrial disease has been the use of deep phenotyping, full characterisation of clinical features, and identification of other organ involvement (appendix pp 2–4). The initial clinical manifestation of mitochondrial disease might be neurological, or not, and can present acutely, subacutely, or evolve slowly.

Acute and subacute neurological presentations

Stroke-like episodes

Stroke-like episodes in individuals with mitochondrial disease are characterised by headache, nausea and vomiting, encephalopathy, focal-onset seizures, or psychiatric symptoms before development of a focal neurological deficit. Epilepsia partialis continua and, rarely, generalised status epilepticus might also occur during the stroke-like episode. Headache and visual disturbance are often experienced during the prodrome. A consensus statement proposed that "a mitochondrial stroke-like episode is a subacute, evolving brain syndrome driven by seizure activity in genetically determined mitochondrial disease".[2]

Although stroke-like episodes were originally described in individuals younger than 40 years, late-onset presentation is now recognised.[3] Stroke-like episodes are the defining feature of MELAS syndrome, with the pathogenic m.3243A→G *MT-TL1* gene variant accounting for up

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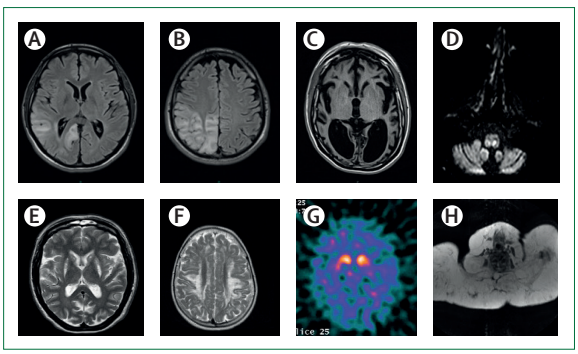


Figure 1: **Neuroimaging features of mitochondrial diseases**
Stroke-like lesions involving the right temporal, occipital (A) and parietal lobes (B) with restricted diffusion (not shown) are seen in a 20-year-old man with m.3243A→G-related mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes syndrome. Severe cerebral atrophy (C) is evident in a 50-year-old man with the m.3243A→G variant who had experienced recurrent stroke-like episodes since his mid-30s. Asymmetrical medullary lesions with restricted diffusion (D) are seen in a 25-year-old woman with the m.9185T→C *MT-ATP6* variant who presented with subacute onset dysphagia, central hypoventilation, and type 2 respiratory failure; her initial clinical phenotype was teenage-onset progressive ataxia and neuropathy. Symmetrical T2-hyperintensities in the basal ganglia (E) are shown in a woman in her mid-40s who had childhood-onset Leigh syndrome related to the m.8993T→C *MT-ATP6* variant. Symmetrical leukodystrophy (F) is identified in a 13-year-old boy with Kearns-Sayre syndrome due to a single, large-scale mitochondrial DNA deletion. Asymmetrical reduction in dopamine uptake (G) is seen on a dopamine transporter PET scan of a 45-year-old man with recessive *POLG* variants who presented with chronic progressive external ophthalmoplegia, ataxia, and parkinsonism. Diffuse lipomatosis (H) is seen in the shoulder girdles and cervical region in a 44-year-old woman with the m.8344A→G variant.

to 80% of cases; pathogenic variants in other mtDNA genes and in the nuclear gene *POLG* are also known to cause stroke-like episodes.[2,4]

On conventional MRI, stroke-like lesions associated with mitochondrial disease typically do not correspond to vascular territories and involve cortex and juxtacortical white matter (figure 1). MRI proton spectroscopy shows a lactate peak both in the affected regions and in ventricular CSF or other apparently unaffected brain areas. Lesions might spread over days, weeks, and even months. Some signal abnormalities resolve completely; however, most severe lesions develop into cortical laminar necrosis, gliosis, and atrophy, potentially leading to progressive physical and cognitive decline. Based on the clinical and MRI features, clinicians should think about a mitochondrial stroke-like episode as a main differential diagnosis to atypical recurrent ischaemic stroke,[5] encephalitis with a negative infectious and autoimmune screen,[6] and posterior reversible encephalopathy syndrome.[7]

Epilepsy

Epilepsy is a common feature of mitochondrial disease[8,9] with both generalised and focal seizures observed. In individuals with m.3243A→G and *POLG* pathogenic variants, seizure activity leading to development of a stroke-like episode is typically refractory or super-refractory to pharmacological treatment, including general anaesthesia—particularly in *POLG*-related epilepsy.[10] Patients with the m.8344A→G variant can have both myoclonic epilepsy and subcortical myoclonus without any EEG correlates.[11,12]

Metabolic decompensation of brainstem function

Leigh syndrome is the most common presentation of paediatric mitochondrial disease,[13] although some children present late and those who are mildly affected can survive into adulthood.[14] Patients with Leigh syndrome can develop subacute brainstem dysfunction when metabolically challenged (eg, during intercurrent infections; figure 1). Features of Leigh syndrome include ophthalmoplegia, ataxia, dysphagia, dysarthria, central hypoventilation, hypersomnolence, and autonomic instability. Brainstem syndrome has also been reported in adult patients who initially presented with neuropathy and ataxia related to *MT-ATP6* pathogenic variants, without a pre-existing diagnosis of Leigh syndrome.[15] Partial recovery of brainstem function occurs in some patients who survive the metabolic crisis.

Subacute visual loss and optic neuropathy

Subacute, painless, and progressive impairment of central vision is a typical presenting feature of the mitochondrial disease Leber hereditary optic neuropathy (LHON). Typically, onset of this disorder is unilateral, with the second eye becoming affected within weeks or months. Male carriers of one of the three primary pathogenic variants (m.3460G→A *MT-ND1*, m.11778G→A *MT-ND4*, and m.14484T→C *MT-ND6*) associated with LHON are more likely to be affected by visual impairment than are women, and smoking and excess alcohol are trigger factors for visual loss.[16] Rapid deterioration of visual function can raise suspicion for inflammatory optic neuritis initially. Therefore, early diagnosis of LHON could avoid the initiation of long-term immunotherapy, which is unnecessary, ineffective, and potentially detrimental. Clinical features of LHON are young age (with peak age of onset in the second and third decade of life), male sex, absence of pain with eye movements, no relative afferent pupillary deficit, maternal family history of blindness, normal MRI and CSF results, and no benefit from steroids. Typical ophthalmological findings during the acute phase of LHON include hyperaemia of the optic disc, oedema of the peripapillary retinal nerve fibre layer, retinal telangiectasia, and absence of leakage on fluorescein angiography.[17] A clinical study using optical coherence tomography showed that thickening of the retinal nerve fibre layer preceded angiopathic changes, measured by an increase in choroidal thickness during the acute phase of LHON.[18]

Chronic neurological presentations

Chronic progressive external ophthalmoplegia (CPEO)

CPEO is one of the most common adult clinical presentations of mitochondrial disease. Only a small proportion of patients (<30%)[19] report diplopia (which can affect reading ability, balance, and movement; and an early onset of

which might result in chronic suppression and amblyopia) due to the insidious nature of disease progression. Depending on the genetic defect, CPEO can occur in isolation or with other neurological features and multisystem involvement. Seronegative myasthenia gravis (without AchR and MuSK antibodies) is an important differential diagnosis, and patients with CPEO can have minor neuromuscular junction electrophysiological abnormalities.[20] Other differential diagnoses[21] include oculopharyngeal muscular dystrophy, congenital myasthenic syndrome, congenital myopathy, and myotonic dystrophy.

Myopathy

Features that can alert the clinician to mitochondrial myopathies include fatiguability, myalgia (often exercise-induced), exercise intolerance, and lactic acidaemia. Many patients with CPEO also develop myopathy, and some mitochondrial diseases might have concomitant peripheral neuropathy that can be axonal or demyelinating, including *POLG*-related disease and mitochondrial neurogastrointestinal encephalomyopathy.[22] Mild bulbar weakness is not uncommon;[23] however, some patients develop severe dysphagia and are at risk of recurrent aspiration. Although an elevated creatinine kinase level (typically >1000 IU/L) is a recognised laboratory finding of mitochondrial disease, rhabdomyolysis manifesting with myoglobinuria is very rare.[24] Severe neuromuscular weakness (including diaphragmatic weakness) requiring ventilatory support is infrequent in adult mitochondrial diseases and is typically associated with specific genetic defects such as TK2 deficiency[25] and the m.8344A→G mutation.

Sensorineural hearing loss

To be suggestive of a mitochondrial disorder, hearing loss is generally young-onset with other systemic features associated with a mitochondrial disease,[26] and a maternal pattern of transmission. Some individuals with specific mtDNA pathogenic variants (eg, a homoplasmic m.1555A→G variant) develop deafness after exposure to aminoglycoside antibiotics. The onset of hearing loss is usually after postlingual development, indicating that cochlear implantation could be useful as a treatment in those patients with profound hearing loss.[27]

Optic neuropathy

Around 60% of cases of autosomal-dominant optic atrophy are due to *OPA1* genetic defects, which have an estimated prevalence of 1 in 25 000 people.[28] Optic atrophy is characterised by a slowly progressive, childhood-onset optic neuropathy with moderate-to-severe loss of visual acuity. At least a fifth of patients with *OPA1* pathogenic variants develop extra-optic nerve manifestations—eg, hearing impairment, CPEO, neuropathy, cerebellar symptoms, or parkinsonism.[29] Additionally, subclinical optic neuropathy can be an accompanying feature in many other mitochondrial diseases—eg, in m.8344A→G-related or m.3243A→G-related disease.

Other chronic neurological presentations

Cerebellar or sensory ataxia, peripheral neuropathy, migraine, cognitive impairment, spasticity, and extrapyramidal movement disorders including tremor, parkinsonism, dystonia, and chorea can also be presenting features of mitochondrial disease. In some patients, the diagnosis of mitochondrial disease is straightforward because of other classic symptoms, such as a combination of CPEO and parkinsonism in *POLG*-related disease, or maternally inherited diabetes and deafness in m.3243A→G-related disease. In other patients, mitochondrial disease is just one of several diagnostic possibilities. Making a diagnosis in these patients can be challenging, particularly when disease onset is late and there is no family history. Moreover, many aspects of mitochondrial disease are still not understood, such as selective tissue involvement and the unique patterns of clinical manifestations that occur with specific gene variants. In such cases, development of large cohorts and natural history studies should be helpful, particularly when combined with mechanistic studies.

Non-neurological features

Management of patients with mitochondrial disease is complicated by the recognition that multiple organs can be involved. Moreover, systemic features might be more prominent than neurological signs (appendix pp 2–4).

Recent advances in diagnosis and biomarkers

Genomics and biomarker development are transforming the diagnosis of mitochondrial disease.[30,31] Securing a precise genetic diagnosis is important since knowledge about genetic variants can facilitate the targeting of treatments, help with management of and surveillance for specific complications, inform counselling for reproductive options, and enable patients to be enrolled into appropriate clinical trials. However, access to diagnostic tests remains inequitable in some countries.

Mitochondrial biomarkers

Validated biomarkers now make it possible to avoid invasive muscle biopsies in some cases.[32] Two novel biomarkers of mitochondrial dysfunction have been identified, FGF21[33] and GDF15.[34] In adults, increased amounts of FGF21 and GDF15 are associated with muscle-manifesting mitochondrial translational defects (pathogenic mt-tRNA variants and mtDNA deletions) and levels of the biomarkers correlate with the severity of the biochemical defect in muscle.[35] Both FGF21 and GDF15 have better sensitivity and specificity for adult mitochondrial disease than do other conventional serum biomarkers, such as creatinine kinase and lactate.[36,37] Studies have shown that coupled measurement of serum FGF21 and GDF15 was more sensitive in diagnosing mitochondrial disease than were muscle histopathological

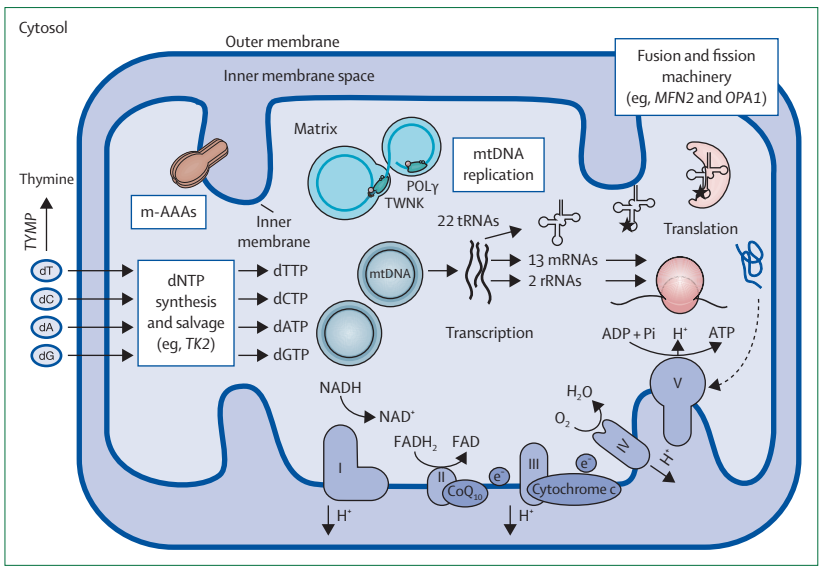


Figure 2: **Illustration of mitochondrial oxidative phosphorylation system and other pathways that are commonly implicated in adult mitochondrial disease**

The mitochondrial oxidative phosphorylation system comprises complexes I–V and two mobile electron carriers, coenzyme Q_{10} and cytochrome c. As high-energy electrons are passed along the complexes, protons are pumped out of the matrix space, creating an electrochemical membrane potential that is used by ATP synthase (complex V) to generate ATP. The mitochondrial genome encodes 13 protein subunits, 22 tRNAs, and two rRNAs; there are multiple copies of mtDNA per cell, ranging from hundreds to thousands depending on the cell type. The replication, maintenance, transcription, and translation of mtDNA and mtDNA-encoded proteins are dependent on many nuclear-encoded proteins. m-AAAs play an important role in the quality control of mitochondrial proteins. Genetic defects in nucleotide synthesis and salvage (eg, *TYMP* and *TK2*), mitochondrial DNA replication and maintenance (eg, catalytic subunit of POLγ and TWNK), fusion and fission machinery (eg, *OPA1* and *MFN2*), and m-AAAs (eg, *SPG7*) perturb mtDNA integrity and mtDNA copy number, leading to the formation of multiple deletions and mtDNA depletion. black stars=amino acid. dA=deoxyadenosine. dC=deoxycytidine. dG=deoxyguanosine. dT=thymidine. dATP=deoxyadenosine triphosphate. dCTP=deoxycytidine triphosphate. dGTP=deoxyguanosine triphosphate. dNTP=deoxynucleoside triphosphate. dTTP=deoxythymidine triphosphate. m-AAAs=mitochondrial ATP-dependent proteases. mtDNA=mitochondrial DNA. Pi=phosphate.

abnormalities.[38] A meta-analysis of data on the use of FGF21 and GDF15 for diagnosis (including five studies of FGF21 and seven studies of GDF15) concluded that FGF21 and GDF15 showed acceptable sensitivity (71% for FDF21 and 83% for GDF15) and high specificity (88% for FDF21 and 92% for GDF15).[39] These findings highlight a supportive role for these two biomarkers in the diagnosis of mitochondrial disease and potentially for monitoring of diseases such as TK2 deficiency.[40] FGF21 is of reduced value for non-muscle-related phenotypes of mitochondrial disease.[36]

New fluid biomarkers for mitochondrial disease are emerging, including in amino acid and one-carbon metabolism. Circulating cell-free mtDNA was found to be increased in m.3243A→G-related mitochondrial disease and could be useful for monitoring disease severity,[41] and the amount of serum neurofilament light chain seems to reflect CNS involvement.[42] Tissue-specific stress responses in the affected organ involving one-carbon cycle, transsulphuration, and mTORC1 remain to be established as specific biomarkers for diagnosis.[43,44] Multibiomarkers, in the form of metabolomic and proteomic approaches, show promise[30,45] but have not yet advanced to clinical implementation. A pilot study showed that systemically low amounts of oxidised nicotinamide adenine dinucleotide (NAD+) in patients with mitochondrial myopathy were restored by the NAD-booster niacin.[46] The potential for using blood NAD+ concentrations as a mitochondrial disease biomarker requires further study.

Genomics

Mitochondrial diseases are the result of a defective oxidative phosphorylation system (figure 2), which comprises subunits encoded by both the nuclear and mitochondrial genomes. Mutations in genes encoding subunits of this system will lead to mitochondrial disease, as will defects in the many other nuclear mitochondrial proteins required for normal assembly and activity of the oxidative phosphorylation system.[1] Nuclear mitochondrial proteins are also essential for the replication, transcription, and maintenance of mtDNA and defects in these proteins can lead to mtDNA depletion or multiple mtDNA deletions (figure 2).

The genetic characteristics of mtDNA differ from those of nuclear DNA: mtDNA is maternally inherited and present in multiple copies in individual cells. Pathogenic variants can, therefore, affect all copies (homoplasmy) or only some (heteroplasmy). In the case of heteroplasmy, the percentage of mtDNA copies affected by a pathogenic variant is an important factor in determining whether a clinical phenotype will manifest (appendix pp 10).

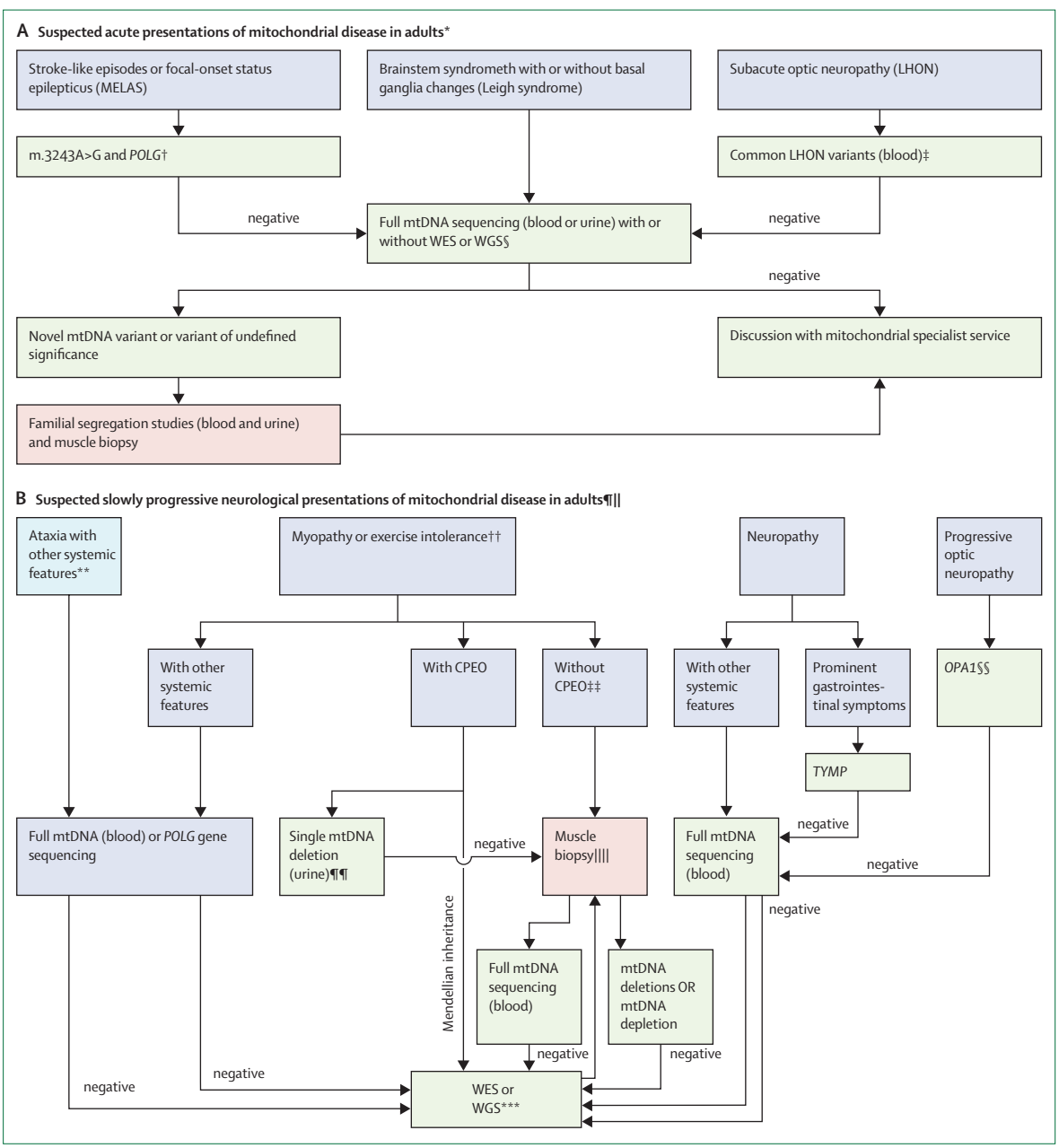
If there is a high clinical suspicion of mitochondrial disease, such as syndromic presentation and multisystem involvement, genetic testing should be prioritised (figure 3). Deep clinical phenotyping and an accurate family history remain essential when considering if genomic variants identified by techniques such as whole exome sequencing (WES) or whole genome sequencing (WGS) can account for the clinical presentation. Systematic enquiries about the clinical histories of other family members should include asking about both neurological and extra-neurological signs and symptoms. Parental consanguinity strongly suggests an autosomal-recessive disorder, but there are exceptions such as maternally inherited mtDNA disease in consanguineous families.[48] Conversely, an absence of family history should not reduce clinical suspicion since de-novo single, large-scale mtDNA deletions, de-novo mtDNA pathogenic variants,[49] and de-novo autosomal-dominant and recessive traits are all known causes of adult mitochondrial disease.[50,51] In clinical practice, assessment of late-onset, non-syndromic neurological presentations in adults for a mitochondrial cause remains a considerable challenge, even after extensive investigations to exclude other conditions.

mtDNA genomics

Approximately two-thirds of cases of adult-onset disease are caused by pathogenic mtDNA variants.[52] The first step in diagnosing adults is typically mtDNA testing, unless there are specific indicators suggesting another gene or mode of inheritance. For the classic syndromic presentations

such as MELAS syndrome and LHON, screening for common pathogenic mtDNA variants (eg, m.3243A→G in MELAS or three primary *MT-ND* gene variants in LHON), will still have a moderate-to-high diagnostic yield (figure 3). Testing sometimes proceeds straight to amplification of a full-length mtDNA amplicon, or overlapping amplicons, for massively parallel sequencing.[30,53] The high depth of coverage with such sequencing provides more sensitive detection of single nucleotide mtDNA variants than with previous methods such as Sanger sequencing, and accurate quantitation of mutant load. Large-scale mtDNA rearrangements are also detected, but if accurate quantitation is required, alternative approaches such as quantitative PCR should be used. Pathological mtDNA variants can be detected robustly from WGS data and, with lower sensitivity, from WES data.[30,54]

Testing for pathogenic mtDNA variants in blood alone can yield false-negative diagnoses. Additional use of non-invasively obtained samples (eg, urinary sediment, buccal swab) can improve the sensitivity of diagnostic mtDNA testing and familial screening, although the age-corrected level of mutant mtDNA heteroplasmy in blood for the common m.3243A→G variant has a stronger correlation with disease burden and progression than in urine.[55]



If mtDNA screening is uninformative in blood, testing of muscle might be needed to fully exclude mtDNA-related disease.[56]

Nuclear DNA genomics

Pathogenic variants in more than 300 nuclear genes cause mitochondrial disease.[50,57] New gene and pathway discoveries and their link to mitochondrial disease will continue, as more than 1100 mitochondrial proteins have been identified to date.[58] In many laboratories, unbiased WES and WGS data have replaced the testing of single genes. The improved detection of copy number variants and mtDNA sequences, and the ability to investigate non-coding regions in the genome, means that the diagnostic yield of WGS is usually higher than with WES. A key consideration in WES or WGS is selection of the gene list to be analysed and whether to use validated and curated gene panels (ie, a clinical exome). International practices differ regarding selection of a narrow phenotype-defined gene list or a mendeliome containing all genes causing inherited disease. Standardisation of gene lists occurs through the use of curated consensus panels such as PanelApp. Phenotypic mimicry (eg, Charcot-Marie-Tooth neuropathy, genetic myopathy, or spinocerebellar ataxia)[20] means that if a relatively narrow mitochondrial gene list is used for initial variant analysis, it is usually advisable to expand the gene list for reanalysis of undiagnosed cases. Early WES analyses were designed primarily to detect single nucleotide variants and short insertions or deletions (indels), and the limitations of these analyses for identifying repeat-expansion disorders such as Huntington's disease, Friedreich's ataxia, and spinocerebellar ataxias have been highlighted. However, new bioinformatic approaches have been developed to address such challenges.[59]

For **PanelApp** see https://panelapp.genomicsengland.co.uk/panels/

Interpretation of genomic sequencing results has been greatly improved by algorithms that prioritise the likelihood of rare variants being pathogenic and by widespread adoption of the American College of Medical Genetics criteria for variant classification.[60] Interpretation of data is usually straightforward when patients are found to have pathogenic or probably pathogenic variants, consistent with their phenotype. However, variants of uncertain importance, without sufficient evidence to determine if they are pathogenic or benign, are common. Some of these variants require further functional testing or other studies to determine causality. Secondary findings of pathogenic variants in genes unrelated to the primary medical reason for testing are found in up to 4% of patients.[61,62] Finally, up to 5% of patients with inherited conditions have phenotypes resulting from pathogenic variants in two or more different genes.[63] The quality of curation of genomic variants is higher when ratified by a multidisciplinary team comprising clinicians and clinical scientists with expertise in the conditions being investigated. For adults with ambiguous genetic results, or for whom no likely genetic diagnosis is achieved, functional testing is a follow-up approach, most usually entailing muscle biopsy.

Figure 3: **Algorithms for diagnosis of mitochondrial disease**

(A) Diagnostic algorithm for suspected acute presentations of adult mitochondrial disease. (B) Diagnostic algorithm for suspected slowly progressive neurological presentations of adult mitochondrial disease. MELAS=mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes. LHON=Leber hereditary optic neuropathy. mtDNA=mitochondrial DNA. WES=whole exome sequencing. WGS=whole genome sequencing. CPEO=chronic progressive external ophthalmoplegia. *Evaluation for multisystem involvement such as sensorineural hearing loss, diabetes, cardiac abnormality, gastrointestinal, renal, or hepatic involvement. †m.3243A→G and *POLG* pathogenic variants account for >80% of cases of mitochondrial stroke-like episodes. ‡m.3460G→A, m.11778G→A, and m.14484T→C pathogenic variants account for >90% of cases of LHON. §Consider other acquired or genetic mimics of mitochondrial disease. ¶Evaluation for multisystem involvement. Ascertainment of family history. ||If myopathy forms part of the overall clinical phenotype, and plasma GDF15 and FGF21 levels are within the normal range, the probability of mitochondrial disease is low. **If CPEO is also evident, follow the myopathy pathway. Isolated cerebellar syndrome is uncommon in primary mitochondrial disease. ††Persistent creatinine kinase concentration >1000 U/L is rare in mitochondrial disease. ‡‡Neurologists face considerable challenges when investigating late-onset, isolated, and progressive myopathy in adults. Many differential diagnoses are possible, including both acquired and genetic causes, and muscle biopsy is important to identify the correct diagnosis. MRI of pelvis and lower limbs show a good diagnostic value for specific genetic muscle diseases; however, no clear pattern of muscle involvement has been identified in primary mitochondrial disease to date. §§Account for at least 60% of dominant optic atrophy cases. ¶¶Accounting for 30–50% of CPEO cases, the ability to detect a single, large-scale mtDNA deletion in urothelial cells has been reported in patients who previously had muscle biopsy.[47] ||||Histopathological evidence of mitochondrial dysfunction can be observed in a muscle biopsy, including COX-deficient and other oxidative phosphorylation complex-deficient fibres, or mitochondrial subsarcolemmal accumulation (ragged-red fibres). However, these changes, usually occurring in only occasional fibres, can be observed in non-primary mitochondrial disorders. ***Identification of variants of undefined significance through WES or WGS might require further functional characterisation by muscle biopsy to prove pathogenicity.

Biochemical assessment of mitochondrial function

Traditionally, investigation of suspected mitochondrial disease focused on detecting evidence of biochemical dysfunction in muscle tissue, including the demonstration of COX-deficient fibres and measurements of oxidative phosphorylation system enzyme activities. Some biopsy findings, such as focal COX deficiency, have imperfect sensitivity and specificity for mitochondrial disease since defects in COX accumulate with age due to somatic mtDNA deletions. Muscle biopsy is typically used after inconclusive genomic testing, and analysis of muscle tissue[64] can provide invaluable information for interpreting the pathogenicity of novel genetic variants; techniques such as imaging mass cytometry offer the potential to study mitochondrial protein signatures at single cell resolution.[65] Although many genetic variants can be studied in blood or non-invasive tissues, muscle-restricted mtDNA variants can only be diagnosed by analysis of muscle.[32] Furthermore, in single, large-scale mtDNA rearrangements, disease onset and progression can be predicted by identifying the deletion size and mutant load within muscle tissue.[66] Finally, muscle biopsy helps confirm other causes (eg, inflammatory myositis, inclusion body myositis, and myofibrillar myopathies) and is a source of tissue for RNA

sequencing, which can lead to a genetic diagnosis in up to 40% of patients who are not diagnosed by WES.[67]

Natural history studies and patient registries

In past years, sparse availability of longitudinal and natural history data for mitochondrial disease created considerable barriers to establishing clear and comprehensive guidance on medical management, and hampered development of clinical trials and patient-centred clinically relevant outcome measures. Publications describing single cases of mitochondrial disease, or retrospective case series of specific genotypes, often report patients with severe and so-called classic clinical manifestations and reflect, therefore, a bias towards severe disease. A greater understanding of the spectrum of clinical phenotypes of mitochondrial disease and disease severity has been provided by cohort-based, observational studies that used standardised methods of data collection.[11,68,69] Substantial progress has been made in describing the nature and frequency of clinical features of patient cohorts, including m.3243A→G,[69–71] m.8344A→G,[11] *MTA-TP6*,[15,72] single large-scale mtDNA deletion,[66,73] *POLG*,[74] *TK2*,[75] *TYMP*,[76] and Leigh syndrome.[77] Moreover, establishing these cohorts has facilitated investigation of a phenotype first approach, furthering our understanding of the diverse genetic causes of specific clinical features of mitochondrial diseases, such as extrapyramidal movement disorders,[78] epilepsy,[8,9] myoclonus,[79] CPEO,[80,81] pregnancy-related complications,[82] and gastrointestinal symptoms.[83] Additionally, our understanding of very rare but clinically important events, such as sudden unexpected death in patients with the m.3243A→G variant,[84] has been augmented by systematic assessment and follow-up afforded by recruitment to these cohort studies. These natural history studies have provided insight into different stages of disease and risk stratification, and surveillance for complications is now routine for common mtDNA-related diseases. Studies of patient cohorts have also provided insights into new disease mechanisms caused by mitochondrial dysfunction—eg, the leukodystrophy observed on MRI in patients with defects in several mt-tRNA synthetases (eg, *AARS2*, *DARS2*, and *EARS2*) that lead to impaired translation of mitochondrial protein[85] indicates a role for mitochondria in myelin metabolism. Furthermore, establishment of patient cohorts has been essential for development of consensus-based international guidelines for the care of patients with mitochondrial disease,[2,86–88] development of clinical phenotype and genomic resources dedicated to mitochondrial disease (including mtDNA variant data in gnomAD and GENOMIT) and selection of outcome measures for clinical trials.[89,90]

Treatment

Current treatment and surveillance for systemic complications

Findings of a randomised controlled study, and data subsequently derived from an expanded access programme,[91,92] led to idebenone becoming the first licensed drug in Europe for treatment of visual impairment in patients with LHON. An international expert group reached consensus that idebenone was indicated in the subacute and dynamic stages of LHON (ie, in the first year after onset) but evidence was considered insufficient to approve the drug for treatment in the chronic stage. In patients treated with idebenone, clinically relevant recovery can occur after 24 months of treatment or, rarely, after an even longer time. Accordingly, idebenone treatment should be maintained for more than 24 months before judging a patient a non-responder.[92] No evidence is available to support the use of idebenone in asymptomatic carriers of pathogenic LHON variants.

No randomised controlled trials have been done of treatments for stroke-like episodes. Use of L-arginine for this neurological presentation remains controversial.[2] A longitudinal open-label study in patients with m.3243A→G-related MELAS syndrome did not attain the predefined primary outcome measure ("the improvement rates of headache and nausea or vomiting at 2 h after completion of the initial intravenous administration"), and L-arginine did not prevent the development of neurodegeneration.[93] A prospective, multicentre, randomised controlled trial would be required to evaluate the efficacy of L-arginine to reduce the recurrence of stroke-like episodes. In an open-label study, taurine supplementation reduced the frequency of stroke-like episodes in ten patients with MELAS syndrome.[94] However, the absence of a control arm in the study questions whether the change in the number of stroke-like episodes was due to the efficacy of taurine, a placebo effect, or simply reflected the natural history of stroke-like episodes, which are paroxysmal and unpredictable. European consensus-based guidance recommends an urgent administration of intravenous anti-epileptic drugs for all stroke-like episodes.[2] Valproate should not be used in patients with *POLG*-related disease, because this drug might precipitate liver failure.[87] However, valproate can be useful for myoclonic epilepsy in other mitochondrial diseases.

Young adults with pathogenic variants in mtDNA-encoded complex I subunits[95] or *MTA-TP6*[15] are at risk of developing subacute brainstem dysfunction. Prompt recognition is crucial, and critical care support might be required in some cases.

With few treatments specific for neurological presentations of mitochondrial diseases, symptomatic management of chronic neurological symptoms is very important. For example, discussing management with an expert oculoplastic surgeon, who has experience in mitochondrial disease, can result in substantial improvements in quality of life for patients with CPEO. Exercise is encouraged because of general health benefits (including cardiovascular fitness) and scant evidence of adverse effects.[96,97]

Some clinical problems, including sensorineural hearing loss, cardiac involvement, and gut dysmotility, are encountered with multiple genetic defects; effective treatments are

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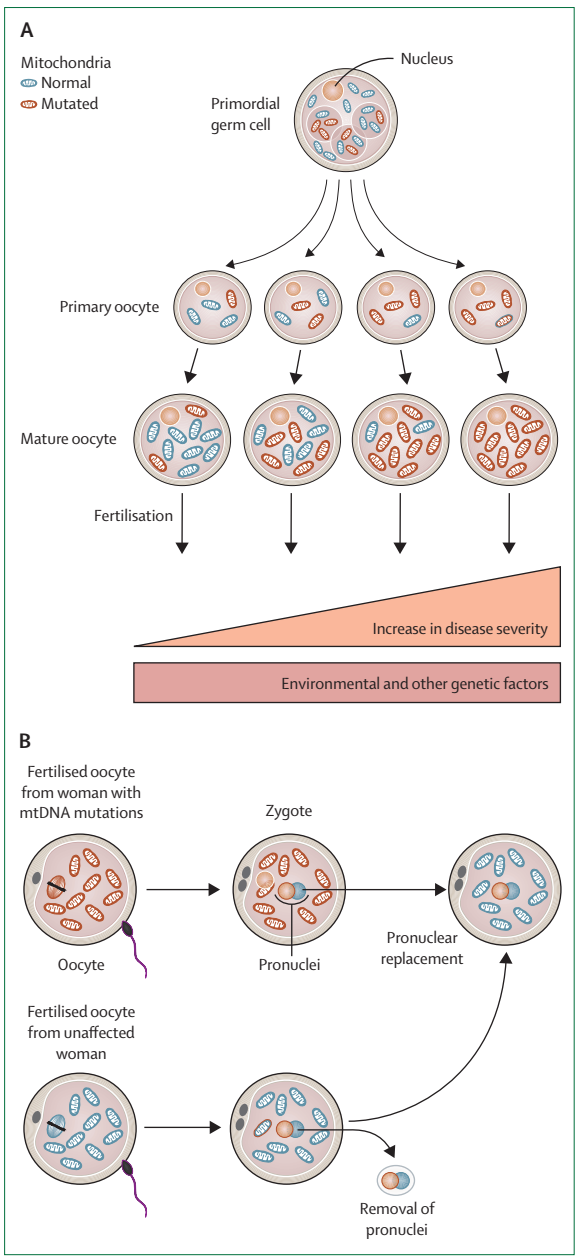

Figure 4: **Mitochondrial heteroplasmy and pronuclear transfer**
(A) The mitochondrial bottleneck explains how there can be extreme divergence in the heteroplasmy between offspring. There is a genetic bottleneck (the restriction in the number of mitochondrial genomes repopulating the female germ line) during development that results in different levels of heteroplasmy in each individual oocyte. This is a major problem when providing genetic counselling for mothers with heteroplasmic variants since the heteroplasmy level will affect the clinical outcome in the offspring. (B) Mitochondrial donation (mitochondrial replacement therapy) involves the transfer of the nDNA from an oocyte or zygote from a woman with a pathogenic mtDNA variant into an enucleated, recipient donor oocyte or zygote. Pronuclear transfer is an in-vitro fertilisation technique that transfers the pronuclei (containing the nDNA) immediately after fertilisation from the patient zygote to the donor zygote. The embryo has the nDNA of the woman with the mtDNA variant (and that of the father) but the mtDNA of the donor woman. nDNA=nuclear DNA. mtDNA=mitochondrial DNA.

available at least for sensorineural hearing loss and cardiac involvement. By contrast, the risk of complications such as diabetes, renal tubulopathy, hepatic involvement, and sideroblastic anaemia seem more genotype-specific (appendix pp 2–4). Therefore, development of personalised strategies based on knowledge of the natural histories of different genetic defects is necessary. In view of the multisystem involvement seen in many patients with mitochondrial disease, multidisciplinary clinics providing advice about non-neurological involvement (eg, diabetes, bowel dysfunction, and cardiac disease), as well as managing the neurological features, are crucial for optimal care.

No trial evidence supports routine dietary supplementation in adult patients with mitochondrial disease.[1] However, specific mitochondrial diseases—such as primary coenzyme Q_{10} deficiency,[98] primary disorders of vitamin cofactor metabolism (eg, thiamine, biotin, and riboflavin), and multiple acyl-CoA dehydrogenase deficiency—are likely to benefit from supplementation.[99] The long-term outcomes of these rare conditions after appropriate supplementation are currently unknown.

Experimental treatments

Several experimental treatments are in development for mitochondrial diseases.[100] The main approaches entail small molecules (including repurposed drugs) to correct the biochemical abnormality, metabolic rewiring or bypass, or gene therapy to target the mitochondrial genome. Some small-molecule approaches have led to clinical trials for the treatment of specific mitochondrial diseases.[100,101]

An area of increasing interest includes disorders associated with abnormalities of mitochondrial nucleoside metabolism leading to mtDNA depletion or multiple mtDNA deletions. For example, in mitochondrial neurogastrointestinal encephalomyopathy due to thymidine phosphorylase deficiency, therapeutic approaches include allogeneic stem-cell transplants, orthotopic liver transplantation, and enzyme replacement therapy;[102] in a retrospective study of thymidine kinase deficiency, deoxynucleoside therapy led to clinical improvement.[103]

Other approaches to treatment of mitochondrial disease include augmentation of mitochondrial biogenesis, restoration of the cellular NAD+ to NADH ratio, increasing mitophagy, metabolic reprogramming, or manipulation of oxidative stress.[100] A pilot study using niacin, a form of vitamin B3, showed that it restored low NAD+ amounts and improved muscle strength, performance, and metabolome in a small group of patients with mitochondrial myopathy.[46] These encouraging results need confirmation but suggest that targeting the NAD+ to NADH ratio might be an important factor in some patients with mitochondrial diseases.

Genetic manipulation techniques could be useful for treatment of mitochondrial diseases. Although techniques such as CRISPR–Cas9 are already established for nuclear disorders, correction of defects in the mitochondrial genome is difficult due to the impermeability of mito-

chondrial membranes. One approach—called allotopic expression—delivers wild-type copies of mtDNA genes using viral vectors that remain in the cytosol. Transport of the protein into the mitochondria is facilitated by addition of a mitochondrial targeting signal. An example of this technique is allotopic AAV2–*ND4* gene therapy for patients with LHON with the m.11778G→A variant.[104] Considerable progress has been made with development of TALENs or zinc finger nucleases that cross the mitochondrial membrane and directly target mutated mtDNA for degradation in experimental models.[105] Another advance is the discovery of an enzyme that can precisely edit mtDNA, although the success of edited bases was very low.[106]

Reproductive options

Although considerable progress has been made in the treatment of mitochondrial diseases, many of these conditions remain severe and life-threatening, with high morbidity. Advances in genomics mean that most patients with mitochondrial disease now have a genetic diagnosis. Therefore, it is important that couples considering a family should be offered reproductive advice, which should be focused on the specific nuclear or mitochondrial genetic defect.

For patients with pathogenic nuclear gene variants, reproductive advice will depend on the inheritance pattern and the availability of IVF techniques such as PGD. Nuclear mitochondrial diseases typically present in childhood, although several disorders present across the age spectrum (eg, *POLG* and other genes involved in mtDNA maintenance). Reproductive options include, where permitted, prenatal testing and preimplantation genetic diagnosis. For some diseases in which the mutant allele is present at high frequency within a population—eg, in consanguineous societies or geographically isolated communities—screening for common pathogenic alleles might be justified.

For patients with mtDNA pathogenic variants, provision of reproductive advice is more complex than for other types of mutation. For example, even the most common sporadic mtDNA pathogenic variant—a single, large-scale mtDNA deletion—is transmitted on rare occasions. Therefore, reproductive options for women with mtDNA pathogenic variants should be discussed at centres that specialise in mitochondrial genetics.[107] Despite some reports suggesting that there is biparental transmission of mtDNA,[108] other studies have shown that this is most likely not the case.[109,110] All reported cases of transmission of mtDNA pathogenic variants have been maternally inherited. For women carrying homoplasmic mtDNA pathogenic variants, the advice might seem simple: they will transmit the variant to their offspring. However, transmission can be complicated by variable penetrance due to either nuclear genetic or environmental factors. For example, in families that carry one of the three common pathogenic variants for LHON, men are more at risk of developing visual loss than are women.

Search strategy and selection criteria

We searched PubMed for articles published in English from Jan 1, 2010, to Nov 1, 2020, using the search terms "mitochondrial disease OR disorder", "neurological feature", "MELAS", "CPEO", "myopathy", "biomarker", "genetic", "treatment", "clinical trial", AND "reproductive option". We further examined the reference lists from relevant articles. The references were chosen based on their originality and relevance to the theme of this Review.

For women with heteroplasmic mtDNA variants, the situation with transmission is more complex due to a so-called genetic bottleneck during development.[111] Rapid intergenerational shifts in the mtDNA heteroplasmy are evident in human pedigrees transmitting pathogenic mtDNA variants due to the restriction in the number of mitochondrial genomes repopulating the female germ line (the mtDNA bottleneck). Since the range of heteroplasmy is a major risk factor in developing symptoms, offspring of a mildly affected women can have either no signs or symptoms or very severe disease (figure 4).

For families with mtDNA pathogenic variants, various reproductive options are available depending on the specific genetic abnormality and whether it is homoplasmic or heteroplasmic.[86] For heteroplasmic mtDNA variants, options such as prenatal diagnosis or preimplantation genetic diagnosis might be appropriate if available. However, there could be difficult decisions about embryo selection or continuation of a pregnancy in terms of a safe level of heteroplasmy. For women with high levels of heteroplasmy or a high frequency of homoplasmic variants, mitochondrial donation (mitochondrial replacement therapy)[112] is now an option in the UK in a highly regulated environment crucial for any new in-vitro fertilisation technique (figure 4).[113]

Conclusions and future directions

Major advances in the past 5 years in our understanding of and ability to diagnose and prevent mitochondrial disease give rise to cautious optimism for improving the lives of patients with mitochondrial disease. Robust guidelines are in use for the management of patients,[114] and targeted treatments now exist for some patient populations. Moreover, developments in in-vitro fertilisation mean new reproductive options are available for families with mitochondrial disease.

For more on **guidelines** see https://www.newcastle-mitochondria.com/wp-content/cache/all/guidelines/index.html

Nevertheless, this progress highlights the need to focus our future research. Elucidation of disease mechanisms is necessary to understand why specific patients have tissue-specific manifestations of mitochondrial disease. New or repurposed drugs are needed, and recognition is required that some approaches—such a gene therapy—might be genotype-specific. Development of new treatments will require not only cohorts of patients for clinical trials but also validated outcome measures that are pertinent to

mitochondrial diseases. Provision of cascade family tracing will be helpful, since early diagnosis is important for the success of disease-modifying treatments and valuable if patients want to consider their reproductive options.

A crucial factor driving research advancement is the close relationship between clinical and basic scientists, the pharmaceutical industry who are developing new treatments, and patient organisations. Mitochondrial disease research should factor in priorities set by patient organisations.

For more on **mitochondrial disease research priorities setting** see https://www.jla.nihr.ac.uk/priority-setting-partnerships/mitochondrial-disease/

Contributors

YSN and DMT proposed the content of manuscript and did the initial literature search. All authors contributed equally to writing and subsequent revision of this manuscript and approved the final version.

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