Recognition, Investigation and Management of Mitochondrial Disease

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Summary

Mitochondria are dynamic organelles present in virtually all human cells that are needed for a multitude of cellular functions, including energy production, control of cell apoptosis and numerous biochemical catabolic and synthetic pathways that are critical for cellular health.

Primary mitochondrial disorders are a group of greater than 200 single gene defects arising from two genomes (nuclear and mitochondrial) leading to mitochondrial dysfunction, and are associated with extremely heterogeneous phenotypes. Neuromuscular features predominate, but often with multi-system involvement.

Clinical suspicion of a mitochondrial disorder should prompt multi-pronged investigation with biochemical and molecular genetic studies. Recent wide scale adoption of next generation sequencing approaches has led to a rapid increase in the number of disease genes.

The advances in unravelling the genetic landscape of mitochondrial diseases have not yet been matched by progress in developing effective therapies, and the mainstay of care remains supportive therapies in a multidisciplinary team setting.

Mitochondrial physiology and genetics

The mitochondrion is an ubiquitous and dynamic cellular organelle with many fundamental roles in maintaining normal cellular function. Since the mitochondrion was first described in the late 19th century by Altmann and Benda,[1] our understanding of the complexities of mitochondrial function, maintenance and replication, and the often catastrophic implications of mitochondrial dysfunction, has greatly advanced.

Mitochondria are composed of a cardiolipin-containing double membrane, with the outer mitochondrial membrane surrounding the highly convoluted inner mitochondrial membrane. The five protein multi-complexes of the respiratory chain are embedded in the inner membrane, while the mitochondrial matrix contains the enzymes of numerous associated metabolic pathways including the tricarboxylic acid (TCA) cycle, part of the urea cycle and the machinery of fatty acid beta-oxidation. Complexes I-IV of the respiratory chain/electron transport chain use the reducing potential of NADH and $FADH_2$ generated by glycolysis, the TCA cycle and fatty acid oxidation to pump protons into the inter-membrane space, thereby generating an electrochemical gradient which complex V dissipates to facilitate the phosphorylation of adenosine diphosphate to generate adenosine triphosphate (ATP), the fundamental "energy currency" of the cell. In addition to energy production, control of cell apoptosis and numerous biochemical synthetic pathways are crucial mitochondrial functions that also contribute to the pathophysiology when mitochondrial function is deranged.

Mitochondrial proteins are encoded in genes held in the nuclear DNA and also in the mitochondrial DNA (mtDNA). mtDNA is a small 37-gene, ~16.5kb DNA molecule found in multiple copies within the mitochondrial matrix.[2] Together, the nuclear and mitochondrial genomes encode more than 1500 proteins involved in mitochondrial structure and function,[3] including several nuclear genes that encode machinery important in the replication and maintenance of the mtDNA.[4]

Primary mitochondrial diseases are known to be caused by mutations in more than 200 of the nuclear genes (figure 1a), inherited in Mendelian patterns (autosomal recessive, autosomal dominant or X-linked), and all the 37 mtDNA encoded genes (figure 1b).[5] As mtDNA is only acquired from the maternal gamete (ovum) at conception, mtDNA mutations are maternally inherited, or may be sporadic *de novo* events.

MtDNA has a high copy number (hundreds or thousands of copies per cell) and normal and mutated mtDNA often coexist in the same cell, a phenomenon called heteroplasmy. Problems only arise if the proportion of mtDNA that is mutated exceeds a threshold. As the level of mutant mtDNA varies

between individuals and between tissues in any one individual, different patients with the same mtDNA mutation can have very different symptoms

Recognition of Mitochondrial Disease

Mitochondrial disorders are thought to affect around 1:5000 of the population.[6] There is a wide heterogeneity in the clinical phenotype, with involvement of either single or multiple organ systems and the consequent clinical features (table 1). The age of presentation also varies widely, ranging from neonatal onset with fulminant lactic acidosis, to relatively mild disease in old age.

Highly energetic tissues including skeletal muscle and nervous system are the most commonly affected due to their greater reliance on mitochondrial ATP synthesis. However, any organ system can be affected and one of the "red flags" alerting clinicians to the potential for a mitochondrial disorder is the association of neurological or muscular symptoms with concurrent or subsequent multi-organ involvement. The occurrence of muscle or central nervous system disease with involvement of two or more other organ systems, or any combination of disease affecting three or more organ systems should prompt consideration of mitochondrial disease. The multitude of potential presenting features means that mitochondrial diseases may be encountered by numerous medical specialists.

Mitochondrial diseases are also progressive, with fluctuation and deterioration expected over time, with the potential for additional organ systems to be involved as time progresses. Progressive involvement of neuromuscular symptoms will often present with neurological regression in the developing child, and progressive loss of skills at any age.

Phenotypic syndromes

Certain clinically distinct clusters of symptoms and features are recognised as phenotypic mitochondrial syndromes. Recognition of a mitochondrial syndrome can lead directly to a specific genetic diagnosis. It is, however, well recognised that the same genetic mutation or deletion can give rise to varied phenotypes, and conversely certain phenotypes can be caused by mutations in a range of different genes.

Leigh syndrome and Leigh-like syndrome are infantile onset mitochondrial encephalopathies associated with bilateral symmetrical brain lesions particularly affecting the basal ganglia, thalamus

and brain stem. Affected individuals usually present in the first year of life with developmental regression, respiratory abnormalities, feeding difficulties and frequently with ophthalmic involvement (nystagmus, retinal dysfunction, ophthalmoplegia).[7,8] The genetic basis of these disorders is diverse, encompassing both nuclear and mtDNA mutations involving at least 89 different genes.[9] Originally a *post mortem* pathological diagnosis, typical *in vivo* MRI patterns are now the cornerstone of diagnosis of Leigh syndrome.

Alpers disease is defined by progressive neurodegeneration, refractory seizures and liver dysfunction. It is usually caused by mutations in the nuclear gene *POLG* which encodes the catalytic subunit of DNA polymerase gamma (polγ) which is required for the correct replication of mtDNA.[10] Dysfunction of polγ leads to progressive depletion of mtDNA with subsequent mitochondrial dysfunction. Valproate toxicity is well recognised and this should be avoided in patients with this diagnosis.

Mitochondrial Recessive Ataxia Syndrome (MIRAS) is one of the ataxia neuropathy spectrum disorders also caused by specific mutations in *POLG*.[11] This group of disorders is characterised by ataxia and neuropathy, variably associated with an encephalopathy with seizures.

MELAS syndrome is the combination of mitochondrial **M**yopathy, **E**ncephalopathy, **L**actic **A**cidosis and **S**troke-like episodes, with the majority of cases being caused by a common mtDNA mutation (m.3243A>G) in the mtDNA gene encoding a mitochondrial leucine transfer RNA (tRNA).[12] Stroke-like episodes are typically heralded by migraine headache, vomiting and seizures. Other clinical features include cognitive decline, ataxia, deafness, optic atrophy, short stature, diabetes and hypertrophic cardiomyopathy. The vast majority of individuals with the m.3243A>G mutation never develop strokes and have other clinical problems, most commonly maternally inherited diabetes and deafness.[13]

MERRF describes the phenotype of **M**yoclonic **E**pilepsy and **R**agged **R**ed **F**ibres, the latter being a histopathological description of the appearances on muscle biopsy. MERRF is often caused by a mutation in the mtDNA gene encoding the tRNA for lysine, and is one of the disorders specifically associated with epilepsy phenotypes, often with ataxia.[14] Some patients have additional visual, audiological and cardiac involvement.

Pearson syndrome is comprised of infantile onset sideroblastic anaemia (often transfusion dependent), lactic acidosis and variable pancreatic exocrine dysfunction.[15] It is associated with a sporadic large deletion in the mtDNA. Many infants do not survive and succumb to liver failure or

overwhelming acidosis in the first few years of life, but those who do live beyond infancy inevitably develop features of the Kearns-Sayre Syndrome.[16]

Kearns-Sayre Syndrome (KSS), also caused by a large mtDNA deletion, may follow Pearson syndrome or present later without preceding anaemia, with onset before 20 years of age with ptosis, progressive external ophthalmoplegia (PEO) and pigmentary retinopathy.[17] Cardiac conduction defects, cerebellar ataxia and cerebral folate deficiency are other features.[16]

Progressive External Ophthalmoplegia (PEO) is a feature of several disorders or can occur in isolation, and is often associated with mtDNA deletions, which may be single (sporadic) or multiple (secondary to autosomal dominant or recessive nuclear gene mutations).

Leber hereditary optic neuropathy (LHON) occurs due to specific mtDNA mutations giving rise in adolescence or adult life to painless visual loss, usually an isolated phenomenon affecting both eyes sequentially.

Neuropathy, Ataxia, Retinitis Pigmentosa (NARP) is often due to mutations in the mtDNA gene *MT-ATP6* encoding a subunit of ATP synthase. Individuals with high loads of the same mutations (typically >90%) present with maternally inherited Leigh syndrome.

Mitochondrial Neuro-Gastro-Intestinal Encephalopathy (MNGIE) presents with gastrointestinal dysmotility, peripheral neuropathy and eye involvement, and is caused by mutations in the nuclear gene *TYMP* that encodes thymidine phosphorylase.[18] This results in a mtDNA depletion syndrome. The enzyme deficiency results in abnormal blood and urinary thymidine and deoxyuridine concentrations, and so blood/urine purine/pyrimidine analysis can suggest the diagnosis.

Myopathy, Lactic Acidosis and Sideroblastic Anaemia (MLASA) is a rare autosomal recessive disorder predominantly affecting the skeletal musculature and bone marrow. It is usually caused by a defect of the YARS2 mitochondrial aminoacyl tRNA-synthetase or the PUS1 tRNA pseudo-uridinylation enzyme, both of which are involved in mitochondrial translation.[19]

Reversible Disorders

While most mitochondrial disorders are considered progressive, a small number display a reversible phenotype. Such disorders include the infantile reversible myopathy due to the mtDNA mutation m.14674T>C/G in the *MT-TE* gene encoding the tRNA for glutamate, and reversible infantile liver disease due to mutations in *TRMU* encoding an enzyme that functions in normal mitochondrial tRNA modification.[20] Prompt recognition of these rare reversible disorders may enable the appropriate

continuation of supportive treatment (such as prolonged ventilation) with the anticipation of subsequent recovery.

Investigation of Mitochondrial Disease

If a mitochondrial disorder is suspected, investigations aim firstly to provide additional evidence (clinical, biochemical, radiological) for mitochondrial disease, then secondly attempt to identify a specific genetic basis for the disease, and thirdly to evaluate for multi-system effects and in particular to detect treatable complications. In practice these three aims are addressed simultaneously.

Multi-system evaluation

Careful clinical evaluation may reveal multi-system dysfunction, and if signs or symptoms become evident specific investigations will be warranted. Table 2 includes examples of investigations that may be indicated in clinical baseline evaluation and on-going monitoring.

Specific Metabolic Biochemistry

Lactate may be elevated in blood and cerebrospinal fluid in mitochondrial disorders, but normal lactate levels do not exclude the possibility of a mitochondrial disorder. Measurement of lactate pre- and post-prandially can be useful in distinguishing mitochondrial disorders from disorders affecting glycogen metabolism. Lactate is the end product of anaerobic respiration (glycolysis), being reversibly converted from pyruvate by lactate dehydrogenase (LDH). Dysfunction of the respiratory chain results in impaired aerobic respiration, while the ratio of NADH to NAD^+ (reflecting the abnormal mitochondrial redox status) is increased, driving the production of lactate. Measurement of the lactate/pyruvate ratio can help distinguish respiratory chain dysfunction from defects in earlier steps in pyruvate metabolism, notably pyruvate dehydrogenase deficiency. Lactate may be artefactually increased if a blood sample is difficult to obtain. Lactate may be increased secondary to tissue hypoxia of any cause including sepsis or significant hypotension. Neonatal lactic acidosis is common after a hypoxic-ischaemic insult, but would be expected to normalise over a few days. Elevated CSF lactate is also seen in non-mitochondrial brain diseases such as encephalitis, however an elevated CSF lactate without evidence of an alternative brain insult or recent seizures is

suspicious for mitochondrial disease. The same applies to lactate detected non-invasively by magnetic resonance spectroscopy of the brain.

Plasma amino acids may show an elevated alanine concentration, again reflecting chronically increased pyruvate and lactate levels. Proline may also be elevated, while decreased citrulline may be seen in MELAS and NARP syndromes.[21,22]

Plasma acylcarnitines may be helpful in differentiating from other causes of lactic acidosis, including long chain fatty acid oxidation defects and organic acidaemias, and may also identify specific metabolites in disorders of flavin metabolism and disorders causing toxic damage to the respiratory chain, such as ethylmalonic acidaemia and HIBCH and ECHS1 deficiencies.[23,24]

Urine organic acid analysis may reveal elevated lactate and associated metabolites; intermediates of the TCA cycle, and ethylmalonic acid, may be seen as a non-specific indicator of mitochondrial dysfunction. 3-methylglutaconic aciduria is seen in a range of disorders including Barth syndrome.[25] Methylmalonic aciduria may be seen in SUCLA2 deficiency and related disorders.[26]

Biotinidase activity detects biotinidase deficiency, which should be ruled out as this is easily treated with biotin supplementation, and is included in routine newborn screening in some countries.

Novel biomarkers that have been reported to be elevated in mitochondrial disease include fibroblast growth factor 21 (FGF21) and growth and differentiation factor 15 (GDF15), but these are neither specific for mitochondrial disease nor are they elevated in all cases.[27,28]

Neuroimaging

Magnetic resonance imaging (MRI) is a powerful tool for evaluating brain involvement. Brain imaging may be undertaken during the investigation of a child with developmental issues, seizures or for other reasons, and certain features may give rise to the suspicion of mitochondrial disease. The central nervous system features seen in neuroradiological examination of mitochondrial disorders are diverse (figure 2). Typical features of Leigh syndrome include bilateral symmetrical signal abnormality in the basal ganglia (figure 2b) and brain stem. White matter may be diffusely abnormal (leukodystrophy) in a number of mitochondrial disorders including complex I (figure 2a) and complex II deficiency;[29] there can be non-specific cerebral atrophy; progressive cerebellar atrophy may be seen in mitochondrial disease but is not specific for this. Specific patterns of brain involvement may be associated with specific genotypes, including leukoecenphalopathy with brainstem and spinal cord involvement with elevated lactate (LBSL, associated with *DARS2* mutations).[30] Infarcts that

do not correspond to an arterial territory are a hallmark of MELAS syndrome (figure 2c,d).[12] Structural brain abnormalities may be seen for example in pyruvate dehydrogenase complex (PDH) deficiency.[31]

Muscle Biopsy

Historically a muscle biopsy (either open surgical, or with large-bore needle biopsy) for histopathological tests and functional enzyme assays has been an important investigation for suspected mitochondrial diseases, although more recently the advent of more rapid genetic testing with next generation sequencing has superseded the need for a muscle biopsy in some situations. However a muscle biopsy may be helpful in differentiating from other causes of myopathy.

Histochemistry

Histochemical findings can be suggestive of mitochondrial disease, including the detection of ragged red fibres on the modified Gomori trichrome stain, or the observation of cytochrome *c* oxidase-negative fibres. Non-specific findings include excess lipid deposition and mitochondrial ultrastructural changes on electron microscopic examination. Recently, a modified immunohistochemical technique known as quadruple OXPHOS immunofluorescence has been reported to provide more quantitative data about oxidative phosphorylation function.[32] Occasionally cardiac muscle biopsies undertaken to investigate an unexplained cardiomyopathy reveal features suggestive of mitochondrial disease, including giant mitochondria and abnormal mitochondrial ultrastructural appearances.

Respiratory Chain Enzymology

Spectrophotometric assays of complexes I-IV in a frozen muscle homogenate assess the function of the respiratory chain complexes and can identify either multiple or isolated complex deficiencies. Combined assay of complexes I+III or II+III indirectly assesses muscle coenzyme Q_{10} (CoQ_{10}) which shuttles electrons between these two complexes, and muscle CoQ_{10} can also be quantified directly. Complex V can be assayed by Polarographic analysis of fresh muscle mitochondria or by Blue native gel assay of frozen muscle homogenate.

Isolated complex deficiencies (e.g. of complex I) are often caused by mutations in a gene encoding either one of the structural subunits of the complex or an associated assembly factor, and identification of a specific isolated complex deficiency can help direct further targeted genetic analysis.

Mitochondrial DNA depletion syndromes are caused by nuclear gene defects affecting genes responsible for the maintenance and replication of the mtDNA, including *POLG, TK2, RRM2B* and *DGUOK* (figure 1a). These result in multiple deficiencies of the respiratory chain complexes. Similarly, disorders affecting mitochondrial DNA transcription or translation can result in multiple complex deficiencies. Since complex II is entirely encoded by nuclear genes it is not primarily affected in disorders of mtDNA (although complex II activity may be decreased secondarily if the muscle is severely compromised).

A skin biopsy may be obtained either as a punch biopsy or at the time of an open muscle biopsy, to obtain cultured fibroblasts. These can be used to assess oxidative phosphorylation function, e.g. by microscale oxygraphy, or to assay PDH and pyruvate carboxylase activity; PDH can also be assayed in the muscle sample.[31]

It should be noted that muscle respiratory chain enzymology may be normal in a patient with genetically confirmed mitochondrial disease, for example where the tissue-specific phenotype does not involve muscle.

Molecular Genetics

The constellation of features from the clinical history, examination and results of initial biochemical and radiological investigations can direct analysis to specific candidate genes (e.g. screening for specific mtDNA mutations in MELAS, LHON or NARP syndromes, or for *POLG* mutations in Alpers syndrome). Particular patterns of respiratory chain enzyme complex deficiencies on muscle biopsy can also suggest groups of genes to be evaluated. The family history can suggest whether a nuclear gene defect (in a consanguineous family with multiple affected individuals on both sides of the pedigree) or a mtDNA defect (matrilineal pattern of inheritance) is more likely.

Nuclear genes can be assessed with DNA extracted from blood. mtDNA mutation load varies from tissue to tissue, with highest levels of mutation heteroplasmy expected in affected tissues, and as a result mtDNA analysis is best performed on DNA extracted from an affected tissue, usually muscle DNA. However, in infants with severe mtDNA-encoded disease it is expected that the blood heteroplasmy level will be sufficiently high that analysis of blood DNA will detect mtDNA mutations.

mtDNA analysis may start with analysis for large scale deletions or rearrangements, followed by specific common point mutation analysis, and finally full mtDNA sequencing. Individual candidate nuclear genes can be Sanger-sequenced directly. However the genetic complexity underlying

mitochondrial disease, with hundreds of candidate genes, means that this is an inefficient approach and increasingly next generation sequencing is being employed to evaluate larger panels of genes or whole exomes or genomes. Furthermore, as we move away from muscle biopsies as the gold standard diagnostic test, novel bioinformatics tools will be needed to help confirm pathogenicity of observed genetic variants.[9]

Parental blood DNA samples are required to evaluate nuclear gene mutations identified in the proband; DNA samples from blood and urinary cellular sediments from the mother may be evaluated in the case of mtDNA encoded disorders to determine maternal levels of mtDNA mutation heteroplasmy. Subsequently it may be appropriate to undertake cascade family screening. Identification of the mode of inheritance can also help inform discussions about recurrence risk for future children and potential reproductive options. Involvement of clinical genetics teams is important.

Management of mitochondrial disorders

The management of a child with mitochondrial disease requires the input of many different healthcare professionals (including metabolic physicians, neurologists, cardiologists, endocrinologists, gastroenterologists, nephrologists, intensivists, ophthalmologists, audiological physicians, community paediatricians, dietitians, nurses, physiotherapists, speech and language therapists and psychologists) collaborating to optimise clinical management, addressing the numerous complications that arise. Ensuring good nutrition, monitoring growth and developmental parameters, providing appropriate support for education and physical mobility needs are all critical aspects of management.

General supportive interventions need to be tailored according to the patient-specific problems that arise, and can span numerous medical specialities (Table 3). Some invasive interventions such as solid organ transplantation may not be considered appropriate in the context of progressive multi-system disease, but may be considered if there is an isolated single-organ failure in limited circumstances.

Specific therapies

There is also a growing evidence base for specific therapies for mitochondrial disorders,[33] although the challenges of undertaking rigorous randomised controlled trials in such disparate and rare disorders is reflected in the relatively small number of high quality trials identified by the Cochrane review process.[34]

Lactic acidosis

Lactic acidaemia if severe can result in acidosis; in such situations appropriate use of sodium bicarbonate to normalise acid-base status is required. Dichloroacetate stimulates PDH activity by inhibition of PDH kinase and can decrease lactate levels. However, the impact on long term outcome is not clear, and long term use may be associated with peripheral nerve toxicity.[34,35]

Treatable disorders

There is a small group of readily treated disorders that respond to specific therapies, and these should be identified promptly and treatment initiated in a timely manner.[5] These include use of CoQ_{10} supplements for CoQ_{10} biosynthesis defects; riboflavin (vitamin B2) in riboflavin-responsive disorders such as ACAD9 or FLAD1 deficiency;[36,37] biotin in biotinidase deficiency; and combinations of biotin and thiamine in high doses for biotin-thiamine responsive basal ganglia disease.[38,39]. Thiamine is given if PDH deficiency is suspected as some defects are thiamine responsive. A deficiency of 5-methyltetrahydrofolate (the major transport form of folic acid) has been reported in a several mitochondrial disorders including KSS and is treatable with regular folinic acid. Supplementation with these therapies may be initiated empirically while awaiting final diagnostic investigations.

Arginine in MELAS syndrome

There is growing evidence for the use of arginine therapy in MELAS syndrome for both acute treatment and prevention of stroke-like episodes.[12] Arginine is required for nitric oxide synthesis, and the observation of low arginine and citrulline levels in patients particularly during acute stroke episodes supports the hypothesis that vasodysregulation contributes to the aetiology of the stroke-like episodes.

Potentially detrimental drugs

Certain drugs have the potential to be detrimental in the context of a mitochondrial disorder and should be avoided or used with caution.[40] These include sodium valproate (risk of hepatotoxicity, and contra-indicated in patients with *POLG* mutations), and anaesthetic agents such as propofol.[41]

Novel therapies under evaluation

Antioxidant therapy

Excessive production of reactive oxygen species occurs when the respiratory chain is dysfunctional, and it is thought that consequent oxidative stress may play an important role in the pathophysiology of mitochondrial disorders.[35] Numerous antioxidants have been used in the treatment of mitochondrial diseases. Therapies include CoQ_{10},[42] and the related compounds idebenone [33] and EPI-743.[43] N-acetylcysteine is a cysteine donor and can replenish glutathione, although evidence for therapeutic efficacy is lacking. Vitamin C, E and chemical analogues are also being evaluated.

Stimulating mitochondrial biogenesis

Approaches aimed at increasing mitochondrial biogenesis have been evaluated, using both pharmacological approaches such as bezafibrate (a PPARα activator) or resveratrol (a SIRT1 sirtuin activator) as well as dietary approaches via the ketogenic diet, which all result in up-regulation of mitochondrial gene expression and subsequent mitochondrial biogenesis.[35] Recently, decanoic acid, a C10 fatty acid produced in response to the ketogenic diet, has been found *in vitro* to stimulate mitochondrial biogenesis,[44] and may have efficacy in primary mitochondrial disease although formal clinical trials are still needed.[45]

Other novel therapies

Other novel therapeutic approaches include: targeting the mitochondrial membrane and its complex lipids;[46] replacing nucleosides in mtDNA depletion disorders that are associated with nucleoside deficiency; [47] and gene therapy approaches including recoding mtDNA-encoded genes to be expressed in the nuclear genome (allotopic gene expression), “conventional” viral-vector mediated nuclear-targeted gene therapies, and selective destruction of mutant mtDNA using restriction endonuclease technologies (see [5]).

Future reproductive options

While detailed comment is beyond the scope of this review, parents of a child with mitochondrial disease, or who are known carriers of either nuclear or mitochondrial gene mutations, may consider a number of options for future children, including antenatal testing, pre-implantation genetic diagnosis [48] or potentially "mitochondrial donation" technologies [49] although these methods have generated medical ethical debate.

Conclusions

Understanding of normal mitochondrial physiology has been furthered through the insights gained from the clinical and laboratory investigation of patients with diverse mitochondrial genetic disorders. Early consideration of the possibility of a mitochondrial disorder, followed by careful clinical assessment and biochemical, radiological and genetic investigation can lead to a diagnosis of mitochondrial disease. Diagnosis will then prompt symptomatic, and in some situations, disease modifying, therapies. Novel therapies in the pipeline aim to further improve the prognosis for children affected by these frequently devastating disorders.

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Tables

Table 1: Diverse clinical features of mitochondrial disease	
Central nervous system	Strokes Seizures Neurological/ developmental regression Neuroradiological features
Peripheral nervous system	Peripheral neuropathy
Eyes	Ptosis Ophthalmoplegia Nystagmus Visual disturbance Retinitis pigmentosa
Hearing	Sensorineural deafness/ hearing impairment
Endocrine	Growth hormone deficiency Thyroid or parathyroid dysfunction Multiple hormonal deficiencies
Pancreatic	Diabetes mellitus Exocrine insufficiency
Hepatic	Liver failure
Gastrointestinal	Enteropathy, dysmotility, pseudo-obstruction
Muscular	Myopathy
Haematological	Anaemia Marrow failure
Renal	Tubular dysfunction Progressive renal impairment Steroid-resistant nephrotic (primary coenzyme Q_{10} deficiency)
Cardiac	Conduction defects Cardiomyopathy
Dermatological	Hypertrichosis Pili torti (some complex III assembly defects)

Table 2: Multi-system evaluation	
Central nervous system	Neuroimaging: MRI, CT for acute stroke episodes Electroencephalogram (EEG)
Peripheral nervous system	Nerve conduction studies, electromyography
Eyes	Ophthalmological referral Visual evoked potentials/ electroretinogram
Hearing	Audiological assessment Speech and language evaluation
Endocrine	Growth parameters Hormone assays (growth hormone, thyroid, parathyroid, adrenal function)
Pancreatic	Blood glucose monitoring, HbA1c Faecal elastase (exocrine function) Amylase, lipase
Hepatic	Hepatic ultrasound Liver function tests
Gastrointestinal	Assessment of swallow Growth monitoring
Muscular	Physiotherapy evaluation
Haematological	Full blood count and film
Renal	Tubular function assays (urine NAG/creatinine and RBP/creatinine) Renal function tests
Cardiac	ECG Echocardiogram

Table 3: Examples of multi-system supportive interventions		
System	***Feature***	***Treatment***
Central nervous system	Seizures	Anticonvulsants
	Stroke-like episodes (MELAS)	Arginine
	Dysphagia	SALT intervention, gastrostomy
Eyes	Ptosis Visual dysfunction	Brow suspension Visual aids
Hearing	Hearing impairment	Hearing aids, cochlear implant
Endocrine	Hormone deficiency	Appropriate hormone supplementation
Pancreatic	Diabetes Pancreatic exocrine insufficiency	Insulin or oral antidiabetic agents Enzyme supplementation
Hepatic	Liver dysfunction, failure	Consider hepatic transplant if isolated liver disease
Gastrointestinal	Dysmotility, swallow dysfunction	Gastrostomy Enteral feeds Consider parenteral nutrition
Muscular	Myopathy	Physiotherapy, appropriate mobility aids and supports
Haematological	Anaemia	Transfusion Iron/ haematinics
Renal	Tubular dysfunction	Electrolyte supplementation Consider renal replacement therapy
Cardiac	Conduction anomalies Cardiomyopathy	Pacing, Implantable defibrillator Medical treatment (Lisinopril, beta blockers) Consider cardiac transplant

Figures

Figure 1 A: Nuclear genes associated with mitochondrial disease (Adapted from [5])

OXPHOS structural subunits

Complex I

NDUFS1 NDUFS2 NDUFS3
NDUFS4 NDUFS6 NDUFS7
NDUFS8 NDUFV1 NDUFV2
NDUFA1 NDUFA2 NDUFA9
NDUFA10 NDUFA11 NDUFA12
NDUFA13 NDUFB3 NDUFB9
NDUFB11

Complex II

SDHA SDHB SDHC SDHD

Complex III

UQCRB UQCRQ CYC1 UQCRC2

Complex IV

COX4I2 COX6A1 COX6B1 COX7B
COX8A NDUFA4

Complex V

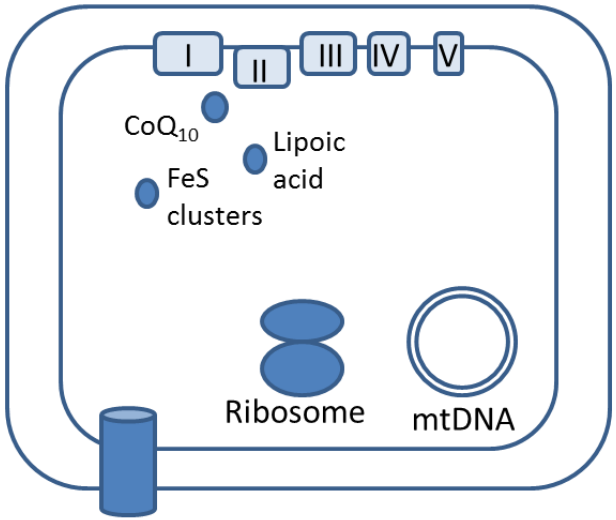
ATP5E ATP5A1

Membrane function, lipid metabolism, dynamics, import

TAZ AGK SERAC1 MFN2 OPA1 GDAP1
DNM1L MFF STAT2 YME1L AIFM1
TIMM8A TIMM50 DNAJC19 GFER
SLC25A3 SLC25A4 SLC25A12 SLC25A13
SLC25A19 SLC25A22 SLC25A25
SLC25A26 SLC25A32 SLC25A40
SLC25A42 SLC25A46 MICU1 QIL1
MPC1

Inhibitors

ETHE1 HIBCH ECHS1 TXN2



Cofactor biosynthesis

PDSS1 PDSS2 COQ2 ADCK3
ADCK4 COQ4 COQ6 COQ7 COQ9
BOLA3 LIAS LIPT1 GLRX5 IBA57
ISCU ISCA2 FXN FDX1L NFU1
ABCB7 SFXN4 LYRM4 NFS1 TPK1
MECR FLAD1 NADK2 NAXE

Mitochondrial translation

ELAC2 PUS1 MTO1 TRMU
GTPBP3 TRIT1 TRMT5 TRMT10C
HSD17B10 NSUN3 TRNT1 PNPT1
MRPS7 MRPS16 MRPS22
MRPS23 MRPL3 MRPL12
MRPL44 GFM1 GFM2 TSFM
TUFM AARS2 CARS2 DARS2
EARS2 FARS2 HARS2 IARS2
LARS2 MARS2 NARS2 PARS2
RARS2 SARS2 TARS2 VARS2
YARS2 QRSL1 GARS KARS
MTFMT MTPAP LRPPRC TACO1
C12orf65 RMND1

OXPHOS assembly factors

Complex I

NDUFAF1 NDUFAF2 NDUFAF3
NDUFAF4 NDUFAF5 NDUFAF6
FOXRED1 ACAD9 NUBPL
TMEM126B

Complex II

SDHAF1 SDHAF2

Complex III

BCS1L HCCS TTC19 UQCC2
LYRM7

Complex IV

SURF1 SCO1 SCO2 COX10 COX14
COX15 COX20 COA5 COA6 COA7
FASTKD2 PET100 CEP89

Complex V

ATPAF2 TMEM70

mtDNA maintenance

POLG POLG2 TWNK TYMP
DGUOK TK2 RRM2B SUCLA2
SUCLG1 MPV17 FBXL4 DNA2
MGME1 ABAT RNASEH1
SAMHD1

Others

TMEM126A SPG7 HSPD1 AFG3L2
TRAP1 DARS LARS NNT POP1
APOPT1 CLPB CLPP LONP1
CHCHD10 OPA3

Figure 1 B: Mitochondrial DNA (mtDNA) genes, all associated with mitochondrial disease.

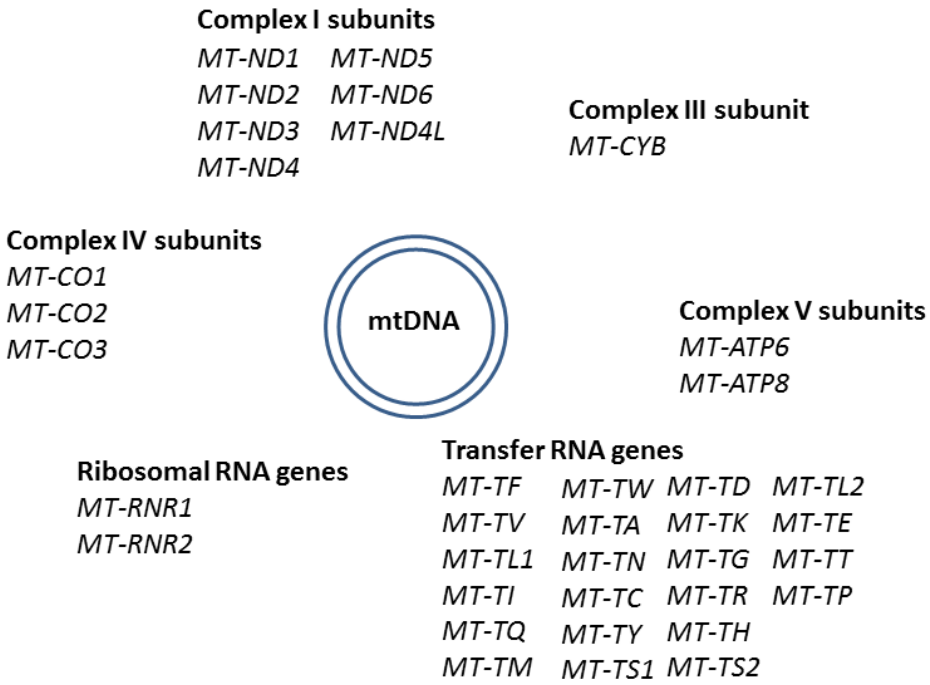


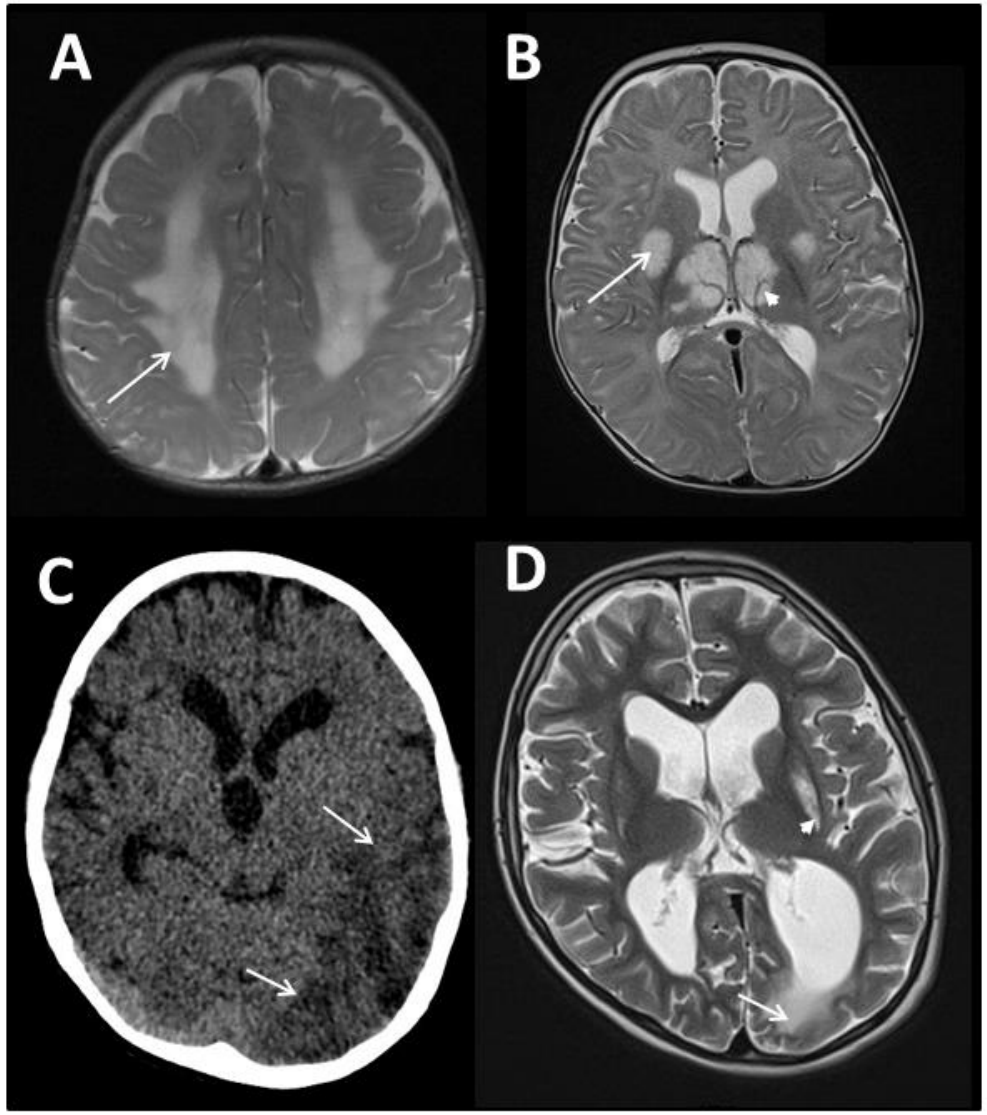
Figure 2: Neuroradiological features in mitochondrial diseases.

A: Axial T2-weighted MRI image from infant with complex I deficiency due to *NDUFV2* mutations showing widespread symmetrical increased white matter signal (leukodystrophy) (arrow)

B: Axial T2-weighted MRI image from infant with Leigh disease due to mtDNA *MT-ND3* mutation, showing increased signal in basal ganglia (arrow) and thalami (arrowhead)

C: Axial CT scan from child with MELAS syndrome showing acute left parieto-occipital infarct with signal hypointensity (arrows).

D: Axial T2-weighted MRI image from same child as (C), obtained several months after acute insult, showing cortical/subcortical volume loss in infarcted region (arrow), ventriculomegaly, and also abnormal striatum (arrowhead).


A
B
C
D