

## Review Article

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# Mitochondrial disorders: Challenges in diagnosis & treatment

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**Mitochondrial dysfunctions are known to be responsible for a number of heterogenous clinical presentations with multi-systemic involvement. Impaired oxidative phosphorylation leading to a decrease in cellular energy (ATP) production is the most important cause underlying these disorders. Despite significant progress made in the field of mitochondrial medicine during the last two decades, the molecular mechanisms underlying these disorders are not fully understood. Since the identification of first mitochondrial DNA (mtDNA) mutation in 1988, there has been an exponential rise in the identification of mtDNA and nuclear DNA mutations that are responsible for mitochondrial dysfunction and disease. Genetic complexity together with ever widening clinical spectrum associated with mitochondrial dysfunction poses a major challenge in diagnosis and treatment. Effective therapy has remained elusive till date and is mostly efficient in relieving symptoms. In this review, we discuss the important clinical and genetic features of mitochondrials disorders with special emphasis on diagnosis and treatment.**

**Key words** Diagnosis - diseases - mitochondrial DNA - mutation - treatment

## Introduction

Mitochondria are double membrane subcellular organelles, responsible for producing the major portion of cellular adenosine triphosphate (ATP) by oxidative phosphorylation (OXPHOS)<sup>1</sup>. The electron transport chain (ETC) consists of about 80 different polypeptides, which are organized into five transmembrane protein complexes (I-V). The proton gradient generated by complexes I, III and IV is released through ATP synthase or complex V, which results in phosphorylation of adenosine diphosphate (ADP) to ATP<sup>2</sup>. In addition to the OXPHOS machinery, mitochondria are also known as metabolic signalling center of the cells<sup>3</sup>,

performing many important biological functions such as regulation of apoptosis<sup>4,5</sup>, maintenance of cytosolic calcium homeostasis, lipid biosynthesis<sup>6,7</sup>, and iron sulphur cluster biogenesis<sup>1</sup>. These are the major source of reactive oxygen species (ROS), which can influence homeostatic signalling pathways, controlling the cell differentiation and proliferation<sup>8</sup>.

Mitochondrial dysfunctions are associated with a large proportion of human diseases, such as neurodegenerative disorders, cardiovascular disorders, neurometabolic diseases, cancer, obesity, *etc.*<sup>9,10</sup>. These are heterogeneous group of diseases with varying clinical features, showing tissue-specific manifestations

and affecting multiple organ systems. During the last two decades, there has been a rise in the studies related to the pathogenicity of mitochondrial DNA (mtDNA) mutations, and their role in the expression of disease phenotype. Although earlier considered to be a rare class of disorders, with about one in one million people being affected, the recent epidemiological studies suggest that at least 1 in 5000 individuals being affected by mitochondrial dysfunction and diseases<sup>11</sup>. Though the prevalence of individual mutations is much higher, approaching 1 in 200 live births<sup>12,13</sup>, only a small proportion of individuals harbouring these mutations develop disease<sup>14</sup>.

At present, there is no definite cure for mitochondrial disorders, with most of the available treatments being directed towards relieving the symptoms<sup>15</sup>. Proper diagnosis of mitochondrial disorders is important for prognosis and to provide counselling. As the underlying mechanisms of mitochondrial disorders are not fully understood, these remain the major challenge in terms of proper diagnosis and treatment. In this review, we present an overview of mitochondrial disorders with special emphasis on diagnosis and treatment.

### Mitochondrial genetics

The mitochondrial genome is a compact, double stranded, circular, 16569 base pairs long molecule, consisting of 37 genes, including 13 polypeptides, 22 transfer RNA (tRNA) and two ribosomal RNA (rRNA) genes<sup>16</sup>. The majority of proteins required for mitochondrial function are nuclear encoded, which are imported and sorted into each mitochondrial compartment<sup>17,18</sup>. The unique characteristic of mtDNA that differs from nuclear DNA (nuDNA) is the presence of multiple copies in a single cell, maternal mode of inheritance, and the absence of introns<sup>19,20</sup>. Mutation rate of mtDNA is much higher than nuDNA, due to very few non-coding sequences and most importantly, its presence in the vicinity of high ROS rich environment (the OXPHOS system) in the inner mitochondrial membrane<sup>21,22</sup>.

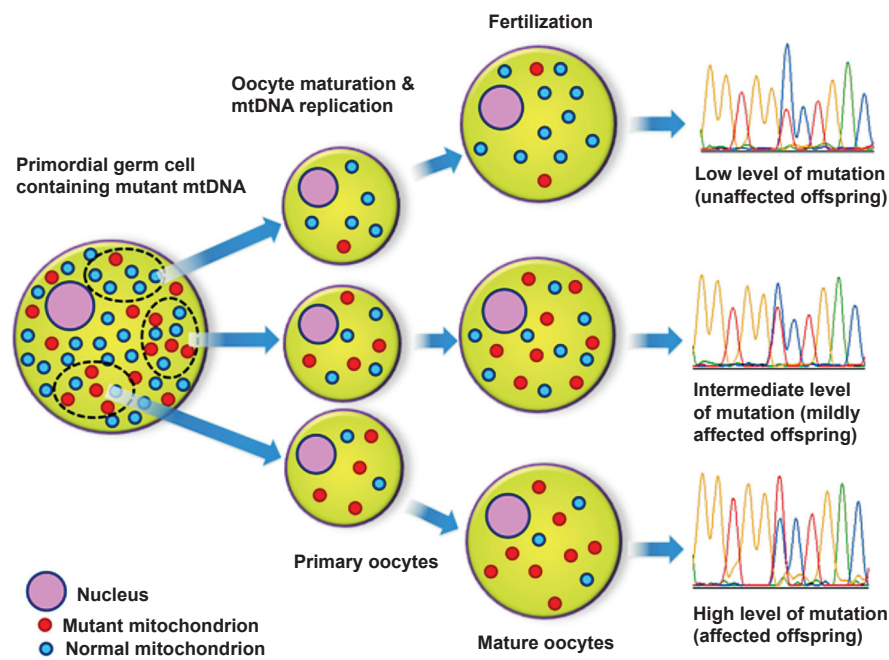
Due to the presence of multiple copies of mtDNA in each cell (1000 to 100000), both wild type and mutant mtDNA molecules can coexist, a condition known as heteroplasmy<sup>23,24</sup>. Most of the pathogenic mutations responsible for expression of the disease phenotype are present in heteroplasmic condition<sup>25</sup>. There are a few exceptions like Leber hereditary optic neuropathy (LHON), where mutations are mostly present in homoplasmic condition in both

symptomatic, and asymptomatic individuals<sup>10,26</sup>. For causing any biochemical defect in the cell, and for the phenotypic expression of diseases, the percentage of mutant mtDNA must exceed the tissue-specific critical threshold level, which is up to 50-60 per cent of mutant to wild type mtDNA<sup>27</sup>. However, in case of some tRNA mutations, the threshold level has been shown to be > 90 per cent<sup>9,28</sup>, while another study suggests < 25 per cent mutant level (C5545T in *MT-TW* gene) in clinically affected tissues<sup>29</sup>. The difference in the level of heteroplasmy is one of the major reasons for clinical variability of diseases in different patients, and in individuals within the same family<sup>30,31</sup>.

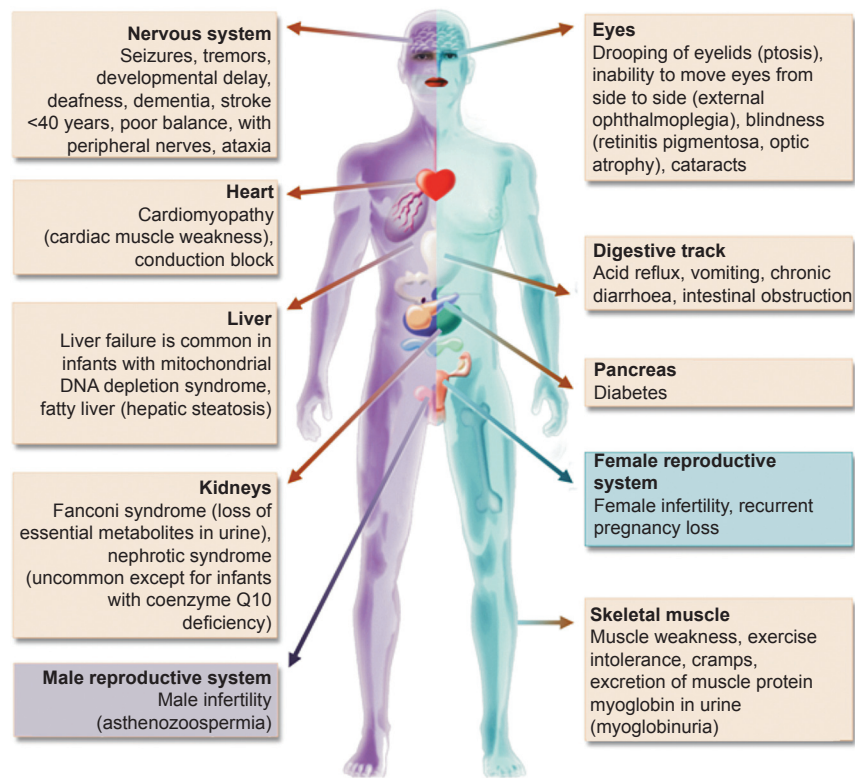
The transmission of disease causing mtDNA from heteroplasmic mother to the offspring shows a high degree of genetic and phenotypic variability between siblings<sup>32</sup>. This variability is very well explained by a phenomenon known as “mitochondrial genetic bottleneck”<sup>33</sup>. Comparison of the heteroplasmic level in offspring with those of oocytes at different stages of development has revealed that the bottleneck occurs in the early stages of oogenesis<sup>34</sup>. During this process, there is a significant reduction in the number of mtDNA molecules (the bottleneck). Following fertilization, heteroplasmic mtDNA mutation present in the oocyte segregates to either of the two daughter cells (Fig. 1). This is a random process, which generates variability in the transmitted mutation load to the offspring; thus, making it possible for an unaffected heteroplasmic female to have children, who are either unaffected or mildly to severely affected<sup>35,36</sup>.

### Mitochondrial DNA mutations and diseases

Till date, more than 300 mutations have been reported, that are known to cause a spectrum of mitochondrial diseases<sup>37</sup>. Failure to produce an adequate amount of ATP or energy is considered the main reason for most of the mitochondrial pathologies, which result in multisystemic disorders. The clinical presentations are extremely severe in high energy demanding tissues, such as skeletal muscle, central nervous system, and heart muscles; however, mitochondrial dysfunction might affect any organ of the body (Fig. 2). The clinical presentation of mitochondrial disorders can be highly suggestive of a particular disease phenotype, with well recognised clinical symptoms, suggesting specific mtDNA defect<sup>38,39</sup> (Table). However, there are large numbers of patients, who are presented with group of clinical symptoms that are suggestive of particular mitochondrial disorders, but do not precisely fit in any disease category.



**Fig. 1.** Schematic picture showing the mitochondrial genetic bottleneck. Selected number of mtDNA molecules are transferred to each cell during the production of primary oocyte. The rapid replication of mtDNA in mature oocyte causes random shift of mutation load between generations and is responsible for the variable levels of mutant mtDNA in affected offspring.



**Fig. 2.** Schematic picture showing the clinical features and the organs affected by mitochondrial diseases. Additional information is also given in the Table.

**Table.** Clinical presentations of mitochondrial syndromes

Syndrome	Common genotype	Age at onset	Clinical features
Mitochondrial encephalopathy with lactic acidosis and stroke-like episodes (MELAS)	Point mutations mtDNA (>80% <i>MTTL1</i> gene) (m.3243A>G)	Late childhood or adulthood	Stroke-like episode with seizures. Other features include intermittent episodes of encephalopathy, vomiting, migraine, diabetes mellitus, cardiomyopathy, SNHI, pigmentary retinopathy, cerebellar ataxia.
Myoclonic epilepsy with ragged red fibers (MERRF)	Point mutation mtDNA ( <i>MTTK</i> ) (m.8344A>G, m.8356C>T and m.8363G>A)	Childhood or early adulthood	Myoclonus, epilepsy, cerebellar ataxia and myopathy; other features include dementia, optic atrophy, bilateral SNHI, pyramidal signs, spasticity, multiple lipomas.
Neuropathy, ataxia and retinitis pigmentosa (NARP)	Point mutation mtDNA ( <i>MTATP6</i> / <i>MTATP8</i> ) (m.8993T>G)	Late child or adult	Peripheral neuropathy, ataxia, pigmentary retinopathy.
Leber hereditary optic neuropathy (LHON)	Point mutation mtDNA ( <i>MTND1</i> / <i>MTND4</i> / <i>MTND6</i> )	Adult	Optic neuropathy with sub-acute bilateral deterioration in vision; 95% of patients harbouring one of three common point mutations: m.11778G>A, m.3460G>A or m.14484T>C.
Chronic progressive external ophthalmoplegia (CPEO)	Large-scale single or multiple deletions. Point mutation mtDNA (m.3243A>G and m.12316G>A)	Early adult	External ophthalmoplegia and bilateral ptosis. Other features may include proximal myopathy, cardiac conduction defects, and dysphagia, etc.
Maternally inherited Leigh syndrome (MILS)	Point mutation mtDNA (m.8993T>C, m.10158T>C, m.10191T>C)	Infancy and early childhood	Myoclonus, seizures, developmental delay, muscle weakness, problems with movement, heart disease, kidney problems and difficulty in breathing.
Pearson syndrome	Large-scale single mtDNA deletion	Early childhood	Sideroblastic anaemia, pancytopenia, exocrine pancreatic failure, renal tubular defects. Survivors develop Kearns–Sayre syndrome.
Kearns–Sayre syndrome (KSS)	Single/large-scale deletions	Later childhood	Progressive external ophthalmoplegia, pigmentary retinopathy, raised cerebrospinal fluid protein, cerebellar ataxia, cognitive impairment, deafness, cardiomyopathy, complete heart block, short stature, dysphagia and endocrinopathies.

Source: Refs 10, 14, 26, 30, 40–45

Presence of mtDNA mutations in protein coding subunits of OXPHOS system, causes isolated biochemical deficiency of that particular complex<sup>46,47</sup>. The mutations of rRNA and tRNA genes may impair overall mitochondrial translation machinery, due to non-availability of functional RNA molecules, causing multiple deficiencies of all complexes<sup>32,48–50</sup>. The disease-causing point mutations are mostly heteroplasmic in nature, displaying considerable clinical heterogeneity<sup>40</sup>. Considering the high mutation rate,

and the presence of a very high number of population-specific polymorphisms, the distinction between a neutral mtDNA variant and disease-causing mutation is often difficult. Even though certain nucleotide changes are not pathogenic, these can still modulate the effect of deleterious mtDNA mutations<sup>51,52</sup>. Apart from nucleotide substitutions, patients with rearrangements in mtDNA have also been reported. The most commonly observed rearrangements of mtDNA are single nucleotide to large scale deletions. Till date,



more than 120 different mtDNA deletion types have been reported to be associated with different diseases<sup>37</sup>. These deletions are always heteroplasmic and sporadic in nature, which are not transmitted to the offsprings<sup>41</sup>. Pearson's syndrome, Kearns-Sayre syndrome (KSS) and chronic progressive external ophthalmoplegia (CPEO) are the most common clinical phenotypes being associated with the single sporadic mtDNA deletions. Occurrence of multiple deletions of varying lengths in a tissue-specific manner is mostly due to inherited nuDNA mutations<sup>42,53</sup>.

### Nuclear DNA mutations in mitochondrial diseases

Mitochondria contain more than 1500 proteins, which may vary in a tissue-specific manner. Due to dual genetic control of nuDNA and mtDNA, mutations in either of these genomes or both could be responsible for mitochondrial dysfunction and diseases<sup>54</sup>. Most of the mutations in nuclear gene can cause mitochondrial dysfunction by disturbing the structural and functional integrity of mitochondrial genome<sup>9</sup>. The major disease-causing nuclear genes are mainly responsible for maintenance, expression and copy number difference of mtDNA, for example; *POLG1* encoding for the catalytic subunit<sup>55</sup>, *POLG2* encoding for the accessory subunit of mitochondrial polymerase gamma, *PEO1* encoding for the mitochondrial helicase twinkle<sup>56</sup>, and thymidine kinase 2 (*TK2*)<sup>57</sup>. The other nuclear genes that have been reported to cause various diseases are those that have an important role in nucleoside transport, salvage or synthesis; such as *SLC25A4*, encoding adenine nucleotide translocator 1 (*ANT1*)<sup>58</sup> and *RRM2B*<sup>59</sup>, encoding for a subunit of the p53-inducible ribonucleotide reductase protein required for maintaining balanced mitochondrial deoxyribonucleoside triphosphates (dNTP) pool.

Mutation in genes responsible for assembly and formation of the cytochrome c oxidase (*COX*) complex such as *SURF1*, *SCO1*, *SCO2* and *COX10* have been reported to be responsible for Leigh syndrome (LS) and fatal infantile cardiomyopathy with severe deficiency in heart and skeletal muscles<sup>60-63</sup>. Similarly, mutations in both nuclear-encoded assembly factors of complex I (*NDUFAF1*, *NDUFAF2*, *NDUFAF3*, *NDUFAF4*, *C8ORF38*, *C20ORF7*)<sup>64,65</sup> and nuclear encoded subunits of complex I (*NDUFS1*, *NDUFS2*, *NDUFS3*, *NDUFS4*, *NDUFS6*, *NDUFS7*)<sup>66,67</sup> have been shown to cause different clinical phenotypes. Mitochondrial translational genes such as; mitochondrial aminoacyl tRNA synthetase (*AARSs*), *DARS2*, *RARS2*, *EARS2*,

*YARS2*, *HARS2*, *AARS2* and *SARS2* are also reported to be associated with a number of clinical phenotypes<sup>68-72</sup>.

### Investigation of mitochondrial diseases

Investigation of mitochondrial disorders requires an integrated approach, such as clinical, biochemical, histological and genetic for unambiguous diagnosis.

### Clinical manifestation of mitochondrial diseases

The clinical diagnosis of patients with mitochondrial disorders poses a major challenge to clinicians due to highly variable clinical features and age of onset. The investigation of any mitochondrial disorder is often complicated, because the same genetic and biochemical defect can present in a variety of phenotypes and *vice versa*<sup>73</sup>. A detailed history and examination of the patient with suspected mitochondrial disease is crucial for both initiation and interpretation of the relevant investigations. Clinical manifestations may affect any system in the body including visual [retinitis pigmentosa, ptosis, progressive loss of vision (LHON), atrophy, optic neuropathy], auditory (sensorineural deafness), muscular (weakness, exercise intolerance, dysphagia, dysarthria), neurological (seizures, migraine, focal deficits ataxia), cardiac (cardiomyopathy, arrhythmias, conduction blocks), gastrointestinal (constipation, pseudo-obstruction), endocrinal (diabetes, short stature, hypoparathyroidism), renal (renal tubular acidosis, Fanconi syndrome, glomerulonephritis) or liver failure mostly observed in early infancy or young childhood in addition to other clinical presentations (Fig. 2). Any combination of these symptoms especially, if maternally inherited, could raise the suspicion of mitochondrial disease.

All patients should have an electrocardiography (ECG) to investigate possible cardiac changes and abnormality. Electromyography (EMG) is mostly performed in cases of myopathy. However, in some cases, EMG may be normal, even in the presence of clinical myopathy and nerve conduction studies can demonstrate either an axonal or a mixed axonal-demyelinating peripheral sensorimotor neuropathy<sup>10</sup>. Computed tomography (CT) imaging is appropriate in an acute setting, to rule out raised intracranial pressure in a child, who presents with seizures or encephalopathy. This also helps identifying basal ganglia calcification, which is one of the common presentations in mitochondrial diseases. Magnetic resonance imaging (MRI) findings are mostly heterogeneous and non-specific, depending on metabolic brain defects, age

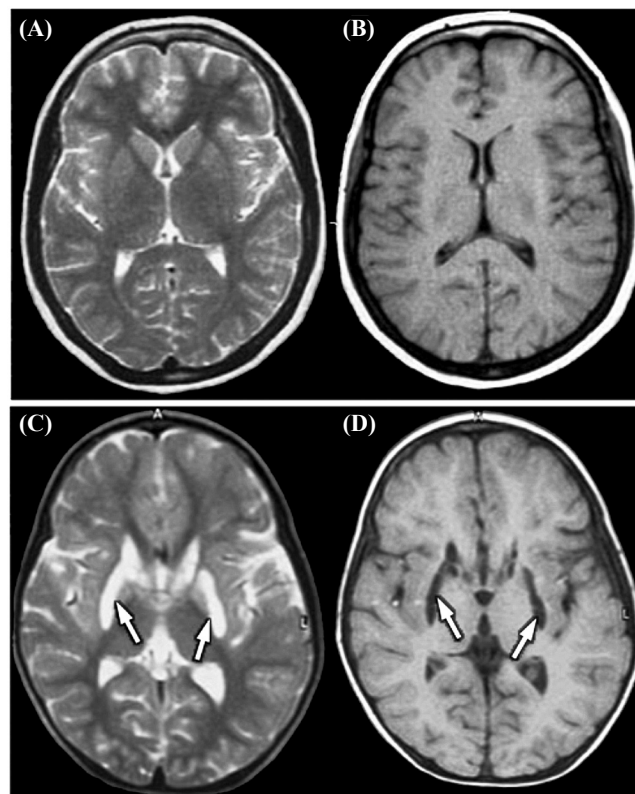
of the patient and severity of the disease<sup>74</sup>. Specific MRI findings have been identified in patients with syndromic phenotypes, such as; symmetric hypodensities of the brainstem, thalamus and basal ganglia mostly seen in LS<sup>75,76</sup> (Fig. 3), asymmetrical, multifocal, and high signal often occurring in the occipital and parietal lobes are observed in mitochondrial encephalopathy, lactic acidosis, with stroke-like episodes (MELAS) cases, which may be permanent or reversible. Abnormalities of the white matter are characteristic of some forms of mitochondrial diseases, like in patients with Kearns-Sayre syndrome (KSS) or mitochondrial neurogastrointestinal encephalopathy (MNGIE). A common pattern of brain MRI imaging such as abnormal signal intensities in brainstem and subtentorial nuclei with lactate peak can be a clue of complex I deficiency<sup>78,79</sup>, while atrophy of the cortex, cerebellum and cerebellar atrophy are other common features observed.

### Biochemical investigations

Simple biochemical examinations of blood would provide very efficient and supporting evidence for clinical investigation. These tests include measuring the levels of lactate, pyruvate, creatine kinase (CK) in the blood, total blood count, thyroid and liver function, bone chemistry, and random blood glucose<sup>38</sup>. CK may show increased level in many cases, but is often found in normal range. Elevation in lactate and pyruvate concentration is usually observed in patients of the mitochondrial diseases<sup>80</sup>. Although elevated lactate level in serum and CSF may be helpful in diagnosis of mitochondrial diseases, it lacks consistency as some patients with confirmed diagnosis do not show increased lactate level. Patients with LS and MELAS usually have elevated lactate, compared to patients with chronic progressive external ophthalmoplegia (CPEO), LHON who rarely demonstrate high lactate. However, presence of elevated CSF lactate together with seizures and stroke like episodes are highly suggestive of mitochondrial disorder and such results should be interpreted with caution<sup>10</sup>. In addition, organic acids and amino acid profiles (urine, plasma and CSF) along with the appropriate clinical features are helpful in making a proper diagnosis.

### Muscle histochemistry

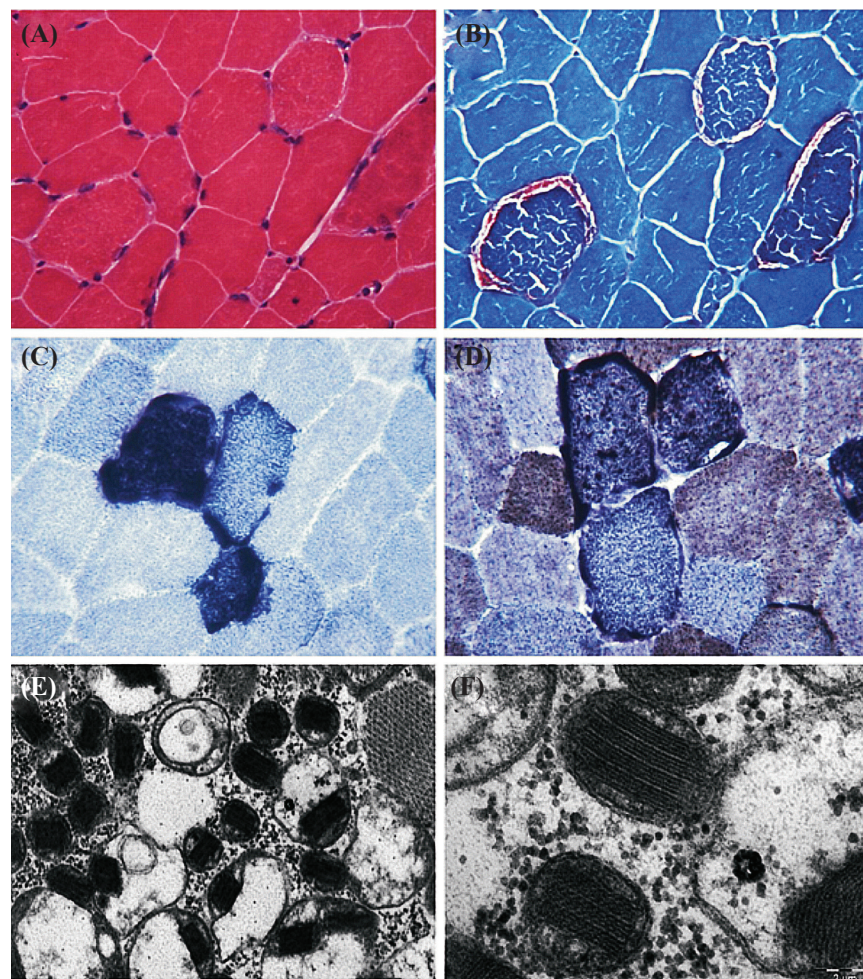
Histological and histochemical findings are one of the most recognized and standard approaches used for the diagnosis of mitochondrial disorders. A fresh biopsy of skeletal muscle or needle biopsy is essential



**Fig. 3.** (A) T2-weighted magnetic resonance (MR) images of a normal child, and (B) Axial T1, and (C) T2- weighted MR images of the patient showing bilateral basal ganglionic lesions, highly characteristic of Leigh disease (shown by arrows), and (D) Axial T1. Source: Ref. 77.

for the histochemical analysis of OXPHOS defect. Hematoxylin and Eosin (HE) staining is generally used for histological examination. These dyes are essential for recognizing various tissue types and the morphological changes and structural information that form the basis for many diseases (Fig. 4A). Presence of “Ragged Red Fibres” (RRF) on staining with modified gomorie trichrome (MGT) is generally indicative of an abnormal subsarcolemmal accumulation of mitochondria (Fig. 4B), which is one of the important features of mitochondrial disorders<sup>40</sup>. Similarly, the activity for succinate dehydrogenase (SDH) is used for identifying complex II disorders (Fig. 4C), and is unaffected by mtDNA mutations<sup>81</sup>. However, presence of RRF in other muscle diseases, and in the muscles of healthy athletes<sup>43</sup> poses a specific challenge in considering this as a gold standard method only for mitochondrial disorders. Presence of cytochrome c oxidase (COX) negative fibres are generally indicative of decrease in complex IV activity (Fig. 4D), and suggestive





**Fig. 4.** Tissue section showing the pathological abnormalities of mitochondrial disorders. (A) Hematoxylin and Eosin (HE) stain; (B) Red ragged fibers appear on modified gomori trichome (MGT) stain suggesting abnormal subsarcolemmal accumulation of mitochondria; (C) Blue red fibers on succinate dehydrogenase (SDH) staining, suggesting an increased number of mitochondria; (D) COX staining shows absence of enzymes of respiratory chain. (E & F) Electron micrograph showing low and high magnification of mitochondria with paracrystalline inclusions (E x10000, F x40000).

of mitochondrial dysfunction<sup>9</sup>. Most of the patients generally show mosaic pattern of COX deficient fibres, which is due to heteroplasmic mutations and difference in the percentage of mutant mtDNA in different cells<sup>73</sup>. Sequential reaction of COX and SDH histochemical activities is helpful, when identifying very low levels of COX negative fibres. The uniform decrease in COX activity in muscles is generally indicative of mutations in nuDNA either encoding COX subunits or COX assembly factors, such as *SURF1*, *SCO2*, *etc.*<sup>60</sup>. However, in many cases the patients having high level of MT-tRNA mutations, or large scale deletions also show global decrease in COX activity<sup>82</sup>. Caution should be taken in diagnosis of elderly patients, who

generally show COX negative fibres due to age related accumulation of mtDNA deletions within individual fibres<sup>83</sup>.

Although the electron microscopic changes in mitochondria, such as abnormal mitochondria (size and shape), abnormal cristae pattern and paracrystalline inclusions (Fig. 4 E & F) provide additional information and aid in diagnosis, but these are not considered as a standard procedure for diagnosis of mitochondrial diseases<sup>39</sup>. Also, certain classes of mitochondrial disorders such as LHON, neuropathy, ataxia and retinitis pigmentosa (NARP) and maternally inherited Leigh syndrome (MILS) due to mtDNA mutations, do not show abnormalities in histopathology. Enzyme

activity for the respiratory chain complexes could be assessed in isolated mitochondria from tissue or cultured cells by spectrophotometry. These complexes are either studied in isolation or in linkage with other complexes, however, it should be taken into consideration that poorly prepared samples or other enzyme deficiencies may also lead to secondary respiratory chain deficiencies<sup>14</sup>.

### Molecular genetic testing

Genetic investigation for patients with suspected mitochondrial disorders should be undertaken after detailed clinical, biochemical and histopathological examination. The information from these sources provides a rational approach for molecular investigations and in understanding the genotype-phenotype correlation<sup>38,81</sup>. Although high levels of mutations can be detected in blood, it is always advisable to use skeletal muscle for detecting mtDNA mutations, especially for deletions or mtDNA rearrangements.

Rearrangements of mtDNA, such as single/multiple deletions and duplications are usually detected by Southern hybridization and long range PCR<sup>10</sup>. The presence of multiple deletions usually points towards defects in the nuclear genes, which have an important function in mtDNA replication and maintenance (*POLG*, *POLG2*, *SLC25A4*, *PEO1*, and *OPA1*). Depletion of mtDNA can be detected by real-time PCR, which is also indicative of nuclear gene mutations, predominantly present in infants with myopathy or hepatocerebral phenotype<sup>84,85</sup>.

Commonly occurring disease causing point mutations, such as A3243G, A8344G, T8993G/C and primary *LHON* mutations can be screened in the DNA, isolated from blood, using restriction fragment length polymorphism (RFLP) or direct sequencing of amplified mtDNA. Most of the diseases causing mtDNA mutations exist in the heteroplasmic state, which can be assessed by either RFLP or real-time PCR using TaqMan assay. A number of techniques such as denaturing gradient electrophoresis (DGGE), denaturing high performance liquid chromatography (dHPLC) and emerging technologies such as Affymetrix MitoChip (2.0) and next generation sequencing methods have made sequencing of entire mtDNA reliable and easy. However, due to highly polymorphic nature of the mitochondrial genome, care must be taken before assigning pathogenicity to a novel mtDNA variant. The criteria to assign pathogenicity to a particular mtDNA mutation proposed by DiMauro and Schon<sup>27</sup> are widely

used by the scientists. These include (i) The mutation must not be a known neutral polymorphism; (ii) The base change must affect an evolutionarily conserved and functionally important site; (iii) Deleterious mutations are usually heteroplasmic; (iv) The degree of heteroplasmy in different family members ought to be in rough agreement with the severity of symptoms; and (v) mutational load should be different in normal and abnormal fibres during single fibre PCR analysis. However, there are exceptions due to occurrence of a number of pathogenic mtDNA mutations that fail to meet the criteria. Whole mtDNA sequencing or screening techniques are increasingly being employed for diagnostic purposes in many laboratories; however, given the highly polymorphic nature of mtDNA, pathogenicity of any new mutation should be assigned and interpreted with caution.

### Management of mitochondrial diseases

Despite considerable advances made over the last decade in understanding the pathogenesis of mitochondrial disorders, there is no effective treatment<sup>86,87</sup>. Only in very rare cases, surgery and transplantations provide momentary relief. Genetic complexity together with high variability in phenotypic expression of these diseases poses a major challenge not only in proper diagnosis; but also in the development of effective therapeutic approaches. Most of the studies for treatments are either limited by the number of patients or very diverse clinical presentation of the disease<sup>15</sup>. The optimal management of mitochondrial diseases can be divided into three major groups: counselling, supportive therapy and pharmacological therapy.

### Genetic counselling and prenatal diagnosis

Clinical and genetic heterogeneity are the major hallmarks of mtDNA disease. For example, A3243G mutation in tRNA leucine can be responsible for severe clinical phenotypes in patients, and is also found in asymptomatic relatives, causing either mild phenotypic abnormality such as deafness with diabetes, cardiomyopathy or severe encephalopathy with death at young age<sup>88,89</sup>. Manifestation of any clinical phenotype depends upon the mutant mtDNA in post mitotic tissues, however, any prognostic advice only relying on blood samples should be given with caution, since the level of mutant mtDNA may increase or decrease with time<sup>38,43</sup>. Nuclear and environmental factors may also influence the expression of mtDNA defect. Hence, it is advisable to interpret the genetic data within the context of the family being investigated.



Due to complexities of mtDNA transmission and segregation, prenatal diagnosis for mitochondrial disorders have not been very successful<sup>90</sup>. The results obtained from chorionic villus sampling (CVS) have not been very reliable for prenatal diagnosis due to the difference in the level of mutant mtDNA in CVS and the foetus<sup>77</sup>. Studies have also shown that the mtDNA mutants may segregate in the placenta; as a result, single CVS samples may not represent the mutant level in the whole placenta. Prenatal genetic diagnosis has been successfully employed in a few mtDNA point mutations, such as T8993G causing NARP and A3243G for MELAS in high risk families<sup>44,45</sup>. Due to difficulties in predicting the heteroplasmic mtDNA segregation within tissues, and final clinical expression, it is always advisable to perform several estimations during pregnancy. For the vast majority of mtDNA mutations, prenatal genetic diagnosis is still hindered due to the inability to predict the clinical severity expected from the amount of mutant mtDNA measured in foetal tissues<sup>91</sup>. Pre-implantation genetic diagnosis (PGD) of mtDNA disorders has also been applied to a very limited extent for a very few common point mutations. PGD generally eliminates the risk of an affected child by selecting an embryo with 0 per cent mutation<sup>92</sup>.

## Treatments

### Exercise therapy

Exercise trainings mostly aim to improve the physical capacity and quality of life in patients by enhancing the mitochondrial function, and decreasing the burden of unhealthy mitochondria. Several studies have shown that the reversal of a sedentary lifestyle in mitochondrial disease with exercise therapy confers benefits to mitochondrial function by improved OXPHOS activity<sup>93</sup>. Also, the benefit of endurance exercises on *COX10* knockout mice showed delay in the disease onset, increased ATP levels, OXPHOS activity, and life expectancy<sup>94</sup>.

### Diet

Caloric needs for the patients with mitochondrial disease are mostly altered compared to normal individuals. Optimizing the quantity and quality of calories has been shown to improve the mitochondrial health in patients<sup>95</sup>. The ketogenic diet (KD) is a high fat and low glucose diet, which stimulates lipid utilization by mitochondrial beta oxidation and ketone body production in the liver<sup>96</sup>. KD has been used for many years in children suffering from seizures, who

are mostly resistant to conventional antiepileptic drugs (AEDs)<sup>86</sup>. Studies also showed a reduction in epileptic seizures in children with epilepsy and RC complex deficiency, however, the diet was not able to control disease progression<sup>97</sup>. The use of KD has been shown to improve the brain energy metabolism by upregulating mitochondrial biogenesis<sup>98</sup>, inhibiting ROS production<sup>99</sup>, increasing neuron glia interaction and ATP concentration<sup>100</sup>. The use of KD has been shown to shift the level of heteroplasmy in cells harbouring mtDNA deletion<sup>101</sup>. In a study on deleter mice model with a late onset of myopathy, carbohydrate restricted KD induced mitochondrial biogenesis, slowing down disease progression and reducing the amount of COX negative muscle fibres<sup>96</sup>. These studies suggest the potential usefulness of KD in treatment of mitochondrial diseases. However, more controlled trials on patients and better elucidation of its mechanism are still warranted.

### Pharmacological treatment

The rationale for using cocktails of vitamins and co-factors is more compelling, when factors in question are decreased either due to deficiency or defect in their transport; for example, diseases due to carnitine or CoQ10 deficiency<sup>102,103</sup>. Some of the common therapies that are provided to patients are discussed here.

**Coenzyme Q10:** Coenzyme Q10 (CoQ10) also known as ubiquinone, has been widely used for the treatment due to its well documented safety and lack of negative side effects even at higher concentrations. The most favourable property of CoQ10 is its dual role as a component of the respiratory chain and one of the most potent ROS scavengers<sup>86,104</sup>. Despite the beneficial effects and widespread use of CoQ10 in mitochondrial diseases, controlled trials of this drug on large cohorts of patients are still missing. The supplementation of CoQ10 showed positive results in patients with primary or secondary CoQ10 deficiency and is advisable to be tried in all patients with decreased CoQ10 concentration<sup>105-108</sup>.

**Creatine:** In addition to CoQ10, creatine is used extensively as an energy boosting compound for the treatment of mitochondrial disorders. A randomized, controlled trial in adult patients with mitochondrial cytopathies showed benefit from creatine intake at an initial dose of 5 g twice a day for two weeks followed by 2 g twice a day for one week<sup>109,110</sup>. A study exploring the effect of the three compounds showed a different mechanism of action, *i.e.* (CoQ10 and

creatine) increased ATP production, (CoQ10 and lipoic acid) scavenging ROS, while only creatine provided an alternative energy source<sup>111</sup>. Given the conflicting results on the use of creatine alone, it is always advisable to give creatine in combination with CoQ10 in patients showing positive response with these supplements<sup>86</sup>.

**L-Arginine:** Arginine is a semi-essential amino acid, involved in growth, urea detoxification, and creatine synthesis. An initial small study demonstrated that intravenous administration of L-arginine (500 mg/kg/dose) decreased the severity of stroke like symptoms, enhanced the dynamics of microcirculation, and reduced tissue injury from ischaemia in patients with MELAS<sup>112</sup>. In a large study, a decrease in clinical severity and frequency of stroke like events was demonstrated in MELAS patients, who were treated prophylactically with oral L-arginine (150-300 mg/kg/d)<sup>113</sup>. However, randomized controlled trials are required to study the effects of L-arginine in treatment of mitochondrial strokes.

**Carnitine:** Carnitine is a cellular compound that plays a critical role in the process of fatty acid oxidation and esterification of fatty acids by transferring long-chain fatty acids across the mitochondrial inner membrane as acyl carnitine esters. These esters are oxidized to acetyl CoA, which enters the Krebs cycle and results in subsequent generation of ATP via oxidative phosphorylation<sup>114</sup>. Primary carnitine deficiency due to defective synthesis is not a typical feature of mitochondrial disorder, but in patients with mitochondrial dysfunction a lower level of carnitine in plasma has been observed. While L-carnitine supplementation is mostly used in patients with mitochondrial disorders to restore free carnitine levels but is rarely used alone. Mostly L-carnitine is used either with valproic acid as it inhibits the carnitine uptake when used alone<sup>115</sup>, or is used together with CoQ10<sup>116</sup>.

**Dichloroacetate:** Dichloroacetate (DCA) is more specifically used as a lactic acid lowering agent. It activates the pyruvate dehydrogenase complex by inhibiting the activity of the pyruvate dehydrogenase kinase, which normally phosphorylates and inhibits the enzyme<sup>117</sup>. DCA has the ability to keep the pyruvate dehydrogenase complex in an active state, which reduces the accumulation of lactate in body tissues. There have been multiple reports about the use of DCA with various degrees of success in lowering the lactic acid level<sup>87</sup>. In a controlled clinical trial, use of 25mg/kg/day of DCA in MELAS patients resulted in peripheral

nerve toxicity, which resulted in discontinuation and termination of study<sup>118</sup>. Due to absence of any beneficial effects and potential role in nerve toxicity, DCA is not recommended to patients with MELAS and should be avoided in cases prone to development of peripheral neuropathy.

**Idebenone:** Idebenone is an analogue of coenzyme Q that facilitates electron transfer. Idebenone has been favourably used in LHON patients. A study on the effects of idebenone on fibroblasts of LHON patients showed marked improvement in the activity of complex I but showed variable effects on cell respiration suggesting that patients might not respond uniformly to this treatment<sup>119</sup>. Randomized controlled trial of LHON patients showed marked improvement in visual acuities and visual recovery with idebenone treatment<sup>120, 121</sup>.

**Other redox agents:** Thiamine (B1), vitamins C and E, and alpha-lipoic acid have been used in mitochondrial disease patients, individually or as part of an antioxidant cocktail, but these have not been tested in controlled trials.

## Conclusion

Mitochondrial dysfunction is associated with an increasingly large number of heterogenous clinical presentations and disorders, such as neuromuscular disorders, cardiovascular disorders, metabolic syndrome, cancer, obesity, *etc.* It is well known that the mitochondrial disorders are complex and difficult to diagnose and treat. We, therefore, emphasized the importance of an integrated approach for appropriate clinical, histopathological, biochemical and genetic diagnosis, management and the treatment of patients with suspected clinical presentations. Proper understanding of mitochondrial genetics and investigative approaches will prove valuable for clinicians and patients aiding proper genetic counselling and prognosis. In the absence of effective treatment, patients with mitochondrial diseases require proper counselling and combination of supplements with pharmacological therapy.

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