Current Perspectives of Mitochondrial Dysfunction and Associated Diseases

Abstract
Mitochondria are morphologically dynamic cell organelles that meet majority of energy requirements of cells. Genetic or acquired mitochondrial dysfunctions may result into significant abnormalities in regular cell functions. Being the most active cell type, hepatocytes possess a larger number of mitochondria for higher energy necessities and hence more vulnerable to mitochondrial dysfunction. Clinically mitochondrial damage has been found to cause various diseases, most of which are untreatable. Heteroplasmic nature of mitochondrial DNA makes diagnosis and treatment of mitochondrial disorders more challenging. Although exercise and gene therapies are extensively being studied, effective treatment strategies for mitochondrial dysfunction disorders are still lacking. This review summarizes clinically well characterized mitochondrial dysfunctional disorders and a few key approaches being used and/or under investigation for diagnosis and treatment of the diseases. Comprehensive catalogs and indices to describe clinically significant mitochondrial aberrations are still under construction. Databases comprised of these catalogs and indices will help to develop targeted diagnostic and therapeutic approaches for mitochondrial disorders.

Keywords: Diagnosis of Mitochondrial Disorders; Dysfunctional Disorders; Hepatopathies; Mitochondria; Mitochondrial Genome; Mitochondrial Disorder Treatment; Mitochondrial Membrane

Abbreviations: CPEO: Chronic Progressive External Ophthalmoplegia; KSS: Kearns-Sayre Syndrome; MELAS: Mitochondrial Encephalopathy Lactic Acidosis Stroke-like Episodes; NARP: Neuropathy Ataxia Retinitis Pigmentosa; LHON: Leber’s Hereditary Optic Neuropathy; MERRF: Myoclonic Epilepsy and Ragged Red Fibers; iPSC: Induced Pluripotent Stem Cells; FIAU: Fialuridine; d4T: Stavudine; AZT: Zidovudine; ddl: Didanosine

Introduction
Mitochondria are intracellular organelles that serve as the metabolic powerhouses of cells since they are their primary source of ATP [1]. Mitochondria play a crucial role in maintaining the high energy requirements of certain cells types such as the hepatocytes, which have more number of mitochondria per cell than most other cell types like small lymphocytes [2]. Besides energy generation, several other characteristics of mitochondria make these essential for cells. Mitochondria are also involved in signaling [3,4], cellular differentiation [5] and cell death [6].

Morphological dynamics of mitochondria
Among intracellular organelles, mitochondria are structurally unique in that their surface consists of an outer and inner membrane. Both membranes create two compartments in the mitochondria called inter-membrane space and inner matrix. Integrity of inner and outer membranes of mitochondria is critical for mitochondrial function and is strictly regulated through interactions between different cellular proteins [7]. Both mitochondrial membranes differ from each other in composition, properties and function [1]. Though basic structure of phospholipids bilayer in the mitochondrial membranes is similar to the cell membranes, phospholipids compositions of the membranes of mitochondria differ from other membranes [8]. Outer membrane of mitochondria contains integral membrane proteins called porins, which allow large sized molecules to freely diffuse across the outer membrane of mitochondria [9]. Other proteins on the outer membrane, such as translocase, allow the movement of larger sized proteins to actively move across the membrane [10]. Other essential mitochondrial outer membrane proteins like TOM20 and TOM22 provide the negative charges to the outer membrane of mitochondria [11]. The inner membrane of mitochondria is extensively folded into cristae, which results in increased total surface area of the inner membrane compared to outer mitochondrial membrane [12]. Inner mitochondrial membrane closely resembles the bacterial membranes in their lipid composition, characterized by increased n-6 saturation because of presence of low levels of triglycerides [13]. Inner membrane is also freely permeable to some molecules, such as oxygen, carbon dioxide, and water, and is associated with numerous essential proteins and enzymes which are involved in aerobic respiration [14]. Together these proteins constitute respiratory complexes that are organized in specific order in inner membrane of mitochondria and take part in synthesis of ATP by mitochondria [15]. In spite of their size and complexity, mitochondria are extremely dynamic cell organelles and by processes of fusion, fission and movement across the cytoskeletal structure they change their shape and size frequently [16]. Mitochondria can divide independent of cell division and also increase in number in cells in response to the energy needs of the cell [17,18].
Frequent fission and fusion of mitochondria in cells permits exchange of mitochondrial genome between fusing mitochondria and hence provide an efficient means of homeostasis and inter-mitochondrial DNA complementation. This allows mitochondria to be morphologically highly polymorphic [12] and functionally independent within cells [17]. Different proteins play different roles in regulating morphological remodeling of mitochondria [19]. Several proteins such as Fis1/Mdv2 [20] or Drp1/Dnm1 [21,22] are localized on outer membrane of mitochondria and regulate fission of mitochondria. Once initiated, fission of mitochondria has been shown to take place in approximately 3 hours [23]. Other proteins like Mgm1/OPA1 [24,25] and Fzo1p/Mfn 1&2 [26] are localized on inner or outer membrane of mitochondria and involved in fusion of mitochondria.

Mitochondrial genome

Human mitochondrial genome is approximately 16,600 base pairs long and codes for 37 genes [27,28] which includes expression of 13 genes for respiratory complexes subunits, 22 for mitochondrial t-RNA and 2 for r-RNA [29]. Mitochondrial protein expression has dual genetic origin, that is, expression from cell nucleus and mitochondrial genome. Many of the vital structural and functional proteins of mitochondria are expressed from cell nucleus, and are transported to mitochondria via specific translocations and signal sequences. Each mitochondrion has 5-12 circular DNA copies, and unique metabolic systems enclosed within double membranes [30]. Mitochondria also possess own DNA replication machinery, which allows the mitochondrial DNA replication in inner matrix of mitochondria independent of cell nuclear DNA division. In contrast to cell nuclear DNA polymerase, which has proof-reading exonuclease mechanism that assists in repairing the errors or mis-matches occurred while DNA replication [31], mitochondrial DNA polymerase lacks nucleotide excision repair mechanisms [32] which results into accumulations of mis-matches and errors in mitochondrial DNA that take place during the DNA replication process or by radiation or chemical damage [33]. Even though a few kinds of mitochondrial DNA repair pathways, such as base excision repair [34], have been shown effective in mitochondrial genome repair, lack of other efficient proof reading and editing pathways and close proximity to reactive oxygen species from respiration complexes on inner membrane of mitochondria leads to increased damage and accumulation of mutations in mitochondrial DNA relative to nuclear DNA which further give rise to heteroplasmic mitochondrial DNA [35]. Penetrance and severity of expression of some mitochondrial disorders can be determined by proportion of accumulated mitochondrial DNA mutations. Some severe mutations may also cause cell, organ or organism death. However, milder mutations and heterogeneity can permit survival [36]. Moreover, introduction of normal mitochondrial DNA to mitochondria with mutated DNA by dynamic networking allows an efficient mechanism of mitochondria survival [37]. In addition, random distribution of mitochondria among daughter cells during cell division causes delivery of different proportions of mitochondria containing normal and mutant mitochondrial DNA to each daughter cell which further allows mitochondrial disease free survival of cells and organs. Mitochondrial DNA sequence heteroplasmy has also been used to determine lineages [38]. Despite the rearrangement of genes in mitochondrial DNA, comparison of DNA sequences in different species has shown high levels of homology. Concurrently, mitochondrial DNA sequences from different species can also be distinguished from each other on basis of non-homologous and rearranged mitochondrial DNA regions [39].

Mitochondrial regulation of cell apoptosis

Apoptosis is programmed cell death, which helps in cell number regulation or elimination of unwanted and potentially dangerous or diseased cells [40]. Apoptosis is tightly regulated by several types of signaling proteins and cascades. Proteolytic activity of caspase-protein family plays an important role in apoptosis [41]. Several mitochondrial proteins have been identified with various biochemical and molecular studies, which have a complex role in direct activation of cellular apoptotic programs in mammalian cells [42]. The outer mitochondrial membrane permeabilization also triggers the caspase proteases activation. Various pro-apoptotic proteins released from inter-membrane space of mitochondria, such as cytochrome c, also promotes caspase activation [43]. Consequently, integrity of outer membrane of mitochondria is highly regulated through complex interactions between numerous pro- and anti-apoptotic proteins in cells [44]. Injury to the outer membranes of mitochondria also results in mitochondrial dysfunction and causes cell death regardless of the activity of signaling cascades, though sometimes under some pathophysiological conditions cells can survive this damage [45].

Mitochondrial dysfunctional diseases

Recently mitochondrial dysfunctions have been found related to an array of human diseases such as hepatic mitochondrial disorders [46], cardiac dysfunction [47] and autism [48]. Both nuclear and mitochondrial genome mutations encoding mitochondrial proteins contribute to mitochondrial dysfunction [49]. Many factors including a threshold effect, and segregation and clonal expansion govern the penetrance of mitochondrial disease. Although with ageing accumulation of mutations increases, heteroplasmic mitochondrial DNA allow appropriate mitochondrial functions in cells until the ratio of mutated mitochondrial DNA to normal mitochondrial DNA increase significantly. At this threshold level clinical symptoms of mitochondrial dysfunction begin to appear [50]. Though there is random segregation of mitochondria into two daughter cells, clinical indicators of mitochondrial disorders may express when mutant load in a specific cell type exceeds the pathogenic threshold. In addition, clonal expansion of specific type of mutated mitochondrial DNA molecules may also cause amplified risk for the manifestation of mitochondrial disorders [51]. Many types of mutations are feasible in mitochondrial DNA. Point mutations may occur in protein-coding genes during mitochondrial DNA replication, and can cause major mitochondrial functional defects. Though being heteroplasmic, these mutations tend to be recessive; however increased levels of these mutations beyond threshold levels, may have deleterious effect on functions of mitochondria [52]. Large-scale deletions and rearrangements of DNA are another kind of mitochondrial DNA anomalies [53]. Aged tissues and individuals with neurodegenerative diseases have shown an exponential accumulation of such deletions in mitochondrial DNA [53,54]. Currently no therapeutic method is
available to replace or correct these dysfunctional mitochondria in cells. A database compiling comprehensive description and clinical properties of all known pathogenic and non-pathogenic mitochondrial DNA mutations is still under construction [55]. Population and mitochondrial genome based studies have revealed that mitochondrial diseases are relatively common [56]. Founder effects and genetic bottlenecks in some geographic areas can cause under- or over-representation of specific mitochondrial DNA disorders [57]. Studies based on childhood and adult mitochondrial syndromes suggest that prevalence of mitochondrial disorders is approximately 1 in 5000, and could be much higher [57]. Increased accumulation of mitochondrial mutations with aging also causes increased genetic and clinical manifestations of mitochondrial disorders [58]. However, the age of onset is also governed by the severity of the biochemical defect caused by mutations [59]. Several other nuclear genetic or environmental factors also play an important role in expression of disease [60]. Mitochondrial DNA mutations associated clinical symptoms are extremely variable and can express at any stage in life (Table 1). There is an increase in evidence supporting the association of mitochondrial DNA mutations to ageing, neurodegeneration, and tumorigenesis [58]. Following are a few examples of some mitochondrial diseases that show early onset in infancy or childhood.

Table 1: Summary of mitochondrial dysfunctional diseases.

<table>
<thead>
<tr>
<th>Age of Onset</th>
<th>Disease</th>
<th>Causes</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early-onset in infancy or childhood</td>
<td>Leigh syndrome</td>
<td>Mitochondrial DNA defects</td>
<td>Progressive neurodegeneration</td>
</tr>
<tr>
<td></td>
<td>Depletion syndrome</td>
<td>Decrease in copy number of mitochondrial DNA.</td>
<td>Muscle weakness; progressive encephalopathy; liver failure</td>
</tr>
<tr>
<td></td>
<td>Kearns–Sayre syndrome</td>
<td>Single or large-scale deletions in mitochondrial DNA</td>
<td>Multisystem failure; external ophthalmoplegia; neurological complications; cardiomyopathy; dysphagia.</td>
</tr>
<tr>
<td>Late-onset in childhood or adult life</td>
<td>Mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes</td>
<td>Mutations in protein coding regions of mitochondria</td>
<td>Brain and visual field defects; seizures; encephalopathy; vomiting; headaches; ataxia; cognitive impairment</td>
</tr>
<tr>
<td></td>
<td>Chronic progressive external ophthalmoplegia</td>
<td>Large-scale mitochondrial DNA deletions</td>
<td>Paralysis of the eye muscles</td>
</tr>
<tr>
<td></td>
<td>Neuropathy, ataxia, and retinitis pigmentosa</td>
<td>Maternally inherited mitochondrial DNA defects</td>
<td>Neuropathy; seizures; dementia</td>
</tr>
<tr>
<td></td>
<td>Leber’s hereditary optic neuropathy</td>
<td>Homoplasmic mitochondrial DNA mutations</td>
<td>Visual loss</td>
</tr>
<tr>
<td></td>
<td>Myoclonic epilepsy and ragged red fibers</td>
<td>Point mutations in mitochondrial DNA</td>
<td>Neurodegenerative disorders</td>
</tr>
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Leigh syndrome: Progressive neurodegenerative disorder due to severe failure of oxidative metabolism. Disease is caused by various mitochondrial DNA defects [61].

**Depletion syndrome:** Depletion syndrome found in several organs depending on specific tissues with mitochondrial DNA depletion, resulting in muscle weakness, progressive encephalopathy or liver failure [55]. Organ transplantation can be therapeutically effective for the patients because of tissue specific nature of the disorder. Such syndromes would also respond well to potential the tissue specific mitochondria replacement therapy, if developed, where healthy and functional mitochondria could be delivered to cells with depleted mitochondrial DNA.

**Kearns-Sayre syndrome (KSS):** Multisystem disorder, caused by single or large-scale deletions [49], including progressive external ophthalmoplegia, neurological complications, cardiomyopathy deafness, short stature and dysphagia.

Following are a few examples of some mitochondrial disorders that show late onset in childhood or adult life. mutations in protein coding regions of mitochondria
a. Mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes (MELAS): brain and visual field defects resulting in severe stroke-like episodes with seizures, encephalopathy, vomiting, migraine-like headaches, ataxia, and cognitive impairment. Several mitochondrial DNA mutations affecting protein-encoding genes or mitochondrial t-RNA expressing genes cause MELAS [61,62].

b. Chronic progressive external ophthalmoplegia (CPEO): progressive paralysis of the eye muscles caused by single or multiple large-scale mitochondrial DNA deletions [49,54].

c. Neuropathy, ataxia, and retinitis pigmentosa (NARP): neuropathy including developmental delay, seizures, and dementia. It has been found that patients with higher mutant load have an early onset in childhood because of maternally inherited mitochondrial DNA defects [36]. This reflects the importance of the levels of heteroplasmy influencing the penetrance of mitochondrial disorders.

d. Leber’s hereditary optic neuropathy (LHON): damage to retinal ganglion cells of the optic nerve leading to visual loss [63,64]. It is caused by homoplasmic mitochondrial DNA mutations, found more in males compared to females.

e. Myoclonic epilepsy and ragged red fibers (MERRF): progressive, neurodegenerative disease caused by point mutations in mitochondrial DNA [65].

Instead of location and size of deleted regions, the levels and distribution of deleted mitochondrial DNA in tissues and organs are more important factors in determination of clinical symptoms [66]. In majority of cases, mutations in mitochondrial DNA impairs the mitochondrial function, which further deprives the cells of its main source of energy required for its optimal function. This results in cell function impairment or death causing loss of tissue or organ function depending on levels of damage occurred at cellular levels.

Besides inheritance of mitochondrial DNA defects, environmental factors may also damage the mitochondria. Several toxic substances internalized by cells can directly or indirectly damage the mitochondria.

**Mitochondrial hepatopathies**

Hepatocytes require, and contain the highest density of mitochondria of all human cell types because of the high metabolic activity. Therefore, hepatocytes are more vulnerable to disorders that affect mitochondrial functions [67]. Disorders affecting normal mitochondrial activities directly regulate hepatocellular metabolism causing impaired liver function, steatosis and cell death. Clinically, genetic and more frequently drug and alcohol-induced mitochondrial damages have been found to cause severe liver diseases, which ultimately result in liver failure and death [68,69] (Table 2). Because of lack of distinct clinical symptoms, the diagnosis of mitochondrial hepatopathies is usually poor and current medical treatments available are largely ineffective. Nevertheless, numerous specific molecular defects (including mutations, deletion or rearrangement of mitochondrial DNA) in hepatic mitochondria have been recognized in recent years. Mitochondrial hepatopathies can be acquired due to alcohol or drug injury to cells or inherited as a mitochondrial genetic defects.

<table>
<thead>
<tr>
<th>Disease</th>
<th>Causes</th>
<th>Effect</th>
</tr>
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<tbody>
<tr>
<td>Neonatal Liver Failure</td>
<td>Defective mitochondrial gene expression in cell nucleus</td>
<td>Respiratory chain defects</td>
</tr>
<tr>
<td>Navajo Neurohepatopathy</td>
<td>Rearrangement in mitochondrial DNA</td>
<td>Cholestasis; cirrhosis; liver failure</td>
</tr>
<tr>
<td>Mitochondrial DNA depletion syndrome</td>
<td>Decreased copy number of mitochondrial DNA</td>
<td>Myopathy; hepatomegaly; progressive liver failure</td>
</tr>
<tr>
<td>Alpers-Huttenlocher Syndrome</td>
<td>Defective expression of mitochondrial genes</td>
<td>Hepatomegaly; jaundice; progressive coagulopathy; hypoglycemia</td>
</tr>
<tr>
<td>Villous Atrophy Syndrome</td>
<td>Deletion in mitochondrial DNA</td>
<td>Intra-cellular respiratory dysfunction</td>
</tr>
<tr>
<td>Pearson’s Syndrome</td>
<td>Mitochondrial DNA depletion and rearrangement</td>
<td>Failure of multi organ system such hematopoietic system, exocrine pancreas, liver, and kidneys</td>
</tr>
</tbody>
</table>

Table 2: Summary of genetic mitochondrial hepatopathies.
Genetic mitochondrial disorders of liver: The clinically mitochondrial hepatopathies are heterogeneous and can cause acute or chronic liver failure, lactic acidosis, and cholestasis. Following are a few examples of primary mitochondrial hepatopathies caused due to mitochondrial functional defects:

1. Neonatal Liver Failure: Occurrence of acute liver failure in few weeks to months old infants due to respiratory chain defects [70]. This is caused due to defective mitochondrial genes expressed in nuclear DNA. Disease is rapidly progressive and causes death within months of manifestation.

2. Navajo Neurohepatopathy: Rearrangement in mitochondrial DNA resulting in respiratory dysfunction [71] causes Navajo Neurohepatopathy. Clinical symptoms include cholestasis, cirrhosis, or liver failure that occur in infancy or childhood.

3. Mitochondrial DNA depletion syndrome: This disease is caused by decreased copy number of mitochondrial DNA and hence insufficient expression of mitochondrial genes from mitochondrial DNA [72]. Besides myopathy, hepatomegaly and progressive liver failure causes death in early age.

4. Alpers-Huttenlocher Syndrome (Delayed-Onset Liver Disease): Defective expression of mitochondrial genes in cell nucleus causes this diseases. It is characterized by hepatomegaly, jaundice, and progressive coagulopathy and hypoglycemia [73]. Studies show that onset of symptoms occurs between 2 months to 8 years of life.

5. Villous Atrophy Syndrome: This syndrome is caused due to deletion in mitochondrial DNA resulting in intra-cellular respiratory dysfunction [74]. Symptoms include severe anorexia, vomiting, chronic diarrhea, and villus atrophy in the first year of life. Hepatic involvement is characterized by mild elevation of aminotransferases, hepatomegaly, and steatosis.

6. Pearson’s Syndrome: Failure of multi organ system (hematopoietic system, exocrine pancreas, liver, and kidneys) occurs due to mitochondrial DNA depletion and rearrangement defects. Liver failure and death before the age of 4 years has been reported [75].

7. Various vitamins, cofactors, respiratory substrates, or antioxidant compounds are being used to by-pass injury to the respiratory function of mitochondria in cells [76,77]. Such clinical management is largely supportive and has very limited success [78]. Involvement of other organ systems limits advantages of liver surgery and transplantation [79,80]. Despite the extensive exploration of mitochondrial hepatopathies in recent years, much remains to be well-educated about these disorders.

Acquired mitochondrial disorders of liver: Besides mitochondrial hepatopathies caused by genetic variations, mitochondrial injuries in liver cells can also be acquired by exposure to alcohol and drugs which can cause serious damage to cells [69]. Hepatocytes are more susceptible to adverse effects of these drugs since detoxification of most drugs takes place in liver. Although hepatotoxicity may be caused through a wide range of sources [81], mitochondrial dysfunction is one major mechanism of injury [82]. Deleterious effects of drugs on hepatic mitochondria can affect mitochondrial DNA or disturb mitochondrial normal respiratory functions. This poses major concern for pharmaceutical companies since it causes failure of a drug or withdrawal of an already marketed medicine [68]. By obstructing mitochondrial respiratory function, injuring mitochondrial membrane, causing leak of pro-apoptotic proteins into cytoplasm or triggering mutations in mitochondrial DNA, drugs can cause necrosis or apoptosis of hepatocytes, leading to cytolysis and liver failure [82]. Depending on susceptibility, diverse hepatic or general symptoms, such as microvesicular steatosis, lesions, hypoglycemia hyperlactataemia, lactic acidosis, myopathy, rhabdomyolysis, peripheral neuropathy or lipoatrophy may occur [83,84]. Severe liver injury instigated by mitochondrial dysfunction in clinical trials can be avoided by careful evaluation of newly developed drugs during the preclinical safety studies.

Following are examples of a few mechanism through which drugs (or their metabolites) can induce mitochondrial dysfunction:

1. Induction of damage to outer membrane of mitochondria and release of pro-apoptotic proteins: Drugs that cause damage to outer membrane of mitochondria trigger the release of pro-apoptotic proteins into cytoplasm, such as cytocytechrome c. Release of pro-apoptotic proteins initiates apoptosis cascades resulting in cell death. Extensive hepatic cell death may result into liver failure. For example, acetaminophen, valproic acid, salicylic acid and others can cause fulminant hepatic failure [85,86]. The molecular mechanisms of induction of membrane damage by drugs are still poorly understood. Pro-apoptotic proteins from mitochondria can also be translocated indirectly from inter-membrane space to cytoplasm [87].

2. Respiration enzyme impairment: Some drugs behave as mitochondrial poisons by directly impairing one or more mitochondrial enzymes required for respiration process to produce ATP [88]. Higher intra-mitochondrial accrual of drugs like amiodarone, perhexiline or tamoxifen causes progressive reduction in mitochondrial respiration [88,89]. This leads to decreased ATP production in cells.

3. Other mechanisms: Several drugs can impair other normal functions of mitochondria, such as damage to fatty acid oxidation [90]. Others can cause more than one kind of impairment to mitochondria in cells [82].

Mitochondrial DNA mutation or copy number depletion: Several nucleoside based anti-viral drugs have been found to either inhibit mitochondrial DNA replication directly by inhibiting mitochondrial DNA polymerase, such as fialuridine (FIAU) [91] or by incorporation and termination of growing chain of mitochondrial DNA during replication, such as stavudine (d4T), zidovudine (AZT) or didanosine (ddI) [92,93]. Some other toxins can manipulate replication causing point mutations or deletions/ rearrangements in mitochondrial DNA.

Several environmental factors may enhance the risk of drug-induced mitochondrial dysfunction [60,94]. Evidence shows that
alcohol abuse can induce underlying mitochondrial dysfunctions, which may render the liver more susceptible to drug-induced mitochondrial injuries [94]. Some microsomal enzyme inducers or viral infections may also predispose cells to drug-induced mitochondrial damages in the liver [95,96]. Release of viral proteins, cytokines and interferon can disturb intracellular homeostasis that causes additive effects to drug-induced mitochondrial function impairment [84].

Unlike most other differentiated cell types, hepatocytes are capable of dividing in response to liver injury. Mitochondria in hepatocytes proliferate in response to cell division and energy needs. However, if mitochondria are considerably impaired, mitochondrial division gets compromised, and cell death and potentially liver failure ensues. Aside from liver transplantation, currently there is no means available to repair mitochondrial damage, or replace dysfunctional mitochondria. Development of a method to deliver healthy and functional mitochondria to hepatocytes that can replace the dysfunctional mitochondria could help treat several mitochondrial hepatopathies caused due to direct or drug/alcohol induced damage to mitochondria.

### Diagnosis and Treatment of Mitochondrial Dysfunctional Disorders

Complications in the diagnosis of mitochondrial DNA disorders arise because of lack of dear correlation between mitochondrial DNA genotype and disorder phenotype. Clinically, family history and histological variations have been found valuable to characterize mitochondrial disorders, but these are not always accurate. Molecular testing supported by clinical, histochemical, and biochemical testing has helped in development of improved rational diagnostic algorithms [97].

Following are a few key histochemical and molecular approaches currently under investigations for diagnosis of mitochondrial disorders:

**Histochemical and biochemical analysis:** Specific histological and histochemical alterations in affected tissue of some patients has allowed to determine some respiratory complex dysfunctions in mitochondria. Accumulation of defective mitochondria with cytochrome c oxidase abnormalities causes appearance of ragged-fibers like structures in affected tissues. Cytochrome c oxidase is an essential component of respiratory complex on inner membrane of mitochondria that takes part in ATP synthesis [98]. Any abnormalities in cytochrome c oxidase cause severe metabolic dysfunctions. Ragged-fibers like structures in tissues appear when mutations in mitochondrial DNA result into expression of defective subunits of cytochrome c oxidase. However tissue biopsies from patients with the MELAS phenotype or other mitochondrial DNA mutations have shown no distinct fiber like structures which limits the use of such histochemical testing. Biochemical evaluation of enzymes involved in respiratory function of mitochondria in affected tissue is also used to diagnose mitochondrial disorders in clinical labs [99].

Assessment of oxygen consumption and ATP synthesis by intact organelles from affected tissue is another approach to evaluate mitochondrial function [100]. The identification of specific mitochondrial enzyme abnormalities further helps to identify the particular molecular defects.

**Molecular genetic analysis:** Point mutations, deletions and rearrangements in mitochondrial DNA can be assayed by several genomic techniques such as Southern blotting [101,102], PCR or sequencing [103,104]. Loss of normal mitochondrial DNA or accumulation of defective mitochondrial DNA is also being determined by real-time PCR in clinical labs. With the help of these molecular techniques, association of several known genes have now been established with myopathic or hepatocerebral disorders. Small size of mitochondrial DNA allows relatively easy screening of entire mitochondrial DNA for rare or novel mutations. Besides some older techniques like denaturing gradient gel electrophoresis [102] and denaturing high-performance liquid chromatography [105], several new emerging technologies such as microarrays [97] have proven very valuable. Newly identified mutations and variations in mitochondrial DNA should be carefully assessed and associated with human diseases because of the extreme heteroplasmic nature of the mitochondrial DNA. Though several guidelines to establish a novel pathogenic mitochondrial DNA variant have been proposed and applied in clinical studies [106], still more canonical criteria are required for accurate identification and establishment of new variants associated with human mitochondrial disorders.

1. Despite of increased understanding of mitochondrial genetics, pathogenesis of mitochondrial DNA variations and associated disorders, currently other than rare helpful cases of transplant or surgery no effective treatment options are available. Attempts for management of mitochondrial dysfunctional disorders has been made with vitamins, cofactors, metabolites or electron acceptors to bypass respiratory complex defects [78]. More dedicated and diseases specific treatments are still under investigation. Following are a few approaches actively being pursued in this direction.

2. Exercise therapy: Aim of exercise therapy is to improve physical capability and quality of life in cases where myopathy is caused by mitochondrial DNA mutations. Although there is no noteworthy change in mitochondrial DNA mutations, evidence shows improved mitochondrial function [107]. Enhanced ATP levels, delayed disease onset and increased life expectancy has been observed in mice undergoing muscle training exercises [108]. Long-term training exercise studies are still under investigation and might help to evaluate the role of exercises on mitochondrial DNA mutations load. In another ongoing study, muscle regeneration is stimulated by proliferating undifferentiated myogenic cells in response to injury by resistance exercise. Once activated, undifferentiated myogenic cells would incorporate muscle cells with normal mitochondrial DNA which may help in shifting heteroplasmy levels significantly to recover muscular function in patients who have high levels of sporadically mutated mitochondrial DNA in mature muscle fibers [109]. Although this approach has been proven advantageous in clinics to some levels, additional studies are required to define the optimal parameters for exercise regime [110].

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3. Gene therapy: Researchers are actively working on a wide number of approaches to develop effective treatment of mitochondrial disorders. Manipulation of heteroplasmy levels in mitochondrial DNA such that balance of mitochondrial genome shifts from mutant to normal may also lead to decreased manifestation of mutations occurred in mitochondrial genome. Genetics-based strategies that would allow propagation of normal mitochondrial genome but inhibit the replication of mutated mitochondrial DNA are also under investigation [111]. Interfering molecules are being identified that could differentiate between normal and mutated mitochondrial DNA. In another study, restriction endonucleases are being tested that could distinguish between mutated and normal mitochondrial DNA. Such approach is limited to the restriction sites generated because of mutations in DNA [112]. Mutated mitochondrial DNA is also being selectively targeted with zinc finger DNA methylase which can bind the DNA in a sequence-specific manner and modify or cleave the mutated genome in mitochondria [113]. Major obstacle in these strategies is delivery of required molecules in matrix of mitochondria across the two mitochondrial membranes. Further investigations are ongoing to establish process of delivery and the ability of such molecules to hinder mutated mitochondrial DNA replication, and their efficiency in vivo.

Similar to many of mitochondrial proteins that are expressed in nucleus and transported to mitochondria, expression and transport of mitochondrial genes in nucleus which are mutated in mitochondrial DNA and causing mitochondria functional defects may help to recover normal functions of mitochondria [114]. Difficulties in mitochondrial localization of such nuclear expressions are anticipated [115]. Recently a mechanism has been demonstrated by which cytosolic t-RNA could be imported in mitochondria [116]. Such techniques may assist in treatment of disorders caused due to defects in genes expressing mitochondrial t-RNAs. Taken together, ongoing investigations to develop successful gene therapies for treatment of mitochondrial disorders are encouraging.

In another study to develop a potential treatment for mitochondrial disorders, reprogrammed cells derived from patients with dysfunctional mitochondria were implanted to repopulate cells with normal mitochondria in tissues [117]. Induced pluripotent stem cells (iPSC) exhibiting normal mitochondrial functions were also screened from a population of iPSCs derived from affected patients. Pluripotent cells were also generated by somatic cell nuclear transfer, that is replacement of nuclear DNA of the egg with nuclear DNA of the diseased somatic cells, and there by another method to obtain potential cells containing healthy mitochondria that can be used to correct mitochondrial disorders. Mitochondrial DNA mutations transmission from mother to offspring vary considerably [118] which creates challenges for providing the genetic counseling to affected families. Though prenatal genetic diagnosis may detect mitochondrial DNA mutation load, clinically major concern arises if heteroplasmy levels reflect phenotypic outcomes at other fetal tissue levels [119]. Currently, mitochondrial gene replacement methods offer great potential for development of techniques to prevent human mitochondrial DNA disease transmission. Transfer of the pronuclei from a fertilized zygote of a female mouse with mitochondrial DNA disease to an enucleated zygote from a healthy female donor has produced healthy offspring successfully [120]. Recently United Kingdom approved a gene-therapy technique, in which the nucleus from a human female egg is transferred to a donor enucleated egg with healthy mitochondria, followed by fertilization of the donor egg [121,122].

Development of study models: Since variations or other environmental factors can be selectively deleterious for mitochondria in some individuals, it may also be important to use novel study models with underlying mitochondrial and/or metabolic abnormalities. This could help in improved prediction of characteristics of injury and development of potential treatments. Pathophysiology of the mitochondrial dysfunction will have to be carefully considered in such study models [123,124].

Conclusion

Several innate and acquired abnormalities may cause inadequate functions of mitochondria that may result in cell death. While variable penetrance and expression of mitochondrial mutations makes the clinical identification and significance of variations more challenging, several targeted population based studies have provided insights into systematic association of mitochondrial abnormalities to clinical syndromes. Despite significant progress in understanding and documenting the mitochondrial DNA defects causing mitochondrial dysfunctions, diagnosis of disorders have become more challenging with increased number of reported pathogenic mutations and constantly evolving guidelines of pathogenicity. Novel experimental approaches for high-throughput screening and mitochondrial DNA disease treatment under investigation are promising. Moreover, research strategies focused on elucidating the molecular mechanisms of mitochondrial dysfunction and complex relationships among mitochondrial DNA variations and associated diverse clinical phenotypes have been also useful in developing therapies for mitochondrial disorders. Though establishing the pathogenesis of mitochondrial disorders will uncover novel targets or molecular pathways that may be exploited for therapies development, meanwhile, approaches to develop generic treatment methods for mitochondrial disorders should be actively pursued. Evidences from current studies under investigation will also provide the foundation for development of targeted therapeutic approaches for mitochondrial disorders.

References


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