Mitochondrial Disorders

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Fran D. Kendall

Abstract

Mitochondrial disorders result in mitochondrial dysfunction and diminished energy production typically presenting with widespread, clinical features in all age groups and ethnicities and are often progressive and degenerative in nature. Because their clinical presentation is so variable and typically leads to widespread multisystem problems, diagnosis based on history and physical findings alone is often difficult, particularly for physicians unfamiliar with this group of diseases. Traditional diagnostic tools, which include invasive muscle biopsies for a variety of histological studies, functional assays, and enzymology, are costly, typically require a surgical procedure, are not definitively diagnostic in all cases and generate data that for the non-mitochondrial specialist is often difficult to interpret. Recent advances with expanded gene testing, in some cases utilizing easily obtainable urine samples, and noninvasive enzymatic testing are proving to increase patient accessibility to testing modalities and will likely lead to more rapid and accurate diagnosis, and ultimately improve management and treatment of affected individuals.

Introduction

Mitochondrial disorders result in mitochondrial dysfunction and diminished energy production and are a known but poorly understood group of diseases with a broad neurodevelopmental impact affecting all ethnicities and age groups. Associated problems range from seizure disorders to memory loss and dementia to weakness and include other system involvement such as cardiac or hepatic symptomatology. Investigative options are complex and often not definitively diagnostic. Developing a clear understanding of clinical presentation and associated radiographic and laboratory abnormalities will allow investigating clinicians to evaluate suspected mitochondrial patients more accurately leading to more timely institution of a therapeutic regimen for improved quality of life of affected individuals.

Fran D. Kendall, M.D. (✉)
VMP, LLC from Virtual Medical Practice,
5579 Chamblee Dunwoody Rd, Suite 110, Atlanta,
GA 30338-4128, USA
e-mail: info@vmpgenetics.com

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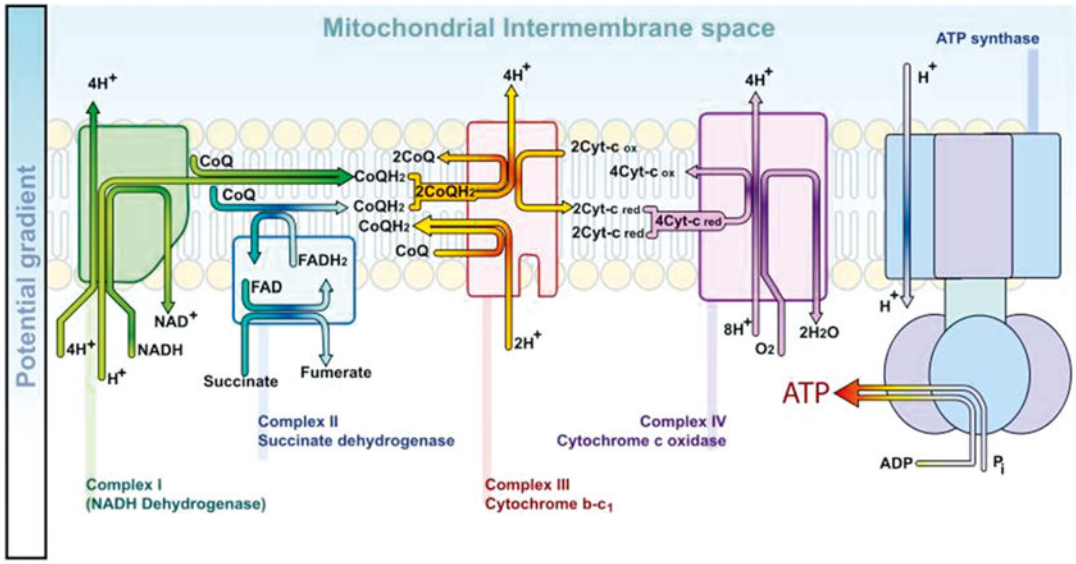

Fig. 58.1 5 Complexes of the electron transport chain

Mitochondria and Mitochondrial Disease Defined

Housed within our body's cells, the mitochondria create energy, or ATP, through the complex chemical reactions that occur in the electron transport chain embedded within the mitochondrial inner membrane. Composed of five complexes, the electron transport chain or respiratory chain generates ATP through an electrochemical gradient utilizing the breakdown products of food in conjunction with phosphate and oxygen (see Fig. 58.1). Over 1500 genes encode for various proteins that ultimately come together like jigsaw puzzle pieces to create energy [1].

Some individuals are born with changes in one of these many genes that ultimately alter their mitochondrial functioning, or sustain injury to the mitochondrial system through other mechanisms, such as the drug toxicity demonstrated with a group of AIDS drugs, either of which can result in decreased energy production and mitochondrial disease symptoms [2]. This secondary mitochondrial dysfunction is due to the mitochondria becoming "sick" or "toxic" due to changes in the cells. However, discussion regarding the mechanism of damage and clinical course of these individuals will not be discussed in this chapter.

Mitochondrial diseases alter the body's ability to adequately convert food into the energy needed for bodily functions through a process known as oxidative phosphorylation. These diseases, which affect up to 1 in 5000 individuals with several more recent studies suggesting a frequency of 1 in 2000, can result in widespread or localized clinical problems [1, 3–6]. Poor mitochondrial functioning has also been linked to the onset of many other disease processes, including Alzheimer's disease, Parkinson's disease, schizophrenia and bipolar disease [7–11]. A recent study has also found that the carrier rate in the general population for the most common mtDNA (mitochondrial DNA) mutations may be as high as 1 in 200 individuals indicating that mitochondrial dysfunction may contribute to an ever broader spectrum of disease and disease processes than was previously considered [12].

Patients affected by mitochondrial disease can present with any number of issues to include vision and hearing loss, seizures, low muscle tone, muscle weakness, migraines, chronic fatigue, developmental issues including autism spectrum disorders (ASD), kidney and liver disease, diabetes and other endocrine problems and autonomic dysfunction. Affected individuals can have some or many of these symptoms and problems. In most cases, the symptoms of mitochondrial disorders progres-

sively worsen over time, particularly when individuals are subject to stressors such as illness or surgery. Although some forms of mitochondrial disease only affect one person in an extended family, most types are inherited, creating a greater impact on families at large.

Genes and Genetics of Mitochondrial Disease

There are over 1500 genes involved in coding for the various proteins and other compounds involved in oxidative phosphorylation or mitochondrial energy production [1]. These genes are contributed by two sets of inherited genetic material; (1) the nuclear genes from both parents providing the vast majority of the information needed for energy production and (2) the maternally inherited mtDNA composed of 37 well defined genes [13, 14].

Hundreds of proteins are encoded for by the nuclear mitochondrial genes and many of these proteins are responsible for the control of electron transport chain structure, function and assembly to include, for example, genes encoding for all four subunits of complex II [15]. In addition, there are also a number of very critical assembly genes found among the nuclear mitochondrial genes such as SURF 1, which encodes for a gene that assembles the various components of cytochrome C oxidase or complex IV of the electron transport chain. In some studies, over 75 % of Leigh disease patients with cytochrome c deficiency have been found to have SURF 1 mutations [16]. Overall, the vast majority of pediatric mitochondrial disease is due to autosomal recessive inheritance affecting nuclear mitochondrial genes. In 1998, Lamont et al. [17] demonstrated that mitochondrial DNA mutations account for less than 10 % of all mitochondrial disorders in children.

Inherited exclusively through the maternal line, the mtDNA is a circular molecule present in 5–10 copies in each mitochondrium and composed of 16,569 bases or 37 genes. In addition, dependent on the cell type, there are hundreds to thousands of mitochondria per cell. These 37 mitochondrial genes encode for 22 tRNAs, 13 polypeptides of the respiratory chain including 7 subunits of complex I, one subunit of complex III, 3 subunits of complex IV and 2 subunits of complex V, and two ribosomal RNAs [13, 14].

Mutations arise in mtDNA de novo or are maternally inherited. In most cases, mtDNA point mutations are inherited whereas large deletions are de novo in nature [18]. However, in my clinical practice I have encountered several cases of mtDNA de novo point mutations including a case of MELAS.

Another unique feature of the mtDNA is heteroplasmy in which mutated mtDNA may be present in varying amounts with wild type DNA within the same cell. When the percentage of mutant mtDNA (mutation load) reaches a certain threshold the function of that tissue may become impaired [18]. The mutation load varies by tissue type, age and specific mutation. Thus, the mutation load varies within and between tissues leading to a broad spectrum of clinical symptoms that can range from healthy and asymptomatic to severely impacted [4]. In certain tissues, like blood, there may be selection against some of these mutations with preferential retention of cells with normal mtDNA. Additional modes of inheritance for mitochondrial disease include sporadic cases, autosomal dominant forms and X-linked disorders.

Clinical Features of Mitochondrial Energy Disorders

When faced with a multitude of issues and problems, mitochondrial disease will often become a consideration at some point during the evaluation of a complex, undiagnosed patient. Findings that are highly suggestive of mitochondrial disease include the presence of widespread, seemingly unrelated multisystem problems (see Table 58.1). Although this list appears daunting, there are some problems to include CNS issues such as developmental delays, hypotonia, seizures, poor growth, chronic fatigue, GI issues and autonomic problems that recur over and over particularly in pediatric patients ultimately determined to have mitochondrial disease making these the most common features seen in clinical practice. Many

Table 58.1 Possible symptoms of mitochondrial disease

Brain		
Developmental delays	Migraines	Seizures
Dementia	Autistic features	Atypical cerebral palsy
Neuro-psychiatric disturbances	Intellectual disability	Strokes
Nerves		
Absent reflexes	Fainting	Neuropathic pain
Weakness (may be intermittent)	Dysautonomia - temperature instability & other dysautonomic problems	
Muscles		
Weakness	Irritable bowel syndrome	Gastroesophogeal reflux
Cramping	Hypotonia	Diarrhea or constipation
Gastrointestinal problems	Muscle pain	Pseudo-obstruction
	Dysmotility	
Kidneys		
Renal tubular acidosis or wasting		
Heart		
Cardiomyopathy	Cardiac conduction defects (heart blocks)	
Liver		
Liver failure	Hypoglycemia (low blood sugar)	
Ears & eyes		
Visual loss and blindness	Optic atrophy	Acquired strabismus
Ptosis	Hearing loss and deafness	Retinitis pigmentosa
Ophthalmoplegia		
Pancreas & other glands		
Diabetes and exocrine pancreatic failure (inability to make digestive enzymes)		
Parathyroid failure (low calcium)		
Systemic		
Failure to gain weight	Unexplained vomiting	Respiratory problems
Fatigue	Short stature	

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adults will present primarily with fatigue, activity and exercise intolerance along with some of the other common issues seen in children to include GI complaints and autonomic dysfunction. Keeping in mind some of the common misperceptions about mitochondrial disease such as the false belief that it only affects children or that affected children only survive into early childhood are important for making the appropriate diagnosis in all patients.

Certain biochemical abnormalities including persistent, significant elevations in lactate, the presence of Kreb Cycle intermediates on urine organic acid analysis, specifically in patients over 1 year of age, or a deficiency in plasma carnitine are all indicators of mitochondrial disorders [19–21]. In one study, 21 out of 48 patients (43.8 %) with a mitochondrial myopathy were found to have elevated esterified carnitine levels with a concurrent decrease in free carnitine. Four of the 21 patients were also shown to have both free and total carnitine deficiencies in plasma (see Table 58.2).

Of note, especially in children, lactate elevations can be falsely increased due to collection artifact or postprandial affect. Determining the concurrent plasma alanine to lysine ratio at the time of plasma lactate collection can assist in deciding if the elevation is in fact an artifact or suggestive of mitochondrial disease. An alanine/lysine ratio >3 is suggestive of a true elevation seen with energy disorders since alanine will remain disproportionately increased in individuals with mitochondrial disorders even hours after

Table 58.2 Metabolic testing abnormalities supportive of OXPHOS disease

Increased blood and/or CSF lactate and pyruvate and/or ratio
Decreased plasma carnitine
Increased blood alanine
Generalized aminoaciduria
Increase lactate, pyruvate, Kreb cycle intermediates, tiglylglycine, and 2-oxoadipic acid on organic acid analysis

Dougherty F. Metabolic testing in Mitochondrial Disease. Semin Neurol 2001;21(3):303–308

a regular meal whereas lysine will usually fall below 150 umol/l.

Brain MRI abnormalities, including specific Leigh disease lesions, will typically place a patient into an energy disorder category while a strong family history of mitochondrial disease should clearly make this diagnosis of high consideration in a patient with undiagnosed clinical symptoms.

How Is Mitochondrial Disease Diagnosed?

Some patients present with a collection of clinical features frequently seen in mitochondrial disease such as failure to thrive, chronic fatigue, gastrointestinal symptoms, autonomic dysfunction and CNS disorders such as seizures or a neurodegenerative course that enable them to be diagnosed by a comprehensive history, examination and minimal testing such as blood and CSF lactate and pyruvate levels and brain MRI, as is the case typically for Leigh disease patients (see Fig. 58.2).

In other cases, patients present with findings that are clearly seen in several of the commonly described mitochondrial disease subtypes known to be associated with specific gene changes. An example is a patient who presents with elevated lactate levels, stroke-like episodes and other progressive problems such as seizures or dementia who is found to have the common tRNA mtDNA 3243 mutation seen with MELAS (mitochondrial encephalomyopathy lactic acidosis and stroke-like episodes). This diagnosis can often be confirmed with a simple blood sample if using next gene sequencing for detection of low level heteroplasmy in mtDNA or in urinary epithelium [22].

In the past, for most others, definitive diagnosis of a mitochondrial disease required the completion of special studies on a tissue rich in mitochondria. The body tissues that house the most mitochondria are the brain, kidney, liver, heart and skeletal muscle. Because collection of brain and heart tissue is impractical, and attainment of kidney or liver tissue for analysis is not only very invasive but potentially damaging and dangerous, muscle tissue became the tissue of choice for investigation of mitochondrial disorders. After collection of the muscle tissue, a number of histological and immunohistochemical and other studies using special instrumentation such as a spectrophotometer would be completed.

Traditional Tissue Studies for the Diagnosis of Mitochondrial Disease

Although testing completed on biopsied muscle material can vary widely depending on the testing institution or facility, the collected muscle is typically used for histological and immunohistochemical studies, mitochondrial respiratory chain enzymology and, in some cases, electron microscopy (EM) studies and muscle DNA testing, as well as other newer functional testing.

Histology

Many of the histological studies utilize standardized pathology protocols for staining with compounds such as a modified Gomori trichrome stain. These techniques identify abnormalities in general muscle structure and detect the presence or absence of various compounds and chemicals that provide clues to the health and function of the biopsied muscle tissue and its mitochondria.

The sub-sarcolemmal accumulation of mitochondria on muscle pathology is considered a hallmark of mitochondrial disorders. This accumulation can be visualized utilizing a modified

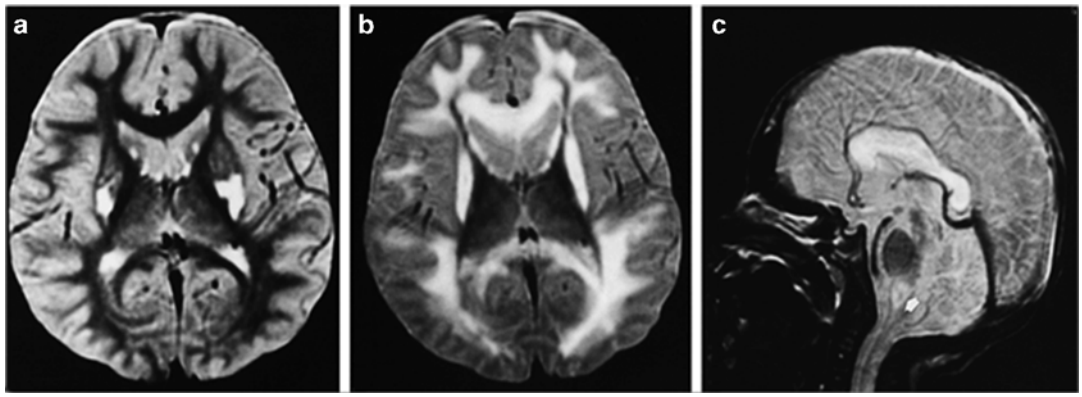

Fig. 58.2 MRI changes in Leigh disease

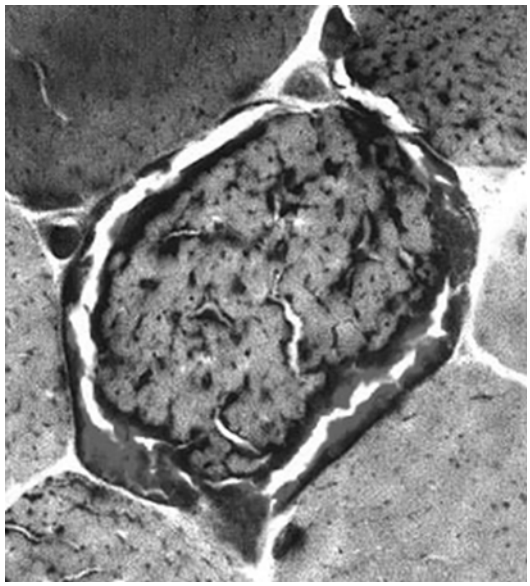

Fig. 58.3 Muscle with ragged red fibers

Gomori trichrome stain (ragged red fibers) or with a stain for succinate dehydrogenase, the latter for the detection of ragged-blue fibers [23, 24] (see Fig. 58.3). Increased SDH (succinic dehydrogenase) staining can also be found in regenerating muscle fibers.

In normal muscle biopsies, virtually all fibers have staining for Cytochrome C oxidase (COX), type I fibers being more darkly stained than type II. Cytochrome oxidase-negative fibers are not evident in healthy individuals under the age of 30 years and their presence is considered pathologic [25]. A diffuse reduction in COX staining can be seen in Leigh syndrome with COX deficiency, as demonstrated in (see Fig. 58.4).

Other histological changes that may be seen with but are not diagnostic of mitochondrial disease include the presence of excessive lipid, particularly in ragged red fibers. Other nonspecific but frequent findings are internal nuclei, atrophic fibers, fiber hypertrophy, fiber type grouping, type I or type II fiber atrophy, glycogen and inflammation [24–27].

Overall, the most common pathologic finding on light microscopy of muscle specimens in mitochondrial disorders is a generalized increase in the number of cytochrome oxidase-negative fibers [28]. However, in many cases, abnormalities on light microscopy are not present at all [24, 29]. In 2004, Patterson demonstrated that 50 % of patients with mitochondrial disorders had normal light microscopy, findings documented previously by Rollins et al. [26, 30], when they evaluated 118 muscle specimens from mitochondrial patients of all ages. Rollins et al. [26] also noted that ragged-red fibers were found in only 2.5 % of cases and cytochrome C oxidase-negative fibers in 7 % of patients. An additional evaluation of 177 children with mitochondrial disorders by Lamont et al. noted ragged-red fibers or cytochrome oxidase negative fibers in 89 % of children with mtDNA mutations in comparison to similar findings in only 17 % of patients with no identifiable mtDNA gene defect [17]. These findings suggest that light microscopy findings are more prevalent in mitochondrial patients with detectable mtDNA mutations. Additional studies

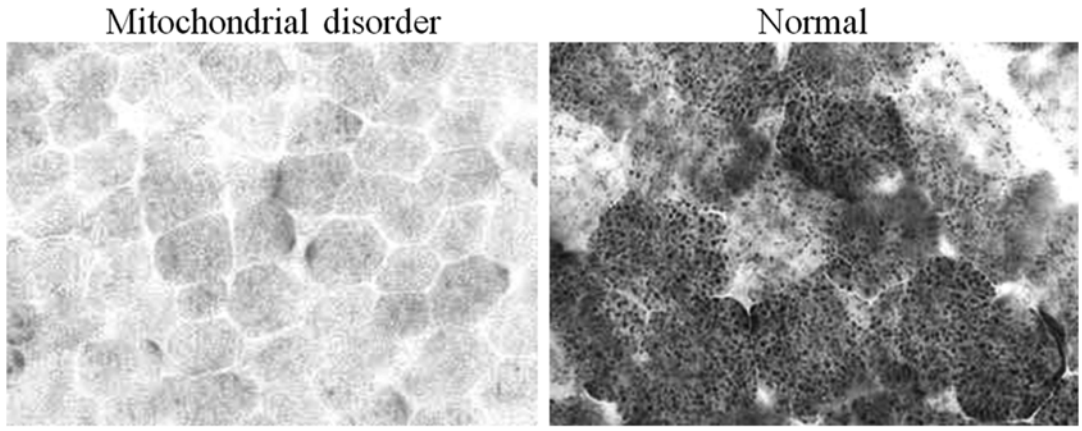

Fig. 58.4 Mitochondrial disorder compared with normal

have found that some patients will develop histological changes over time indicating that detectable abnormalities may develop with disease progression therefore making them less likely to be seen in children, in general, when compared to adults [25]. These various studies indicate that while muscle pathology may contribute to the diagnosis of mitochondrial disease and is more commonly abnormal with mitochondrial DNA mutations and in adult patients, the presence of normal histological findings does not exclude a mitochondrial disorder, particularly in children [30].

Immunohistochemistry

In some laboratories, additional mitochondrial enzyme immunohistochemistry studies using, for example, mtDNA-encoded respiratory chain subunits such as COX II (Subunit II of complex IV) is utilized to detect the specific presence or absence of very specific subunit proteins.

Electron Microscopy

Although used frequently in the past, a recent study reviewing the utility of electron microscopy (EM) in the diagnosis of mitochondrial patients found that it did not provide additional diagnostic information when evaluating suspected mitochondrial cases [31]. However, in children, ultrastructure changes on EM have been reported to be present in 30–44 % of patients with suspected mitochondrial disorders in several previous studies [28, 32]. When evaluating 20 pediatric patients, Kyriacou and Mikellidou detected ultrastructural findings in 33 % of them who had no detected abnormality on light microscopy [28]. EM findings included an increase in mitochondrial number (48 %) or size (7 %), increased lipid (8 %), glycogen droplets (12 %), increased mitochondrial matrix, abnormal cristae, and paracrystalline inclusions [28, 32, 33]. However, many of these findings can be seen with other disorders including muscular dystrophies, neurogenic atrophy, inflammatory myopathies, chronic steroid use and in other metabolic disorders making them nondiagnostic and merely a point of data that may suggest mitochondrial disease [25, 27]. As such, detectable changes on EM, or lack thereof, do not eliminate the possibility of a mitochondrial disorder and many mitochondrial experts no longer utilize this technique as part of a standard evaluation.

Mitochondrial Enzymology

Most mitochondrial diagnoses are not made on histological or immunohistochemical findings but rather on the detection of enzymatic deficiencies as detected by polarographic or spectropho-

tometric assays. Because the detection of a causative gene mutation can be difficult in most children up until very recently with the expansion of molecular tools that now can detect at least 50 % of mitochondrial gene changes, enzyme analysis has traditionally been considered important for the diagnosis of mitochondrial disease [17, 34, 35].

Utilizing fresh tissue only due to a disruption of mitochondrial membrane potential in frozen samples, polarographic assays measure mitochondrial substrate oxidation detecting an overall estimate of electron transport chain activity [35–37]. Because the mitochondria tend to proliferate in patients with mitochondrial disease creating a false elevation of overall electron transport chain activity, the use of the polarographic assay can result in a false negative diagnosis [37]. Completed on either fresh or frozen tissue, spectrophotometric assay, which is used by most mitochondrial diagnostic laboratories, measures the activity of each individual complex in the electron transport chain. By adding an electron donor or acceptor to a muscle homogenate containing mitochondria, the rate of oxidation or reduction is measured [36, 37]. Although there is marked variation among complex activity in different tissues and between individuals, there is a constant ratio of electron transport chain enzymes to each other in all human tissues allowing the development of standards by comparing each enzyme with the activity of another enzyme in the ATP generating system [32, 36, 37]. To prevent missing a generalized enzyme deficiency, complex activity is also compared to another mitochondrial enzyme not a part of the electron transport chain, typically citrate synthase, a Kreb cycle intermediate located in the mitochondrial matrix [36, 37]. Interpretation of testing results can be challenging particularly given the fact that decreased respiratory chain activities have been reported in a number of non-mitochondrial disorders [38].

Fresh vs. Frozen Samples

Over the years, there has been considerable discussion regarding whether fresh or frozen biopsy material should be utilized for the most accurate testing results with spectrophotometric assays. Although some reports over 20 years ago noted reduction in complex I activity in frozen specimens, this decreased activity has been seen linked primarily to the initial processing and handling of the tissue [39]. In personal experience owning a mitochondrial diagnostic laboratory, we found that complex I activity was more robust in fresh samples but also well within normal ranges in frozen samples. As such, most mitochondrial experts find frozen sample results reliable when collected in hospital or laboratory settings that frequently process such samples utilizing standardized collection protocols. Of note, however, some additional studies such as respirometry, discussed below, cannot be completed utilizing frozen tissue samples. However, the logistics of having patients evaluated only in centers completing fresh muscle sample is unrealistic in most cases making the use of frozen tissue the only acceptable mode for tissue diagnosis in many cases.

Other Tissue Assays

More easily obtainable skin fibroblasts or other non-muscle tissues such as liver and heart have been utilized on many occasions to measure electron transport chain activity in patients. One study noted, though, that half of all children with deficiencies in their muscle have normal enzyme activity in cultured fibroblasts likely due to the fact that cells with electron transport chain defects are fragile and die in culture [35]. However, modification of culture medium, to include the addition of uridine and pyruvate, or alteration of technique has allowed the survival of abnormal mitochondria, preventing false positive results and increasing the accuracy of skin fibroblast assays [36, 37, 40]. Some mitochondrial disorders affect specific tissues, such as the heart and liver, with electron transport chain abnormalities non-detectable in muscle specimens [41, 42]. As such, in certain cases, enzymatic assays should be targeted to the affected tissue preventing false negative results in biopsied muscle tissue.

Additional Tissue Studies: Respirometry, Blue Native and Clear Native Gels and Western Blot Testing

Over the last several years, additional testing tools are being utilized by some testing laboratories with the aim of increased understanding of mitochondrial functionality. Overall, these various techniques can provide additional information regarding the functionality of the mitochondrial OXPHOS system in a given individual, findings that might not have been detectable utilizing the standardized histology, immunohistochemistry and enzymology studies. Details are summarized in Table 58.3 regarding the utility of each technique.

Table 58.3 Additional muscle tissue assays for mitochondrial disease diagnosis

High Resolution Respirometry	Measures integrated activity of all complexes in the electron transport chain system in cells or isolated mitochondria under in vivo like conditions [43–45]
	May identify up to 30 % of mitochondrial defects not detectable with traditional enzymatic assays [46–48]
Blue Native Gel Electrophoresis (BNGE)	Detects mitochondrial complex assembly defects using electrophoresis in the presence of the dye Coomassie Brilliant Blue [49–52]
Clear Native Gel Electrophoresis	Detects mitochondrial complex assembly defects using Coomassie Brilliant Blue for improved identification of Complex III defects
	Less resolution than BNGE for all other Complexes [51]
Western Blot Testing	Detects the presence or absence of specific proteins in the various Complexes [53, 54]

Problems with Traditional Invasive Testing

Over recent years, many problems have come to light with the use of traditional mitochondrial muscle testing. Muscle studies are, obviously, invasive and the need for general anesthesia in children and a surgical procedure for all individuals places many fragile patients at risk for associated complications, even death.

Although there is significant variability in testing cost depending on the laboratory providing service and what is ordered as part of an evaluation, the final cost varies widely and ranges from $5000–10,000 for basic histology and enzymology to a staggering $50,000 plus in some laboratories who complete additional DNA testing and sequencing, blue native gels and other functional assays.

In addition to cost and safety issues, it is well known that the detection of mitochondrial enzymatic abnormalities on muscle tissue is not definitively diagnostic in all cases. It has been suggested that upwards of 50 % of abnormal mitochondrial enzymology findings on biopsies may be false positives (unpublished data). Abnormal mitochondrial enzymology has been seen in many other disease processes including Alzheimer's disease, Parkinson's disease and other disorders including some reported cases of Prader-Willi syndrome making it clear that other disease processes can alter mitochondrial enzyme activity and may cloud the true underlying primary pathology in a given patient [11, 55].

Many patients previously labeled as primary mitochondrial respiratory chain disease based on abnormal muscle enzymology have been rediagnosed with a variety of other diseases. An example is described below:

Vignette: The patient presented as a 4 year 1 month old boy with a biochemical OXPHOS defect, ASD, speech and language issues, hypotonia, GERD, FTT, seizures and behavioral issues. He was born head first at 32 weeks gestation by a vaginal delivery weighing 1800 g and 46 cm long to a 25-year-old G1 P0 mother following a pregnancy notable for a viral illness at 15 weeks, exposure to x-rays as a dental assistant throughout the pregnancy,

and onset of premature labor at 32 weeks leading to delivery.

Following delivery, the patient had Apgars of 8 at 1 min and 9 at 5 min. He remained in the neonatal nursery for 3½ weeks due to issues with weight gain, feeding, temperature instability, and apnea. The remainder of his first year of life was notable for the onset of developmental delays. He did not roll over until 14 months, sit alone until 16 months of age, and did not walk until 4 years of age and has not developed speech. He has been diagnosed with autism. Additional problems include hypotonia, gastro esophageal reflux, poor feeding and secondary growth issues, and recent onset seizures.

Chromosome 15 deletion studies, type I myotonic dystrophy, Fragile X and chromosome microarray studies were normal. Muscle biopsy and metabolic testing noted normal plasma lactate and pyruvate, plasma and urine amino acids, urine organic acids, acylcarnitines, carnitine, CPK, creatine and guanidinoacetate studies, leukocyte CoQ level, and CSF testing. Extensive muscle histology studies were negative. Muscle OXPHOS enzymology noted a complex I and possible complex III defect. Blue native gel and clear native gel studies reported decreased super complex formation. High resolution spirometry was also reported to be abnormal. mtDNA sequencing and other gene studies were normal. The patient was classified as a highly probable mitochondrial disorder.

However, because of his clinical findings to include a seizure disorder and several dysmorphic features including a large tented mouth and widely spaced teeth and the lack of gene diagnosis in the context of an isolated OXPHOS defect with no other biochemical abnormalities (such as lactate, CPK, carnitine etc.) we pursued additional epilepsy gene testing and identified a de novo TCF4 mutation consistent with Autosomal Dominant Pitts Hopkins syndrome.

Because of these problematic cases, definitive diagnosis of mitochondrial disease is often being reserved by many mitochondrial specialists for those patients with a confirmed mitochondrial gene mutation, a challenging requirement for patients given that until very recently most disease causing mutations had yet to be elucidated [17].

New Trends in Mitochondrial Disease Diagnosis

Recent trends in new testing development are providing alternative approaches to a traditional muscle biopsy evaluation. These techniques include non-invasive enzyme testing utilizing skin cells and lymphocytes, next generation (gen) sequencing for the detection of low level heteroplasmic mtDNA mutations in blood, and expanded nuclear gene testing which now screens hundreds of the known mitochondrial genes utilizing a blood sample [56, 57].

Researchers have recently developed an assay that measures mitochondrial complex I and IV activity, the particularly prevalent targets in mitochondrial defects, in buccal swab samples. Using a cohort of 164 suspected mitochondrial disease patients, when buccal swabs were analyzed by combined micro-spectrophotometry and enzyme immunocapture, 96 patients showed significant deficiency in either complex I or IV activity, compared to values from 68 patients without defect and 63 age-matched unaffected control subjects. Moreover, their findings showed that of 32 patients with demonstrated complex I or IV deficiency in skeletal muscle, 26 (81 %) had an analogous deficient buccal complex activity as compared to values obtained from the unaffected controls, supporting the validity of this approach [58]. They have also begun to corroborate their complex I findings with a novel in-gel assay of complex I activity using patient buccal extracts. In addition, they are expanding their assays to include complexes II and III activity measurements. Although this less invasive approach appears to have diagnostic utility in identifying mitochondrial dysfunction in large numbers of children or adults suspected of having mitochondrial disease without the cost and risks associated

with more traditional invasive procedures as demonstrated by its use in a suspected MERRF patient [59] it is still in its infancy but gaining acceptance as a good screening tool for the initial evaluation of patients with possible mitochondrial disease. In addition, the use of a noninvasive study allows the advantage of repeat testing for the purpose of monitoring disease progression and possible response to a variety of treatment modalities.

Although many mtDNA mutations, such as the 3243 A>G MELAS mutation, are usually measurable in blood leukocytes, the level of mutation declines over time [60]. Thus even in severely affected patients presenting with MELAS, the level of m 3243 A>G may be very low, or even escape detection. This has led to the use of muscle tissue for years as the gold standard for the measurement of heteroplasmy in mitochondrial DNA mutations to both confirm diagnosis and predict the incidence of specific clinical features or severity of disease. However, the development of several new methods of heteroplasmic detection utilizing a range of noninvasive tissues has proven to be useful and is leading to the elimination of the invasive biopsy. In many cases, the use of next generation sequencing, a technique that allows the sequencing of a large number of amplicons (pieces of DNA formed as the product of amplification events such as PCR) in parallel available through a number of laboratories, enables the detection of heteroplasmic mtDNA mutations down to a level of 1–2 %, eliminating the need for muscle testing [61]. In addition, a study looking at MELAS patients found that screening of m. 3243 A>G mutation load in urinary epithelium was a better predictor of outcome than the present gold standard of skeletal muscle [22]. Because of advances in various diagnostic techniques, muscle biopsy is being utilized less frequently and if often the last investigatory study utilized for patients with suspected mitochondrial disease.

Until very recently, confirmation of a mitochondrial disease through identification of a causative gene mutation occurred infrequently. In a study reviewing 118 children, a genetic defect of any type was identified in only 5 % of patients investigated [62]. Out of the 1500 known mitochondrial genes only the mtDNA and a select few nuclear genes could be screened for on a routine basis up until 2010. However, several laboratories have developed expanded gene panels looking at hundreds of the nuclear encoded mitochondrial disease related genes utilizing blood specimens making confirmatory diagnostic testing likely to occur in more and more cases moving forward.

Management and Treatment

To date, there is no standard of care approach for the treatment and management of patients with mitochondrial disease. However, the general approach to these patients includes three general categories of management: preventative care, symptomatic therapy for chronic and acute issues, and vitamin and cofactor treatment.

Preventative Care

Preventative care is targeted towards the pre-symptomatic identification of clinical problems associated with mitochondrial disease and the implementation of various protocols or regimens to minimize worsening or onset of associated issues.

For the pre-symptomatic identification of associated problems most mitochondrial specialists complete yearly or as needed multisystem screening studies that include basic chemistries, CBC, CPK and a variety of metabolic studies such as lactate levels. In addition, depending on the patient, brain MRI's, EEG's and EKG's and echocardiograms are included in these evaluations. The goal is to identify a disease process early in its course with improvement in long-term outcome for the patient [21].

The introduction of exercise, nutrition, hydration and energy conversation regimens into a mitochondrial patient's daily routine can lead to improved stabilization and reduction of associated problems. For example, an exercise routine can improve overall endurance and strength bat-

tling the common problems of fatigue and weakness but must be approached conservatively. Allowing for good hydration and rest periods before and after an exercise routine improves the positive aspects of physical activity without exacerbating the underlying problems. In our practice, we utilize detailed school protocols for children and young adults that educate the community and allow affected patients to function more effectively in the school environment by, for example, introducing rest periods into their curriculum or allowing students to have access to snacks and hydration throughout the day, practices which improve their overall functionality [63].

Symptomatic Therapy for Chronic and Acute Issues

Symptomatic therapy is seemingly relatively clear cut indicating introduction of treatment aimed at specific problems. For example, a patient with a seizure disorder will be treated with anticonvulsants. However, the multisystem nature of the problem set facing these individuals often leads to fractured approaches with various physicians focusing only on one aspect or problem without looking at the larger picture. As such, a clinician who has an extensive knowledge of the various issues confronting these patients can often spearhead care and provide a more comprehensive approach to management leading to an improved quality of life.

Patients with mitochondrial disorders, like most patients with metabolic diseases, are subject to episodic metabolic decompensation and crises surrounding intercurrent illnesses, surgery and other stressors. To address these times, most mitochondrial specialists develop acute care management protocols highlighting the most common problems or findings that will surface for these patients during these times. For example, our sick time protocols address risks for metabolic acidosis from elevated lactate or dehydration and make recommendations to treating physicians for fluid and other interventions aimed at stabilizing the acutely ill patient as quickly as possible.

Surgery presents a specific set of risks for patients with mitochondrial disease given that a number of anesthetic agents have been shown to cause mitochondrial toxicity and intra and postoperative issues for patients exposed to those medications. Specifically, Propofol, a popular intravenous maintenance anesthetic, is a complex I inhibitor and can uncouple oxidative phosphorylation. In addition, the propofol infusion syndrome is thought to result from the inhibition of transport of long-chain acylcarnitine species with an indirect effect on complex II activity. Several reports have documented a number of perioperative complications in mitochondrial patients receiving Propofol including respiratory depression, cardiac dysfunction and occasionally severe neurological deficits. More significant, however, are reports of late, profound respiratory depression and/or CNS white matter degeneration in mildly affected patients who had uneventful courses during surgery [64].

These findings along with concerns regarding other anesthetic agents has led to the development of surgical protocols for the peri- intra- and post-op care of mitochondrial patients. These protocols, examples of which can be found on the Mito Action and UMDF (United Mitochondrial Disease Foundation) websites, also include recommendations for fasting and monitoring guidelines to reduce additional stressors in an already vulnerable patient population.

Vitamin and Cofactor Treatment

Although associated with variable response in mitochondrial patients, the “mito cocktail”, a combination of vitamins, supplements, antioxidants and cofactors is a mainstay of therapy and frequently recommended by mitochondrial physicians to increase mitochondrial efficiency and cellular energy production. While alterations in the basic cocktail may vary from patient to patient, the primary ingredients utilized by most experts include Coenzyme Q10, L-carnitine, creatine, B vitamins, L-arginine and a variety of antioxidants including alpha lipoic acid. See Table 58.4 for comprehensive information.

Table 58.4 Mito cocktail ingredients, dosages, and side effects

Ingredient	Dosage	Possible side effects
Thiamine (B1)	50–800 mg daily, by mouth	Allergic reactions (rash, hives), stomach upset
Riboflavin (B2)	100–400 mg daily, by mouth	Bright orange urine
Niacin (B3) (nicotinic acid)	50–100 mg daily, by mouth	Stomach upset, flushing in the face and neck, headache, and liver problems
Pyroxidine (B6)	10–250 mg daily, by mouth	Increased sensitivity to light, rash, stomach upset, allergic reactions, and tingling or numbness in the hands or feet
Cobalmin (B12) (cyancobalamin)	100–1000 mg daily, may be taken as an injection	Itching, diarrhea, headache, anxiety
Vitamin C (ascorbic acid)	100–500 mg up to three times daily	Nausea, stomach upset, diarrhea, kidney problems
Vitamin E (tocopherol)	200–400 IU (international units) daily	Nausea, diarrhea, bleeding, bruising easily, headache, fatigue; high doses not advised for people with diabetes or heart disease
L-Carnitine (Ievocarnitine, Carnitor®, carnitine)	30–100 mg/kg/day by mouth in two to three divided doses	Stomach upset, diarrhea, body odor, rash, increased risk of seizures in people with a history
Alpha lipoic acid (thioctic acid)	60–200 mg up to three times daily by mouth	Nausea, vomiting, rash, headache
Selenium	25–50 mcg daily	Risk of selenium toxicity in higher doses
Biotin (vitamin H)	2.5–10 mg daily	Stomach upset
Sodium succinate (Na succinate)	6 g daily	None reported
Vitamin K1 (phytonadione or phylloquinone)	1–25 mg daily by mouth	Jaundice, allergic reactions (rash, hives, swelling of the airway); contraindicated for use with blood thinners (Warfarin)
Coenzyme Q10 (ubiquinone)	5–15 mg/kg/day by mouth	Nausea, stomach upset, headache, loss of appetite, dizziness; benefits may take months to appear
Coenzyme Q10 (idebenone) (a synthetic product similar to CoQ10)	90–300 mg daily by mouth	Stomach upset, nausea, vomiting, diarrhea, headache, dizziness, confusion
Creatine	Up to 5 g, divided, twice daily	Stomach upset, nausea, muscle cramping
Ribose	15 g up to four times daily by mouth; may also be used with exercise to prevent muscle cramping	Stomach discomfort, nausea, diarrhea, headache, hypoglycemia
Uridine	150 mg	Stomach discomfort, diarrhea
Folic acid	1–10 mg by mouth daily	Stomach discomfort, diarrhea
Folinic acid (leucovorin)	400–800 mcg	Stomach upset

Adapted and reprinted with permission from "Mitochondrial Cocktail Information Sheet," provided by America's Compounding Center (www.accrx.com) and Living Well with Mitochondrial Disease by C Balcells. This is not a complete list, but a sample of the most commonly prescribed compounds

Note: Dosages of the following vitamins and supplements are individual based on age, weight, symptoms, and other factors. Please, never begin taking any of these compounds without the advice and involvement of a physician. In addition, keep careful records of your prescription and update it frequently

Coenzyme Q 10, a natural component of the electron transport chain, assists mitochondrial energy production by carrying electrons from cytochrome to cytochrome allowing for ATP production. Flooding the body with extra CoQ 10 theoretically allows for improved energy production and protection from free radical damage. Although ubiquinone is the common form of CoQ10 found in most over-the-counter products, it must be converted to ubiquinol to become bioactive. Because ubiquinol is more readily absorbed, we often recommend this form for children and individuals with gastrointestinal issues.

L-carnitine transports fatty acids across the mitochondrial membrane for Beta oxidation. Although many patients with mitochondrial disease are carnitine deficient we typically prescribe carnitine supplementation for all mitochondrial patients, regardless of their levels, to increase free carnitine within cells with the intent of improving overall energy production.

Creatine is a nitrogenous organic acid that occurs naturally in many animals including humans and helps to supply energy to all cells in the body, primarily muscle but also brain. This is achieved by increasing the formation of adenosine triphosphate (ATP). Because creatine phosphate is converted into creatinine, a toxic by-product of normal muscle metabolism excreted through the kidney, many mitochondrial experts will recommend the use of creatine monohydrate since it is believed that this form has less side effects. Nonetheless, good hydration and monitoring kidney function tests is critical with the use of creatine supplementation. In addition, most patients gain weight on creatine supplements creating yet another negative side effect that limits its use in this patient population.

Vitamins B1 (Thiamine) and B2 (Riboflavin) are also used frequently as cofactors for PDH (pyruvate dehydrogenase) deficiency (B1) and complex I and II biochemical abnormalities, respectively. Of note, there are known thiamine dependent PDH mutations so B1 supplementation should be used in all cases of suspected or known PDH deficiency.

L-arginine has been used effectively in treating stroke-like episodes in MELAS patients by increasing nitric oxide causing vasodilation and preventing the inappropriate constriction of blood vessels leading to these metabolic strokes. Both children and adults with MELAS are treated with maintenance doses of L-arginine and acutely during times of active stroke [68].

Many patients take a combination of other antioxidants such as alpha lipoic acid, vitamin C, vitamin E and selenium. These "free radical scavengers" prevent and repair cell damage incurred by the naturally occurring by-products of energy production. Because mitochondrial patients have less effective energy metabolism they generate an increased free radical load with increased potential for free radical damage. The use of supplemental antioxidants is believed to reduce this increased risk of damage from a poorly functioning OXPHOS pathway.

Despite the almost consistent use of some variation of the "mito cocktail" by all mitochondrial disease experts, we have little documentation to support long term improvements in patients utilizing these supplements. As such, the mitochondrial community has been unable to secure consistent insurance coverage for the expensive supplements resulting in, at times, a quite substantial out of pocket expense for most patients [69].

Future Treatments

Although we have no consistently effective treatment for patients with mitochondrial disorders to date, Edison Pharmaceuticals has developed EPI-743, an orally bioavailable small molecule for the treatment of Leigh syndrome and other inherited mitochondrial diseases.

EPI-743 is a member of the para-benzoquinone class of drugs. It serves as a cofactor for the novel drug target– NADPH quinone oxidase 1 (NQO1). Through a redox-based mechanism, EPI-743 augments endogenous glutathione biosynthesis– essential for the control of oxidative stress.

For the EPI-743 Phase 2A trial, ten (10) children in Rome, Italy with Leigh syndrome were treated for 180 days. All ten subjects responded favorably. After 90 days all subjects demon-

strated arrest and reversal of the disease; after 180 days the reversal persisted in all but one subject. In this one patient, treatment was discontinued by the choice of the patient's parents and the patient reverted to his original status. All patients also had a normalization of disease-relevant biomarkers. These results were published online in the September 10, 2012, publication of the Molecular Genetics and Metabolism journal. At the same time, Edison announced that EPI-743 had received orphan drug status from the European Medicines Agency (the European regulatory agency akin to the FDA in the United States). Based on the findings of this Phase 2A trial, Edison Pharmaceuticals is currently enrolling 30 (or more) children at four different sites in the US in its Phase 2B trial.

Prognosis

As a general rule, mitochondrial diseases show a progressive course over time. However, their course can be quite variable depending on the sub-type of disease affecting a given patient.

For example, patients with classic Leigh disease show a rapidly progressive course typically resulting in death by several years of age in the most severe cases but there are later onset forms of the disease. In the infantile form (about 50 % of cases), symptoms may become apparent within 2 years of life. In this situation, there may be hypotonia, regression of neuropsychomotor development, ataxia, seizures and breathing dysfunctions. In the juvenile form, patients have mainly an extrapyramidal syndrome with dystonia and stiffness that can be fairly rapid in onset as is described in this patient below. Recognizing the phenotypic variability in a disorder such as Leigh disease will prevent misdiagnosis and failure to receive appropriate treatment.

Vignette: A 5 year 10 month old boy presented with recent onset ataxia, loss of functioning, and bilateral basal ganglia lesions. He was born at 35 weeks by a Caesarean Section after 18 h of labor to a 28 year old G1P0 mother following a pregnancy notable for onset of premature labor resulting in delivery. Initially following delivery, he did well but developed an apneic episode during a bathing resulting in a 2½ day NICU stay and several days of ventilator support. However, he did well otherwise and was sent home at 3 days of age on bottle feeds.

His early development was normal. He rolled over at 6 months, sat alone at 9 months, walked independently at 13 months and said his first word at 14 months of age. He remained healthy and active, an avid soccer player, until age 5 years 5 months when he developed fatigue and flexing of his left hand and arm. One month later, he developed a shuffling gait, loss of coordination with onset of unsteadiness and overall poor movement in his lower extremities. At times his legs would "lock up".

A brain MRI noted markedly abnormal symmetrical signals in his basal ganglia. An EEG was normal. Plasma amino acids, lactate, acylcarntines, CMP, VLCFAs, urine organic acids, arysulfatase, ferritin, lead level and ceruloplasmin, were all unremarkable. A CPK was 246 with nL reported to be 0–190.

At presentation to our office 5 months after the onset of his problems he was wheelchair bound and had developed slurring of his speech with loss of some fine motor skills. He was unable to hold utensils well.

Additional evaluation noted chronically elevated CPK values, increased lactate, abnormal enzyme testing on buccal swab mitochondrial enzyme analysis and a homoplasmic mtDNA change that is of unclear clinical significance. PDH and mitochondrial nuclear gene panels were unremarkable. Exome sequencing is currently pending. He has been diagnosed with juvenile onset Leigh disease. Since diagnosis, he has shown a progressive course with continued loss of skills including speech and is now G-tube dependent. He is about to begin the EPI 743 drug trial.

Other disorders have a slower, more smoldering course resulting in disability but not necessarily shortened lifespans. However, intercurrent ill-

nesses such as the flu or other stressors including surgery can affect a patient's long-term outcome by worsening underlying problems or leading to onset of others due to tissue and organ damage during episodes of decompensation.

Vignette: A 33 year old young woman presented with ptosis, CPEO, RP, and weakness at presentation. She was born head first by a vaginal delivery at 36 weeks gestation weighing 2400 g, and 48 cm long to a 26 year old G1P0 mother at an uncomplicated pregnancy. Following delivery, she had problems with temperature instability and poor feeding resulting in placement of an IV for fluids with an ultimate transition to breast feeds and discharge at 7 days of age.

Developmentally, her early milestones were notable for failure to walk until 18 months of age. Nonetheless, she did well academically graduating from college with a 4 year degree.

By age 13–14 years old, the patient began developing progressive ptosis, having a sling procedure at age 20 years. In her mid to late 20s she developed right exotropia, general fatigue and muscle weakness in her core and neck. Over the next several years she also developed dysphagia to solid foods as well as GE reflux requiring treatment.

She underwent a muscle biopsy which identified ragged red and ragged blue fibers by modified trichrome stain and SDH, respectively, and COX-negative fibers. mtDNA deletion duplication study done in her blood sample was negative. However, mtDNA deletion studies completed on her muscle tissue noted a large mtDNA deletion, confirming her mitochondrial disorder.

In my clinical practice of over 20 years, approximately 20–30 % of my patient population has shown a more rapidly progressive, debilitating course leading to early death. Those individuals often fall into several general categories: (1) those patients with progressive brain lesions as seen with Leigh disease; (2) patients with intractable seizure disorders; (3) individuals with multisystem body failure and (4) patients who show a worsening of their clinical features measured in weeks and months and not years. For the other 70–80 % of the patients, they typically struggle with multisystem body problems that progress over time typically measured in years ultimately leading to considerable morbidity during the course of their lifetime if not increased mortality.

Conclusions

Mitochondrial disorders have a far larger footprint and impact than was previously noted with an incidence of at least 1 in 5000 individuals [1, 3–6]. Recent studies have also shown than 1 in 200 newborns carry one of the ten common mtDNA mutations, perhaps contributing to the onset or development of many commonplace diseases such as diabetes and heart disease [12]. However, traditional diagnostic tools for mitochondrial disease have been invasive, costly and, in many cases, not definitively diagnostic. In addition, although several sets of diagnostic criteria have been developed over the last few decades based on combinations of clinical, laboratory, pathologic, biochemical, and genetic findings, they have not proven all that effective or practical in the clinical setting. In many cases, the diagnosis of a mitochondrial disorder remains a clinical diagnosis based on the acumen of the treating physician. However, innovative new techniques are revolutionizing mitochondrial medicine providing less invasive and more definitive diagnostic options for the evaluation of suspected mitochondrial patients. This will undoubtedly broaden the testing availability to a wider range of patients and lead to earlier and more accurate diagnosis that will result in improved management and treatment of affected individuals with diminished morbidity and mortality for this patient population.

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