Mitochondrial encephalomyopathies: advances in understanding

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Summary

Mitochondrial encephalomyopathies encompass a group of disorders that have impaired oxidative metabolism in skeletal muscles and central nervous system. As the field of mitochondrial medicine takes shape and physicians in all specialties become increasingly aware of respiratory chain or oxidative phosphorylation (OXPHOS) related disorders, their prevalence remains largely unknown. The unique features of the mitochondrial genome and the dual control over this important cellular apparatus makes the clinical presentation variable and diagnosis difficult. There is a confounding variation in phenotype and genotype, and the natural history of the disorders in individual patients is not accurately predictable. Only recently have things begun to fall into place and some phenotypes defined. Diagnosis requires a complex battery of clinical studies coupled with diagnostic findings on muscle biopsy (abnormal structure, histochemistry, or enzyme studies) or DNA testing. However, a reasonably confident diagnosis can be made by viewing the clinical presentation in the light of family history and some basic, routinely available laboratory investigations. This review tries to give a brief account of mitochondrial structure, function and genetics, and clinical presentation, evaluation, and treatment in suspected cases of mitochondrial encephalomyopathies.

key words: encephalomyopathies • mitochondrial disorders

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BACKGROUND

No longer are the mitochondrial disorders as ‘mysterious’ as they were a decade earlier. With advances in molecular genetics we are beginning to understand the possible mechanisms that underlie disorders of mitochondrial function and the wide range of human diseases that can be caused by them. This review intends to give the readers an idea about mitochondrial defects, their clinical consequences, the diagnostic tools available, and treatment options in consideration.

MITOCHONDRIAL STRUCTURE AND METABOLISM

Nearly all the energy for cell function in aerobic tissues is provided by cellular respiration, and mitochondria are the subcellular organelles in which this process takes place. Structurally, it consists of two membranes: a smooth, highly permeable outer membrane which gives access to the intermembrane space and a relatively impermeable inner membrane which is folded into cristae. This is osmotically sensitive and regulates the access of ions and metabolites to the mitochondrial matrix. Respiratory chain enzymes are found within the folded inner membrane.

The most important fuels for the generation of adenosine triphosphate (ATP) in most mammalian cells are glucose and fatty acids. Glycogen is the main storage form of glucose and is catabolized to glucose by glycogen phosphorylase. Glucose is in turn catabolized to pyruvate in the cytoplasm by the enzymes of glycolysis. Under aerobic conditions, pyruvate is transported into the mitochondrion and metabolized to acetyl coenzyme A by the pyruvate dehydrogenase complex. Acetyl-CoA then enters the citric acid cycle. Long-chain free fatty acids cannot freely enter the mitochondria. They attach to carnitine and enter the mitochondrial matrix through the inner mitochondrial membrane and undergo degradation through β-oxidation. The citric acid cycle and β-oxidation both produce reduced forms of NADH (nicotinamide adenine dinucleotide) and FADH₂ (flavine adenine dinucleotide). Each contains a pair of electrons that can be transferred to molecular oxygen. The energy liberated in this process is used to generate ATP. This step of transferring electrons from reduced NADH and FADH₂ to oxygen by electron carriers, which also results in production of ATP, is called oxidative phosphorylation (OXPHOS) or the respiratory chain. As already mentioned, the process takes place within the inner membrane of the mitochondrion. Oxidative phosphorylation is not a single-step process, but involves 5 multimeric enzyme complexes (complexes I to V) and two redox carriers, coenzyme Q and cytochrome C.

The term ‘mitochondrial (mt) disorders’ was originally proposed to include all the clinical phenotypes associated with primary defects in mitochondrial energy output. However, this term is currently restricted to defects in the terminal component of the mt energy pathway, i.e., the OXPHOS system [1]. Faulty OXPHOS can be caused by single or multiple defects involving any of the five complexes of the respiratory chain. The multimeric enzyme complexes involved in OXPHOS are the product of complementation of two separate genetic systems, the nuclear and the mt genome. To put it simply, some of the units of these multimers are coded for by nuclear DNA and others by mtDNA. Explaining further, complex I has 42 subunits, complex II has 4, complex III 11, complex IV 13, and complex V has 14 subunits. More than 97% of these units are nuclear encoded. Mitochondria codes for 7 subunits of complex I, 1 subunit of complex III, 3 of complex IV, and 2 subunits of complex V. Complex II is entirely coded by nDNA. Our new awareness of mtDNA-related diseases and the peculiar features of mtDNA have added novel concepts of mitochondrial genetics. Following is a brief description of mtDNA and its unique features.

MITOCHONDRIAL GENETICS

Human mtDNA is a small (16.5 kilobase, a miniscule amount compared with the 3 million kilobases of the nuclear genome), circular, double-stranded molecule that has been sequenced in its entirety. It is entirely maternally inherited and encodes for 22 tRNAs, 2 rRNAs, and 13 polypeptide subunits of respiratory chain enzymes, already mentioned in the foregoing discussion. Several features of mtDNA may be related to its frequent association with disease. mtDNA mutates 10 times more frequently than nDNA and has no introns, so that a random mutation will usually strike a coding DNA sequence. In addition, mtDNA has neither protective histones nor an effective repair system, and is constantly exposed to oxygen free radicals generated by oxidative phosphorylation [2]. There are several distinctive features of mtDNA that will facilitate our understanding of mtDNA-related diseases.

Polyplasmy

Multiple mitochondria are present in every cell and each mitochondrion contains multiple genomes, so that each cell contains hundreds or thousands of copies of mtDNA. At cell division, mitochondria (and mtDNAs) distribute haphazardly between daughter cells [3].

Heteroplasmy

In normal tissues, all mtDNA molecules are identical, a situation known as homoplasmy. If there is a mutation in mtDNA, this may affect all genomes, another example of homoplasmy, or it may result in the coexistence in the same cell or in the same tissue of two populations of mtDNA, wild-type and mutant: this situation is called heteroplasmy [4].

Threshold effect

mtDNA mutations escape the rules that govern the expression of dominant or recessive nuclear genes. A minimum critical number of mutant mtDNAs will be necessary to impair energy metabolism severely enough to cause dysfunction of that particular organ or tissue, a concept known as the ‘threshold effect’ [5]. The threshold effect is, however, a relative concept. The number of mutant mtDNAs needed to cause cell dysfunction will vary from tissue to tissue depending on the vulnerability of any given tissue to impairments of oxidative metabolism. Thus the presence of a certain mutation in 80% of liver mtDNA may be clinically silent, whereas the same percentage of mutant mtDNAs may be symptomatic in muscle or the brain.
Maternal inheritance

The mode of transmission of mtDNA differs from Mendelian inheritance [6]. All mtDNA is derived from the oocyte, which means that a mother carrying an mtDNA mutation will transmit it to all her children, males and females, but only her daughters will pass it on to their progeny.

Mitotic segregation

At cell division, a proportion of mutant mitochondrial genomes may shift in daughter cells, and the phenotype may change accordingly. The clinical relevance of mitotic segregation is probably best exemplified by Pearson’s syndrome and Kearns Sayre syndrome, both of which are characterized by deletions in mtDNA. Children who present in infancy with Pearson’s syndrome usually survive this fatal form of sideroblastic anemia only to develop Kearns Sayre syndrome later in life. The clinical improvement of the blood dyscrasia in these patients is probably due to a gradual decrease in the number of mtDNA deletions in the rapidly replicating blood cells. Conversely, the proportion of deleted mtDNAs increases with time in the muscle.

Genetic code

The genetic code of mtDNA is different from the universal mammalian nuclear genetic code. This difference will be of special importance when considering the potential of gene therapy in mt disorders. If transfer of an mtDNA gene to nDNA is contemplated, then the ‘language’ will have to be changed from mitochondrial to nuclear [7].

Despite the obvious importance and ‘autonomy’ of mtDNA, mitochondria depend on the nucleus (nDNA) for the synthesis and importation of most constitutive proteins. mtDNA also requires nDNA-encoded factors for its replication, transcription, and translation [8]. By virtue of this dual genetic control, mitochondrial diseases can be due to defects in either mtDNA or nDNA. Mitochondrial diseases can therefore be genetically classified as given under:

Genetic classification of mitochondrial disorders:

A. Defects in mitochondrial DNA;
   • Point mutations (maternal inheritance),
   • Deletions and duplications (usually sporadic).
B. Defects in nuclear DNA (Mendelian inheritance);
   • Defects in genes encoding mitochondrial proteins,
   • Defects in mitochondrial protein importation,
   • Defects in intergenic communication.

As mitochondria are present in all organs, a bewildering array of clinical manifestations can result from their dysfunction. Usually, tissues characterized by high energy demand, such as muscle, brain, and heart, are the most severely involved. (mt encephalomyopathies and mt encephalocardiomyopathies) A detailed description of all the disorders due to mitochondrial dysfunction is beyond the scope of this review. Focus will be on mitochondrial encephalomyopathies (ME).

CLINICAL PRESENTATION

Ever since Holt et al. [8] described large scale deletions and Wallace et al. [9] the first point mutation in mtDNA of patients, a host of other mutations have been identified on the mtDNA. Mutations with the potential to cause a lethal impairment of oxidative phosphorylation are variable if they are heteroplasmic. Although mutations have been found in each type of mtDNA gene, tRNA mutations predominate [2]. In spite of the unraveling of the genetic mysteries underlying mitochondrial disorders, their diagnosis is still a challenge. The clinical heterogeneity and involvement of apparently unrelated organs are clues to the diagnosis of a mitochondrial disorder. It must be borne in mind that in some diseases, mitochondrial dysfunction may be merely a secondary phenomenon. Attempts have been made to classify these disorders clinically, but a significant proportion of patients do not conform to any particular syndrome. Moreover, the clinical features evolve with time and assignment to a particular phenotypic group may be possible only after several years.

As can already be derived, the relationship between the genotype and phenotype is not always a mathematical one. This compounds the difficulty in classifying these diseases. However, some correlates have been identified between specific mtDNA mutations and certain phenotypes. There are a few features common to most of these disorders, e.g. worsening of symptoms by unusual exercise, extremes of temperature, hypoglycemia, infections, and emotional stress. This is most likely due to increased metabolic demands imposed by these conditions. In general, when the clinical presentation is in childhood, the patient will be short statured and the course of the disease more severe. Certain features may be in common, e.g. sensorineural hearing loss, dementia, neuropathies, glucose intolerance, and elevated serum and CSF lactic acid. Ragged red fibers (RRFs) on muscle biopsy are of great significance. It may be of relevance to mention here that, as a rule, RRFs are seen in patients with impairment of mt protein synthesis (deletions and mutations in tRNA genes or quantitative defects of mtDNA) and not in patients with point mutations in structural genes, as happens in LHON or NARP. Because, presumably, oxidative phosphorylation is impaired in both types of disorders, it is postulated that decreased protein synthesis may somehow act as a signal for mt proliferation [10]. Consistent phenotypic features have also been described in some clinical syndromes. A clinical classification has been proposed based on the age at presentation [11].

A. Presenting in infancy

1. Subacute necrotizing encephalopathy (Leigh’s disease)

This is a clinical and pathological syndrome with several biochemical causes. It has been described in association with defects of pyruvate carboxylase, pyruvate dehydrogenase, and several other components of the respiratory chain. The clinical presentation is heterogeneous. The onset in more than half of cases is in the first year of life (mostly before the sixth month). However, cases with onset in early adulthood have also been reported. Infants present with developmental delay and failure to thrive. Sucking is poor, and often congenital lactic acidosis is present. The latter manifests as vomiting, anorexia, irritability, excessive crying, generalized seizures, and myoclonic jerks. Cardiorespiratory failure and death usually occurs within the first year itself. If the onset is in the second year of life, the child presents
with difficulty in walking, ataxia, dysarthria, cognitive decline, tonic spasms, respiratory disturbances such as episodic hyperventilation (especially during infections), periods of apnoea, and gasping, and quiet sobbing. Other features which could be seen are external ophthalmoplegia, nystagmus, gaze palsy, dysphagia, and involuntary movements of limbs, e.g. dystonia, choreiform movements, and ataxia. Mild cases showing developmental delay may be mistaken for cerebral palsy. Peripher al neuropathy may occur in some cases. The disease may be episodic and quite protracted, with exacerbations of symptoms during fever or infections. CSF is usually normal, but proteins may be increased. The clinical boundaries of Leigh’s disease have not been defined precisely. Bilateral striatal necrosis, a familial disorder of infancy and early childhood associated with dystonia, visual failure, and other neurological defects, is probably a variant of Leigh’s disease. The same may be true of an obscure syndrome of progressive dementia due to a thalamic lesion showing necrosis, vascular proliferation, and gliosis.

On pathological examination there are bilateral symmetrical foci of spongy necrosis with myelin degeneration, vascular proliferation, and gliosis in the thalami, brain stem, and spinal cord. The basal ganglia are usually affected early. Peripher al demyelinating neuropathy may occur. The distribution and histological appearance of the CNS lesions characteristically resemble those of Wernicke’s disease (a thiaminosis), except that the lesions of Leigh’s disease are more extensive, at times involving the striatum. Mamillary bodies tend to be spared. Neuro-imaging (CT/MRI) shows lesions in the basal ganglia, midbrain, and brainstem [12]. The lesions are usually symmetrical, with the putamen being affected more severely than the caudate. On muscle biopsy, RRFs are uncommon, though deficiency of cytochrome oxidase can be demonstrated.

Leigh’s syndrome demonstrates the concept of heteroplasmy and the threshold effect very well. To elaborate further, maternally inherited Leigh’s and NARP (to be discussed later) share the same T8993G mutation on the ATPase gene. A quantitative correlation between heteroplasmy and disease is seen in that with the increase in the load of mutant gene to more than 90% of total mtDNA, there is a shift from NARP, an adult-onset, slowly progressive syndrome, to Leigh’s disease, an early-onset, rapidly fatal phenotype. Retrospective analysis of 50 cases of Leigh’s syndrome not due to pyruvate dehydrogenase or cytochrome oxidase (COX) deficiency showed that 12 were associated with nt8993 mutation [13]. Patients with NARP and Leigh’s syndrome may coexist in the same family. NARP representing the oligosymptomatic expression of the nt8993 mutation.

Genetics: The commonest mutations in Leigh’s syndrome are point mutations, notable ones being the T8993G and T8993C mutation in ATPase6 gene. As mentioned before, T8993G is also seen in NARP disease. Nuclear gene defects, following a Mendelian pattern of inheritance, have also been reported, primarily in the SDH flavoprotein subunit.

2. Progressive infantile poliodystrophy (Alpers syndrome)

Poliodystrophies are degenerative diseases involving the cerebral gray matter. At an early stage, the pathology is focal, but later, widespread glioneuronal spongy degeneration is reported. These disorders are usually of autosomal recessive inheritance. The onset is characteristically with prominent seizures of various types, usually before the age of 6 months. A severe bout of seizures may be followed by marked dementia and pyramidal deficits. Liver involvement may occur late in the course of the disease.

Genetics: Severe depletion of a nuclear gene following a Mendelian autosomal recessive pattern of inheritance has been seen [14].

3. Lethal infantile mitochondrial encephalopathy

A disease of the neonatal period, it usually presents with cardiorespiratory failure, hypotonic muscle weakness, lactic acidosis, and failure to thrive. Muscle biopsy shows ragged red fibers and severe cytochrome oxidase deficiency on histochemistry. Biochemical analysis may show defects in complexes I or IV. The tissue distribution of biochemical defects runs in close parallel to the pattern of systemic involvement.

Genetics: Deletions or point mutations are not known so far. Depletion of mtDNA has been reported in a nuclear gene [15].

4. Pearson’s marrow-pancreas syndrome

This is a rare sporadic disorder characterized by refractory sideroblastic anemia and exocrine pancreatic dysfunction [16]. Anemia is detected soon after birth. Peripheral erythrocytes are macrocytic or may be normocytic. This is associated with neutropenia and thrombocytopenia. Reticulocyte count is low. Bone marrow is hypercellular or normocellular and the vacuolization of both erythroid and myeloid precursors is striking. Marrow hemosiderin content is increased and ring sideroblasts have been noted in most cases. Episodes of lactic acidosis frequently occur, eventually followed by hepatic or renal failure [17]. Death usually occurs in infancy. The few children who survive develop Kearns-Sayre syndrome [18].

Genetics: Deletions and duplications, with a sporadic pattern of inheritance, have been reported in the COXIII gene.

B. Presenting in childhood/ adolescence

1. Kearns-Sayre syndrome (KSS) and chronic progressive external ophthalmoplegia (CPEO)

Kearns and Sayre were the first to describe the association of progressive external ophthalmoplegia, pigmentary degeneration of the retina, and defects in cardiac conduction. This presumably sporadic disorder is defined by a triad of (1) onset before 20 years, (2) chronic progressive external ophthalmoplegia, and (3) pigmentary retinopathy. In addition, any of the following may also be present: heart block, cerebellar syndrome, or cerebrospinal fluid protein concentration ≥100 mg/dl [19]. Patients with these manifestations can be classified into 3 groups according to the age at onset and severity of clinical symptoms. The most severe is KSS characterized by infant, childhood, or adolescent onset and significant multisystem involvement. Some patients present in infancy with an atypical variant called Pearson’s syndrome (to be discussed
later). CPEO refers to a disorder of intermediate severity with adolescent or adult onset and variable involvement of tissues other than the eyelids and eye muscles [20].

Retinal changes in KSS are believed to be due to primary degeneration of the retinal pigment epithelium followed by secondary outer retinal degeneration. Clinically, a salt and pepper pattern of pigmentary clumping is seen in the posterior fundus, associated with a relatively benign course. Decreased visual acuity and night blindness are seen in only 40% of the patients. Defects in cardiac conduction are due to degeneration of the HIS Purkinje system and progressive infranodal block. This risk of early progression to complete heart block and sudden death is high, with a poor prognosis even after placement of a pacemaker. MRI shows predominantly white matter damage. Autopsy studies have revealed spongiform changes, predominantly of deep cerebral and cerebellar white matter, brain stem, and the dorsolateral spinal cord.

Genetics: Deletions (a large deletion of 15 base pairs has been reported) and duplications in the COXIII gene are the most frequent and follow a sporadic inheritance pattern.

2. Mitochondrial encephalopathy with lactic acidosis and stroke-like episodes (MELAS)

The MELAS syndrome is characterized by (1) stroke-like episodes with CT/MRI evidence of focal brain abnormalities, (2) lactic acidosis, RRFs in the muscle biopsy specimen or both, and (3) at least two of the following: focal or generalized seizures, dementia, recurrent headaches, and vomiting [21]. Most patients with MELAS develop normally until they experience intermittent nausea, vomiting, and migrainous headache followed by their first stroke-like episode. The episodes result in focal neurological defects, e.g. transient cortical blindness, hemianopia, focal paresis/paraplegia, and/or aphasia. Despite dramatic computerized tomographic (CT) abnormalities, the recovery is initially good. With recurrent episodes there is only partial recovery, with eventual deterioration. CT/MRI abnormalities are not located in classical vascular territories and angiography has not revealed significant vascular disease [22]. The proposed mechanism for vascular injury is that under conditions of stress, certain areas of the brain with particularly high rates of oxidative metabolism cannot meet the additional energy demands and a ‘metabolic infarct’ results. Autopsy studies of the CNS in patients with MELAS have shown spongy degeneration, predominantly affecting the cortex, with relative sparing of deep white matter. Prognosis is poor, with most patients dying within a few years of diagnosis [23].

Genetics: Point mutations in tRNA genes are known. Notable ones being A3243G in tRNA{Leu} (UUR), G1642A in tRNA{Val}, A3252G in tRNA{Leu} (UUR), T3271C in tRNA{Leu} (UUR), T3291C in tRNA{Leu} (UUR), and T7512C in tRNA{Val}. An A3243G mutation is the most common and tRNA{Leu} (UUR) the most commonly involved gene in MELAS. Like most point mutations, these are also maternally inherited.

3. Myoclonic epilepsy with ragged red fibers (MERRF)

Fukuhara et al. described the association of epilepsy and ragged red fibers in 1980 [24], and it is now recognized to be perhaps the most distinctive mt disease, presenting as progressive myoclonic epilepsy or ataxia with ragged red fibers on muscle biopsy. A child or young adult presents with myoclonus, which may be elicited by startling or movement of the limb. Seizure type may vary (drop attacks, focal, tonic-clonic), but are often photosensitive. Ataxia tends to worsen progressively. Myopathy is usually mild or not apparent. Other features include dementia, corticospinal tract degeneration, peripheral neuropathy, optic atrophy, and deafness. Central hypoventilation syndrome with respiratory failure and myoclonic epilepsy have also been reported [25]. Pathological studies of brain have shown spongy degeneration and laminar necrosis and/or infarcts of the cortex and subcortical white matter of the cerebral or cerebellar hemisphere [26].

Genetics: The most commonly associated mutation with MERRF is an A to G transition at nt8344 in the tRNA gene of mtDNA [27]. Another mutation at nt8356 in the same gene has been described in two families [28]. Other clinical phenotypes associated with the nt8344 MERRF mutation include Leigh’s syndrome, myoclonus or myopathy with truncal lipomas, and proximal myopathy [29]. The mutation in blood is as abundant as in muscle, providing a useful diagnostic test.

4. Neuropathy, ataxia and retinitis pigmentosa (NARP)

This is a maternally inherited syndrome characterized by a multisystem disorder including developmental delay, retinitis pigmentosa, ataxia of mixed tabetic-cerebellar type, sensorineuropathy, dementia, seizures, and proximal weakness. Its severity corresponds to the amount of aberrant DNA in the mitochondrial genome. As already mentioned in the description of Leigh’s syndrome, NARP shares the nt8993 mutation with it. Within kindred, the mitochondrial aberration may manifest itself as a mild developmental delay, NARP, full-blown Leigh’s syndrome, or early death with lactic acidosis. These differences in severity are thought to result from the protective effects of even small amounts of the normal mt genome. The clinical picture of NARP is quite similar to that of Refsum’s disease (heredopathia atactica polyneuritiformis), but in contrast to it there is no change in the level of phytic acid.

Genetics: Point mutations, in common with maternally inherited Leigh’s syndrome, are known, e.g. a T8899G and T8993C mutation in ATPase 6 gene.

5. Leber’s hereditary optic neuropathy (LHON)

This is characterized by acute/subacute sequential loss of vision with variable recovery in both eyes. During the acute stage the optic disc is swollen with peripapillary telangiectasis in many cases and is followed by optic atrophy within 2–3 months. 80–85% patients are males with age at onset between 18–30 years. Associated features may include hyperreflexia, cerebellar ataxia, peripheral neuropathy, cardiac conduction abnormalities (pre-excitation syndrome), or psychiatric disorders [30].

Genetics: Point mutations G11778A and T14484C in the ND4 gene and G144559A and T14484C in the ND6 gene have been described most commonly.
6. Myoneurogastrointestinal disorder and encephalopathy (MNGIE syndrome)

This is an etiologically heterogeneous disorder characterized by PEO, mitochondrial myopathy, peripheral neuropathy, dementia with progressive leukodystrophy [31], with prominent involvement of gastrointestinal tract presenting as chronic diarrhea and intestinal pseudo-obstruction.

Genetics: Multiple deletions in the nuclear genome causing defects in intergenomic communication have been reported. Recently, functional mutations have been described in the thymidine phosphorylase gene [32]. The pattern of inheritance is Mendelian, usually autosomal recessive.

DEVELOPMENTS IN UNDERSTANDING

With so many variables involved in the development of mt encephalomyopathies, the relationship between the genotype and phenotype is not always a mathematical one. Adding a new dimension to the understanding of mt encephalomyopathies is the evidence of a ‘genetic bottleneck’ in the transmission of mtDNA from mother to offspring. This bottleneck influences the dose of intergenerational mutant mtDNA transfer. There is now direct evidence that a large number of mtDNA units segregate at the bottleneck [35]. As a result, relatively few mtDNA units are thought actually to contribute to the next generation [34]. This extreme reduction is followed by a rapid expansion of the mtDNA copy number [35]. The eventual amplification or reduction of a mutation would therefore be determined by the size of the ‘bottleneck’ [36]. Much of the segregation occurs by the time oocytes are mature. In fact, animal studies suggest that most of the segregation occurs during the oogonial stage of oogenesis before the development of primary oocytes [37]. The results so far imply that the mtDNA genotype of a mature oocyte or early embryo is a good predictor of the mtDNA genotype of the whole organism, and this has important implications for counseling carriers.

Histochemistry, immunohistochemistry, and in situ hybridization techniques have been used to understand the link between mtDNA mutations, impairment of oxidative phosphorylation, and the clinical phenotype. Another recently emerging tool to assess the intrinsic pathogenicity of severe mtDNA mutations are the hybrids. These are generated by fusing immortal human cell line-derived cells devoid of mtDNA (rho− cells) with the patient’s enucleated cells (cytoplasts). Cells are made devoid of mtDNA through prolonged exposure to ethidium bromide [38]. Cybrid cell lines with varying proportions of mutant and wild-type mitochondrial genomes are generated and the effect is studied on oxidative phosphorylation, mitochondrial protein synthesis, and the visual correlates thereof. The threshold point of each mutation can also be ascertained. The proportion of mutant mitochondrial DNA required for the occurrence of a deleterious phenotype varies among persons, among organ systems, and within a given tissue. The threshold effect depends on the delicate balance between oxidative supply and demand. Cybrid studies have shown that the threshold effect is higher (92%) for point mutations as in MERRF or MELAS than for deletions [39–40]. This corroborates with the clinical observation that small amounts of wild-type genomes are sufficient to protect patients with a MERRF or MELAS mutation from expressing these diseases [41].

Cybrid studies have also justified the theory that the ragged red fibers (RRFs) so often seen in mt diseases represent the crossing over of the threshold level. When the population of mutant mtDNAs crosses this level, the respiratory rate and mitochondrial protein synthesis are depressed. RRFs have been shown to contain more abundant mtDNA mutations and usually lack cytochrome-oxidative activity and have increased succinate dehydrogenase. The enzymes are coded by mtDNA and nDNA, respectively. NADH-TR detects a reductase which is present not only in mitochondria, but also in sarcoplasmic reticulum. Tubular aggregates derived from sarcoplasmic reticulum are therefore heavily stained by NADH-TR and not with SDH. A combination of these two stains therefore allows differentiation between tubular aggregates and mitochondrial accumulations. RRFs were first reported in 1972 in muscle biopsies of patients with progressive neuromuscular disease [42]. The name was derived from the subsarcolemmal and intermyofibrillar accumulations of red material on fresh frozen sections of muscle stained with modified Gomori’s trichrome stain. Electron microscopy of these aggregates has revealed increased numbers of mitochondria and atypical mitochondria, some with paracrystalline inclusions [41]. Only recently have studies shown that occasional ragged-red fibers are non-specific findings and are indicative of a mitochondrial reaction to different types of insult. However, they provide an important clue while interpreting muscle biopsies [43].

Recently some other previously known disorders have been found to be associated with mt defects, such as Friedreich’s ataxia, hereditary spastic paraplegia with ragged red fiber myopathy, and Wolfram syndrome. Many new syndromes are being added, with a variable constellation of symptoms.

EVALUATION OF THE PATIENT

While evaluating a patient, it is the clinical syndrome, family history, and any corroborating evidence of an mt disorder or its genetic representation that is important. A methodical, systematic approach has been recommended when dealing with a suspected case of mt encephalomyopathy [20].

Diagnostic tools

The ultimate diagnostic tools for these disorders are, of course, biochemical and molecular studies. But the need for a sophisticated set-up to conduct these types of analyses has tended to limit the investigation and diagnosis of mt encephalomyopathies to a few specialist centers. Fortunately, a battery of routinely available clinical investigations are now being increasingly used when an mt defect is suspected. The findings of these investigations in conjunction with the clinical picture is effectively used to diagnose mt disorders even in non-specialist centers. A brief account of these diagnostic tools is given here.

Lactate, Pyruvate, and Lactate/Pyruvate (L/P) ratio

Lactic acidosis has been recognized in mt disease for many years, but it is not an invariable finding [44]. If present, mt disease cannot be presumed to be its only cause [45]. An elevated fasting lactate is reported in only 40% of patients
Therefore, an isolated fasting blood lactate alone is not a good screening. It may be of benefit to subject the patient to mild physical stress with bicycle ergometry in order to demonstrate an abnormal rise in venous lactate levels. In some patients, the lactate level may only be elevated during periods of metabolic stress, such as fever or infections. This makes it necessary to assay lactate repeatedly. Used selectively and interpreted cautiously, measurements of venous lactate appear to be a useful marker in certain categories of mt disease, such as Leigh’s syndrome and 3243/MELAS, among other mt encephalopathies, but it is of little value in CPEO, even with additional CNS features. CSF lactate measurement is important in patients with a predominantly CNS disease. At times these patients may have a normal venous lactate level whilst their CSF lactate is elevated. In general it has been noticed that the more destructive encephalopathies, having focal low density and/or calcification on imaging (e.g. 3243/MELAS, KSS and Leigh’s syndrome), are invariably associated with increased CSF lactate, whilst less destructive forms, such as 3244MERRF and complex II deficiency that show atrophy alone on imaging, may have normal or only mildly elevated CSF lactate. The venous lactate-to-pyruvate ratio is suggested to be of value in the differential diagnosis of lactic acidosis, with an elevated L/P ratio indicative of mt disease. A note of caution to be remembered is that a normal L/P ratio can also occur in mt disease. Measurement of the ketone body ratio of \( \beta \)-hydroxybutyrate to acetoacetate has also been advocated by some. Alanine concentration is particularly helpful in diseases where pyruvate metabolism is disturbed.

Electromyography

EMG has not proved to be a very important indicator of muscle involvement in mt disease. Here again, the finding of a normal/near normal EMG in a patient with significant muscle weakness is highly suggestive of mt disease.

Electroencephalogram

EEG abnormalities appear consistent within certain groups, but by and large, EEG findings are not sufficiently specific.

CT/MRI

Of the various abnormalities described in mt disease, the significant ones are calcification of the basal ganglia and low density (on CT) or high signal (on T2-weighted MRI). Basal ganglia calcification has been particularly noted in CTs of patients with a 3243 MELAS mutation. Low-density basal ganglia areas on CT and its equivalent finding of high signal on T2-weighted images have been reported in Leigh’s disease and also in KSS. In typical 3243 MELAS, areas of low density on CT (high signal on T2 MRI) are commonly seen. Though non-specific, such findings should nonetheless alert the clinician to the possibility of mt disease.

Table 1. Major diagnostic criteria [11].

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<td>1.</td>
<td>Clinically complete RC encephalomyopathy</td>
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<td>2.</td>
<td>&gt;2% RRF in a skeletal muscle biopsy</td>
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<td>3.</td>
<td>Presence of one or more of the following indicators of depressed RC enzymatic activity:</td>
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<td>• &lt;20% activity of age-adjusted mean on biochemical or polarographic assessment of one or more RC complexes</td>
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<td>• &gt;2% COX-negative fibers in an open muscle biopsy if &lt;50 years of age or</td>
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<td>• &gt;5% COX-negative fibers if &gt;50 years of age</td>
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<td>4.</td>
<td>Identification of a nDNA or mtDNA alteration of undisputed pathogenicity</td>
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Table 2. Minor diagnostic criteria [11].

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<td>1.</td>
<td>Some clinical symptoms with muscles or CNS involvement, but no complete encephalomyopathy</td>
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<td>2.</td>
<td>At least one of the following bio-optical indicators of increased mitochondrial content or of mitochondrial abnormality in muscle:</td>
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<td>• 1–2% RRF if aged 30–50 years</td>
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<td>• Any RRF if &lt; 30 years of age</td>
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<td>• Widespread electron microscopic abnormalities</td>
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<td>3.</td>
<td>Presence of one or more of the following indicators of depressed RC function:</td>
</tr>
<tr>
<td></td>
<td>• 20–30% activity of age-adjusted mean on biochemical or polarographic assessment of one or more RC complexes</td>
</tr>
<tr>
<td></td>
<td>• Antibody-based demonstration of a defect in RC complex expression</td>
</tr>
<tr>
<td>4.</td>
<td>Identification of mtDNA alteration previously recognized as being associated with the presenting syndrome and fulfilling some, but not all, of the six criteria set out in the text</td>
</tr>
<tr>
<td>5.</td>
<td>One or more metabolic indicators of impaired oxidative phosphorylation:</td>
</tr>
<tr>
<td></td>
<td>• Elevated lactate, pyruvate, and/or alanine content (CSF and/or blood)</td>
</tr>
<tr>
<td></td>
<td>• Increased CSF protein if KSS is suspected</td>
</tr>
<tr>
<td></td>
<td>• Impaired metabolism in muscle or brain, demonstrated by (^{31})P-MRS or PET</td>
</tr>
<tr>
<td></td>
<td>• Reduced VO(_2)max, AVO2D, or lactate threshold on ergometry, not explained by detraining</td>
</tr>
</tbody>
</table>

[46]. Therefore, an isolated fasting blood lactate alone is not a good screening. It may be of benefit to subject the patient to mild physical stress with bicycle ergometry in order to demonstrate an abnormal rise in venous lactate levels [47]. In some patients, the lactate level may only be elevated during periods of metabolic stress, such as fever or infections. This makes it necessary to assay lactate repeatedly. Used selectively and interpreted cautiously, measurements of venous lactate appears to be a useful marker in certain categories of mt disease, such as Leigh’s syndrome and 3243/MELAS, among other mt encephalopathies, but it is of little value in CPEO, even with additional CNS features. CSF lactate measurement is important in patients with a predominantly CNS disease. At times these patients may have a normal venous lactate level whilst their CSF lactate is elevated. In general it has been noticed that the more destructive encephalopathies, having focal low density and/or calcification on imaging (e.g. 3243/MELAS, KSS and Leigh’s syndrome), are invariably associated with increased CSF lactate, whilst less destructive forms, such as 3244MERRF and complex II deficiency that show atrophy alone on imaging, may have normal or only mildly elevated CSF lactate. The venous lactate-to-pyruvate ratio is suggested to be of value in the differential diagnosis of lactic acidosis, with an elevated L/P ratio indicative of mt disease. A note of caution to be remembered is that a normal L/P ratio can also occur in mt disease. Measurement of the ketone body ratio of \( \beta \)-hydroxybutyrate to acetoacetate has also been advocated by
**Muscle histology and histochemistry**

Histological and histochemical analysis of skeletal muscle remains one of the most important investigations in the diagnosis of mt disease. Gomori’s trichrome stain is used to demonstrate the classic ragged red fibers. The histochemical stains most commonly used are cytochrome oxidase (COX) and succinate dehydrogenase (SDH). The latter is especially useful in demonstrating the abnormal subsarcolemmal accumulation of mitochondria (SSAMs).

From the foregoing discussion, the complexity of the diagnostic process, especially in non-clear-cut cases, is obvious. Walker et al. [11] have proposed an approach which delineates major and minor criteria, the former being very supportive of a diagnosis of mt encephalomyopathy, the latter being less definite. (Tables 1 and 2). Based on these, they have defined definite, probable, and possible categories. The identification of two major criteria or one major criterion and two minor criteria allows definite diagnosis of mt encephalomyopathy. The identification of one major criterion and one minor criterion or at least three minor criteria allows a diagnosis of probable mt encephalomyopathy. The identification of either one major criterion or one minor criterion in addition to the clinical criterion indicates possible mt encephalomyopathy.

**Prenatal diagnosis**

Sampling of chorionic villi for prenatal diagnosis may show mutant mtDNA that corresponds to tissue levels in aborted fetuses, but the use of this information seems not to be dependable in predicting the severity of illness because of the erratic distribution of mtDNA.

**TREATMENT**

The treatment of mitochondrial encephalomyopathies is based on attempts to minimize the energy demands on dysfunctional mitochondria and to biochemically manipulate the mitochondrial milieu in order to permit them to function under the best possible circumstances. Certain compounds that can act as artificial electron acceptors have had favorable results. Vitamin K<sub>3</sub>, vitamin C, and coenzyme Q work on these principles. Riboflavin, vitamin B<sub>2</sub>, is a precursor of flavin monophosphate and flavin adenine dinucleotide (components of complexes I and II) and has been put to beneficial use in mt disorders due to complex I deficiency. Use of corticosteroids has also had favorable results, probably by induction of antiphospholipase compounds. Kimura et al. documented significant clinical, biochemical, and radiological improvements following sodium dichloroacetate (DCA) therapy, which reduces the circulating lactate and pyruvate concentrations by stimulating the activity of the pyruvate dehydrogenase complex (PDHC), in three children with mitochondrial encephalomyopathy [51]. However, Zeman et al. administered DCA to 7 children with mitochondrial myopathy and found no significant improvement in clinical course [52].

**CONCLUSIONS**

The past few years have seen a great advancement in understanding mitochondrial structure, metabolism, and genetics. This has resulted in a greater appreciation and identification of mitochondrial disorders. Though still challenging, some coherence has started to emerge among the diverse fields of clinical presentation, mt genetics, and biochemical effects. This should logically result in better evaluation and treatment. A clinical classification based on the age at the time of presentation and the identification of some major and minor criteria by Walker et al. may be considered a significant contribution in the clinical evaluation of a patient.

The authors feel that though much has been accomplished in the study of mt encephalomyopathies, it needs to be translated into clinical practice. That clinicians have started considering the possibility of a mitochondrial defect in various settings is significant enough to predict more work in this field. The challenge lies in diagnosing such cases in a routine hospital setting with commonly available diagnostic tools. Only when the disease moves out of the domain of highly specialized, sophisticated centers can its real incidence and significance be ascertained. In the meantime, the emerging molecular correlates will enable giving the prospective outcome of certain clinical phenotypes.

**REFERENCES:**


