Diagnosis and Treatment of Childhood Mitochondrial Diseases

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Mitochondrial cytopathies are caused by genetic alterations of nuclear- or mitochondrial-encoded genes involved in the synthesis of subunits of the electron transport chain. Mutations of mitochondrial DNA are associated with a wide range of clinical presentations [1–4]. The ubiquitous nature of mitochondria and the role of the mitochondria in cellular metabolism result in the potential for any tissue in the body to be affected [5–7,8••,9]. Although some children with mitochondrial disease present with life-threatening lactic acidosis in the newborn period, the majority of children come to clinical attention for nonspecific problems, including failure to thrive, developmental delay, seizures, hypotonia, and loss of developmental milestones. The diagnosis of these disorders is made through careful clinical evaluation, coupled with biochemical, morphologic, and molecular biologic techniques. Genetic counseling is difficult due to unique aspects of mitochondrial genetics. Despite advances in our understanding of mitochondrial biochemistry and genetics, treatment options remain limited.

Introduction

Mitochondria are important in cellular metabolism and transport, and function in a variety of degradative and synthetic functions. Mitochondria participate in the process of oxidative phosphorylation (OXPHOS), the transformation of energy (from the breakdown of nutrients) in the presence of oxygen to adenosine triphosphate (ATP). The process of OXPHOS is accomplished by sequential metabolic reactions occurring on the inner mitochondrial membrane. Enzymes mediating this process are collectively known as the electron transport chain (ETC). Four major enzyme complexes (complexes I through IV), an ATP synthetase activity (complex V), and associated protein cofactors make up the ETC. The various substrates are metabolized and produce reducing equivalents that enter the respiratory chain (Fig 1.). Disorders of OXPHOS are caused by a malfunction in one or several of the five enzymes that comprise the ETC.

Disorders of OXPHOS may affect the brain (central, peripheral, and autonomic nervous systems), muscles, kidneys, heart, liver, eyes, ears, pancreas, skin, and other organ systems. In some patients, only one organ is clinically involved, whereas in others multiple organs are involved and cause clinical symptoms. The conditions caused by defects in OXPHOS can range from subclinical to lethal. Despite the wide range of clinical presentations, a number of distinctive syndromes have been identified.

Mitochondrial DNA, Replication, and Translation

Mitochondria contain the enzymes, complexes, and proteins needed for the production of ATP and its exportation into the cytoplasm. Mitochondria contain their own DNA that is separate from nuclear DNA. The human mitochondrial (mt) genome is a 16.5-kb, double-stranded, circular molecule whose sequence is known [10]. It is a highly compact molecule, composed of 37 genes encoding 22 transfer (t) RNAs, 2 ribosomal (r) RNAs, and 13 protein subunits of the ETC. There are no introns. Mitochondria divide and replicate under the control of nuclear factors.

The genetic code for translation of mitochondrial proteins is different from the cell universal code involved in the translation of nuclear-encoded genes. Although the mitochondria encode 13 polypeptide subunits of the ETC, the majority are nuclear encoded and imported into the mitochondria from the cytosol to assemble together with the mitochondrial (mt) DNA-encoded subunits into holoenzymes. Only complex II is encoded entirely by nuclear DNA (Table 1).

Unique Aspects of Mitochondrial Genetics

There are several differences between nuclear DNA and mtDNA. Each mitochondrion contains two to 10 copies of mtDNA. A cell may harbor hundreds to thousands of mitochondria and mtDNAs, which results in unique genetics. The mtDNA is maternally inherited. The mtDNA can exist in a cell as a mixture of mutant and normal mtDNAs (heteroplasmy). The mtDNA do not recombine, and can undergo replicative segregation during meiotic or mitotic

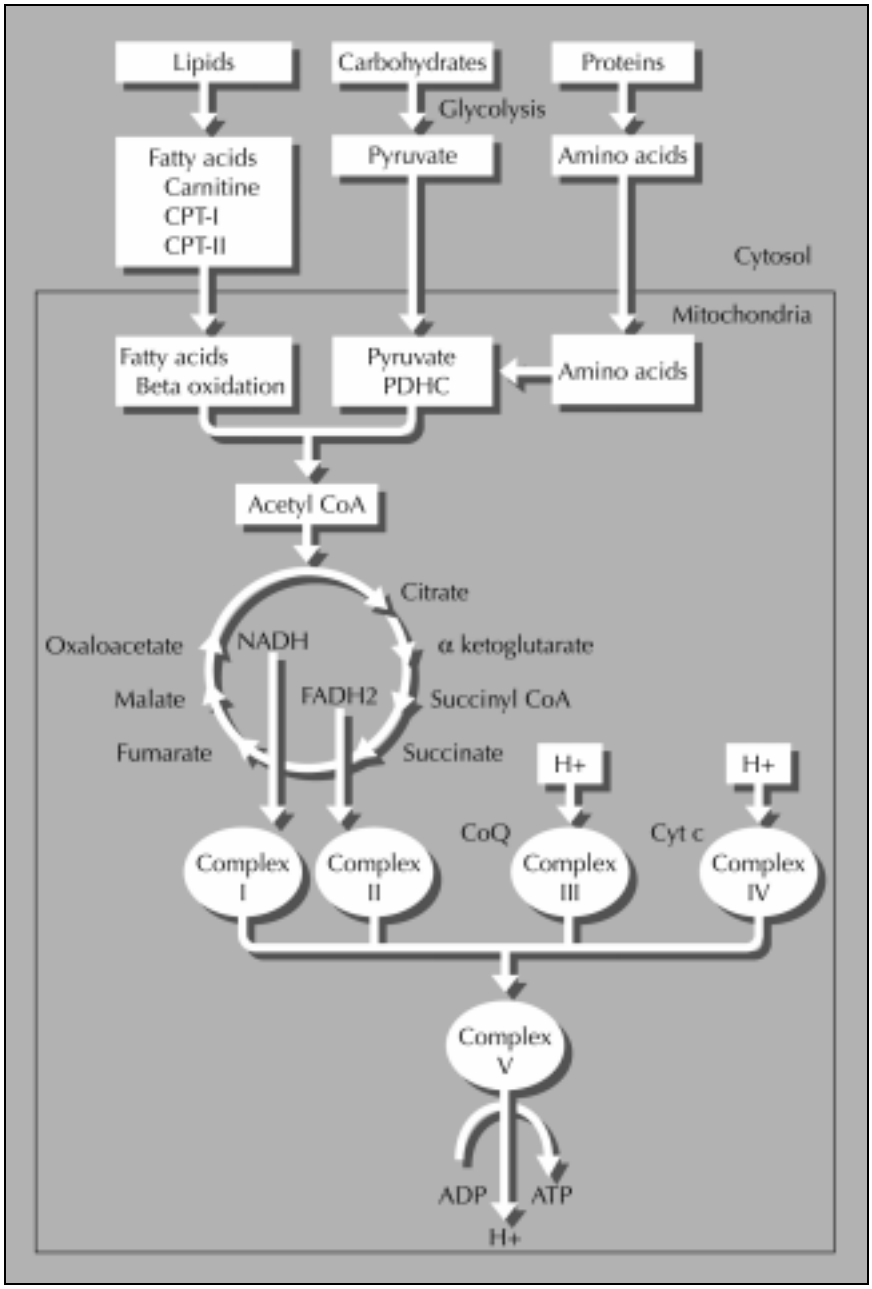

Figure 1. Simplified schema of mitochondrial oxidation and related pathways. Breakdown products of carbohydrates (via breakdown of pyruvate), proteins, and fats from acetyl CoA, which is metabolized by the Krebs cycle to provide H+ for the electron transport chain (ETC) to synthesize adenosine triphosphate (ATP). The oxidative decarboxylation of pyruvate is achieved by the pyruvate deyhdrogenase complex (PDHC). Through sequential reactions known as the Krebs cycle, high-energy electrons are extracted in the form of NADH and FADH2. These high-energy electrons flow through a series of carriers in the ETC to molecular oxygen, where the final oxidative step occurs with formation of H_2O. As high-energy electrons flow down the carriers in the ETC, the energy released is used to pump protons across the inner mitochondrial membrane. This electrochemical gradient is used to generate ATP via the action of complex V (ATP synthetase).

division to give pure genotypes (homoplasmic). When a cell divides, both mutated and nonmutated mtDNA are randomly segregated in the daughter cells.

The severity of a defect due to a mtDNA mutation depends on the nature of the mutation, the proportion of mutant mtDNA present within a cell, and the tissue threshold for expression. The expression threshold depends on the severity of the OXPHOS defect and the relative reliance of each organ system on mitochondrial energy production, with the central nervous system (CNS) being most sensitive to mitochondrial defects, followed by skeletal muscle, heart, endocrine organs, and kidney. Because mitochondria replicate more often than nuclei, the proportion of mutant and wild-type mtDNA may change within a given cell cycle.

Because of increased replication cycles, there is a greater chance for replication-related mutations. The mtDNA has a high mutation rate. Nucleotide substitutions can alter the structure of one of the 13 genes (missense mutation), or one of the tRNA or rRNA genes (protein synthesis mutation). The mtDNA is a target for mutations due to a lack of histones and absence of introns, and also because of heteroplasmy, which allows lethal mutations to persist. The mitochondria produce over 95% of the cell's free radicals, which can lead to further damage of the mtDNA genome.

Oxidative Phosphorylation Disorders

Genetically inherited defects of mtDNA or nuclear-encoded genes that lead to impaired OXPHOS give rise to a

Table 1. Genetic origin of the OXPHOS protein subunits

Complex	Subunits	Nuclear encoded	Mitochondrial DNA encoded
I	41	34	7 (ND1, ND2, ND3, ND4, ND4L, ND5, ND6)
II	4	4	0
III	11	10	1 (cytochrome b)
IV	13	10	3 (COXI, COXII, COXIII)
V	14	12	2 (ATPase 6, ATPase8)

ATP—adenosine triphosphate; COX—cytochrome oxidase; OXPHOS—oxidative phosphorylation.

variety of clinical diseases due to failure of energy (ATP) synthesis. Disease symptoms appear when the mitochondrial energy-generating potential falls below an energetic threshold of the organ or tissue. The amount of mtDNA mutation is one factor that determines whether the defect will be clinically expressed. Typically, the highest levels of mtDNA mutations occur in postmitotic tissues (skeletal muscle, neurons). The threshold for expression may approach 60% for mtDNA deletions [11], and up to 95% for tRNA mutations [12].

Impaired function of the OXPHOS system leads to a disturbed oxidation/reduction state, shift of the pyruvate to lactate ratio towards lactic acidosis, and impairment of the Krebs cycle. A postprandial increase of ketone bodies may be seen due to buildup of coenzyme A and shuttling of substrates towards ketogenesis. Fats, in the form of free fatty acids and triglycerides, may build up. This can often be seen histologically as macrovesicular hepatic steatosis and lipid myopathy. On electron microscopy, the mitochondria may appear swollen and enlarged with loss of membrane integrity.

Clinical Manifestations of Mitochondrial Cytopathies

Disorders due to mitochondrial mutations are heterogeneous, and clinical manifestations range from single organ involvement to multisystem disease (Table 2). The same mutation or different mutation in the same mtDNA gene may give rise to very different clinical phenotypes, whereas the same phenotype may be due to different mutations.

Variability in clinical manifestation is due to several factors, including the proportion of heteroplasmy, varying thresholds of biochemical expression for both the mutation and the tissue involved, and the modifying effect of nuclear mitochondrial genes.

Class I mutations: disorders of nuclear genes

Mutations in nuclear-encoded OXPHOS subunits are increasingly being identified [13]. In general, syndromes due to defects of nuclear genes are much more stereotypical than syndromes due to mtDNA mutations.

Myoneurogastrointestinal encephalopathy syndrome

Myoneurogastrointestinal encephalopathy syndrome (MNGIE) features myopathy, neuropathy, and intestinal pseudoobstruction [14]. Other findings include external ophthalmoplegia, malabsorption, intermittent diarrhea, chronic malnutrition, gastrointestinal scleroderma, proximal limb weakness, muscle atrophy, polyneuropathy, encephalopathy, and sensorineural hearing loss. Hypodensity of the cerebral white matter has been described on magnetic resonance imaging (MRI) tests [15]. Biochemical findings include lactic acidosis after moderate glucose loads, or increased excretion of hydroxybutyric and fumaric acids. Muscle biopsy reveals ragged-red ocular and skeletal myopathy, abnormal meningeal and peripheral nerve blood vessels, and partial defect of cytochrome-c-oxidase. Mutations in the thymidine phosphorylase gene on 22q13.32-qter are implicated [16,17].

Leigh syndrome due to cytochrome oxidase deficiency

The clinical features of Leigh syndrome (subacute necrotizing encephalomyelopathy) are due to several biochemical defects [18], including pyruvate dehydrogenase deficiency (X-linked), cytochrome-*c*-oxidase deficiency (autosomal recessive), and OXPHOS defects (maternal inheritance). The prognosis for early-onset disease is poor, with death in the first years of life. The symptoms are extensive and include muscle weakness, hypotonia, clumsiness, tremor, Babinski reflex, absent tendon reflexes, abnormal eye movements, sluggish pupils, blindness, hyperventilation, apnea, and respiratory failure. Multisystemic disease may include liver and cardiac dysfunction. Biochemical findings may include intermittent lactic acidosis, high blood pyruvate, or high blood lactate. Neuroimaging in Leigh syndrome demonstrates a necrotizing encephalopathy with grey matter degeneration and brain stem necrosis [19,20]. Ragged-red fibers (RRFs) are absent on muscle biopsy. Recently, the nuclear-encoded genes *Surf-1* (on chromosome 9q34.1) and *SDH* (on chromosome 5p15) have been identified and mutations found in patients with these forms of Leigh syndrome [21••,22••]. Additional genes on chromosomes 5q11.1 and 11q13 have been identified.

Table 2. Characteristic features of mitochondrial cytopathies*

System	Clinical Features
Central nervous system	Seizures, ataxia, stroke in the young, myoclonus, other movement disorders, dementia, migraine, mental retardation, certain pyschiatric features
Ear	Sensorineural hearing loss, aminoglycoside-induced deafness
Eye	Optic atrophy, pigmentary retinopathy, cataracts, vessel tortuosity, visual loss
Neuromuscular	Ptosis, CPEO, weakness, hypotonia, neuropathy, recurrent myoglobinuria
Autonomic	Temperature instability; vital sign abnormalities
Bone marrow	Sideroblastic anemia, pancytopenia
Kidney	Fanconi syndrome, renal tubular dysfunction, tubulointerstitial dysfunction
Endocrine	Diabetes mellitus, hypoparathyroidism, hypothyroidism, Addison's disease, growth deficiency, multiple hormone deficiencies
Heart	Cardiomyopathy, arrhythmias, conduction block
Gastrointestinal	Cyclical vomiting, pseudoobstruction, pancreatic failure, liver failure, valproate-induced liver toxicity, failure to thrive, chronic diarrhea
Skin	Mottled pigmentation of sun-exposed areas, dry, thick, brittle hair

*The presence of features in multiple systems increases the likelihood of mitochondrial disorder.
CPEO—chronic progressive external ophthalmoplegia.

Class II mutations: mitochondrial DNA point mutations

Leber hereditary optic neuropathy

Ophthalmologic features of Leber hereditary optic neuropathy (LHON) include optic atrophy, sudden central visual loss, swollen optic disk, and a large, central visual field defect. Inconsistent signs include hyperreflexia, extensor plantar reflexes, incoordination, and peripheral neuropathy. A variant of LHON with early-onset dystonia and bilateral basal ganglia lesions is associated with *mtG14459A* mutation [23]. The onset of LHON is in the second to third decade of life. Muscle biopsies may be normal, and there is no lactic acidosis except in the *14459* mutation. The majority of cases, at least 50% to 70%, are due to A to G transition in *mt11778*; however, many other missense mutations have been recognized [24].

Mitochondrial encephalopathy with lactic acidosis and stroke-like episodes

Patients with mitochondrial encephalopathy with lactic acidosis and stroke-like episodes (MELAS) may present with episodic sudden headaches, intermittent, atypical migraine headaches, intractable seizures including myoclonic, generalized tonic clonic, and others, hemiparesis, stroke-like episodes, dementia, and encephalopathy [25]. Childhood onset after normal early development is common. Other findings may include myopathy, episodic vomiting, progressive bilateral sensorineural hearing loss, bilateral cataracts, hemianopsia, and cortical blindness. Patients with MELAS have elevated resting serum lactate and elevated cerebrospinal fluid (CSF) lactate. MRI may demonstrate posteriorly located strokes that do not conform to a vascular territory, and basal ganglia lucencies. Muscle biopsy may demonstrate RRFs or subsarcolemmal pleomorphic mitochondria. A3243G mutation in the tRNA leucine gene is the major mutation; however, other mutations can cause the same phenotype, including those at other locations.

Myoclonic epilepsy with ragged-red fibers

Patients with myoclonic epilepsy with ragged-red fibers (MERRF) present with myoclonic epilepsy, ataxia, spasticity, generalized seizures, optic atrophy, and dementia [26]. They may also have muscle weakness, myopathy, neuropathy, or sensorineural hearing loss. The muscle biopsy shows ragged-red muscle fibers. Biochemical aberrations may include elevations of serum pyruvate, or pyruvate and lactate, and reduced activities of complexes I and IV. The majority is due to a single point mutation, G8344G $tRNA^{lys}$; however, the mutation T8356C is seen with some frequency.

Neurogenic atrophy, retinitis pigmentosa

Neurogenic atrophy, retinitis pigmentosa (NARP) may represent a milder form of maternally inherited Leigh syndrome. Patients with NARP may experience developmental delay, dementia, seizures, ataxia, retinitis pigmentosa, proximal neurogenic muscle weakness, and sensory neuropathy. The muscle biopsy may demonstrate features of neurogenic atrophy, no RRFs, and no histochemical evidence of mitochondrial myopathy. Patients may have inconsistent lactic acidosis. The genetic defect is due to T8993G in *ATPase 6* gene. T to C transition in this position is observed in maternally inherited Leigh syndrome, with features similar to that observed in *Surf-1* gene mutations [27].

Class III mutations: mitochondrial DNA depletion and duplications

Mitochondrial DNA depletion syndromes

Mitochondrial DNA depletion represents a class of mitochondrial disorders involving quantitative, rather than qualitative, errors of mtDNA in affected tissues [28•]. First

described in 1991 [29], patients show variable levels of mtDNA depletion in the affected tissues; unaffected tissues have normal levels of mtDNA.

Infantile onset is common after normal birth and presents with hypotonia, renal dysfunction (Fanconi syndrome), and hepatic failure with lactic acidosis. Liver histology discloses fatty changes with fibrosis and bile duct proliferation. Muscle biopsy may be negative or show cytochrome oxidase (COX)-negative fibers in a patchy distribution. Death typically occurs by the first year of life. Muscle or liver biopsies from these patients lack mtDNA, often containing less than 5% of the amount found in age-matched controls. Autosomal recessive inheritance seems likely with a high recurrence rate.

The milder variant presents with a slowly progressive mitochondrial encephalomyopathy beginning in childhood, associated with lesser levels of tissue depletion in skeletal muscle. In these cases, the liver may be the only affected organ. The genetics of mtDNA depletion involve a defect in the control of mtDNA copy number [30].

Methods of Diagnosis

The evaluation of a child with a suspected mitochondrial disorder can be complex and expensive. Tragically, a sibling may be affected before the diagnosis has been made in the proband. The diagnosis is based on collective evidence that a mitochondrial disorder is present. This includes a suggestive clinical history in which a child presents with either catastrophic disease and involvement of three organ systems, or two or more organ systems are affected with slow or relapsing deterioration. There may be abnormal metabolites in blood, urine, or CSF, a family history suggestive of maternal or autosomal inheritance, abnormal brain stem functioning, or specific abnormalities on MRI or muscle biopsy. Certain features are especially frequent in these diseases and should raise suspicion about a mitochondrial disorder, even when seen in isolation. These include strokes before the age of 40 years, especially involving the occipital lobes, chronic progressive external ophthalmoplegia (CPEO) in a nonmyasthenic patient, and sensorineural hearing loss. Certain clinical features when seen in combination are strongly suggestive of mitochondrial disease. The combination of a myopathy and CNS involvement such as ataxia, deafness, or seizures is suggestive.

Many cases appear sporadic and do not fit into a diagnostic category. Birth and developmental history may be normal before the onset of recurrent metabolic crises that may be heralded by an intercurrent viral infection. Patients may present with a subacute encephalopathy with progressive dementia and seizures. The nonneurologic range of organ involvement in mitochondrial cytopathies is vast, and many patients with renal tubular acidosis, gastrointestinal dysmotility, endocrinopathies, and cardiomyopathies may have mtDNA mutations.

Initial assessment should consist of a thorough medical history and physical examination, with emphasis on the neurologic examination. The history should focus on birth history, presence of seizures, type and response to anticonvulsants, developmental delay, retardation, dementia, hypotonia, ataxia, dystonia, neuropathy, ptosis, and migraines. Intractable or myoclonic seizures may be seen with increased frequency in this population. A history of neurosensory findings such as visual impairment (optic atrophy, nystagmus, pigmentary retinopathy) or hearing loss may also be suggestive. Mitochondrial cytopathies affect muscle only (myopathy), manifest primarily as CNS features (encephalopathy), or can be multisystemic .

Inheritance

When considering the diagnosis of a mitochondrial cytopathy, it is important to obtain a detailed family history. In some cases, there will be clear evidence of maternal or autosomal inheritance. One must pay attention to possible "soft signs" in maternal relatives such as short stature, deafness, migraine headaches, or diabetes mellitus, as well as history of early, unexplained childhood death or disability. Sometimes, the collective medical symptoms of the family, rather than of one individual, will suggest mitochondrial disease [31]. Many mitochondrial disorders are due to nuclear-encoded gene defects and are transmitted by Mendelian inheritance (autosomal dominant or recessive). A few mitochondrial cytopathies associated with single mitochondrial deletions are sporadic [32].

Noninvasive screening tests

There are a number of noninvasive evaluations that can be performed in the initial evaluation, or once a mutation has been identified, to screen for clinical or subclinical involvement in other tissues or organs. An electrocardiogram or echocardiogram may demonstrate cardiomyopathy and cardiac conduction defects, the most common cardiac features of mitochondrial disorders. Ophthalmologic examination may disclose the presence of retinal pigmentary abnormalities or optic atrophy. An electroretinogram (ERG) may be indicated.

Biochemical studies

Several laboratory studies may be useful to screen for impaired energy metabolism, such as serum lactate, pyruvate, plasma amino acids, complete blood count, electrolytes, carnitine, acylcarnitine profile, ammonia, and creatine phosphokinase (CPK). Renal tubular acidosis as part of a Fanconi syndrome may be seen. There is no one specific screening test. Elevated lactate is suggestive, but not specific, for mitochondrial disorders. Many children do not have elevated serum lactate or may have elevations only under certain conditions, such as after glucose loading, illness, or exercise. Blood lactate values may be spuriously elevated when a tourniquet is used or as a result of a child struggling with the venipuncture. In

these cases, arterial lactate level may be more reliable. In infants and young children with encephalopathy, CSF lactate may be elevated.

Other abnormal studies may include elevations of pyruvate and elevated alanine. The lactate to pyruvate ratio is as important as each component individually, such that a ratio of greater than 20 is suggestive of defect of OXPHOS, whereas a ratio of less than 20 suggests a defect in the Krebs cycle.

Serum CPK values are usually normal in mitochondrial disorders except in mitochondrial depletion. In both congenital and infantile forms of mtDNA depletion, the creatine kinase concentration may be greater than 1000 IU and should alert the physician to a possible diagnosis.

Defects in fatty acid metabolism may be associated with elevated plasma-free fatty acids, hypoketonemia, hypocarnitinemia, and dicarboxylic aciduria. Intermediates of the Krebs cycle may suggest mitochondrial fatty acid oxidation disorder. Values may be abnormal only during a concurrent stressor. Many of these studies are more informative if performed after a brief fast.

Electrophysiologic studies

Electroencephalogram (EEG) results may be normal, show evidence of seizures, or show generalized slow waves consistent with an encephalopathy. The finding of polyspike and wave discharges may be seen in patients with MELAS and MERRF. Abnormalities in brainstem transmission can suggest mitochondrial disease and may underlie a subclinical manifestation. However, electrodiagnostic features in the mitochondrial cytopathies may be normal, but when abnormal they are not specific and cannot be used to distinguish one group of disorders from another. Some patients may have evidence of myopathies, neuropathies, or combined pictures on EMG. Evidence of a sensorimotor or axonal neuropathy may be demonstrated.

Brain magnetic resonance imaging and spectroscopy

Magnetic resonance imaging and spectroscopy are important tools in the diagnosis of children suspected of having a mitochondrial disorder, and may be used to monitor therapy [33]. MRI may be especially useful in children where nonspecific neurologic symptoms are common, mtDNA defects may be absent, and biochemical and morphologic findings can be marginal.

There is a spectrum of abnormalities seen that will vary based on the metabolic brain defect, stage of the disease, and age of the patient. Only two studies have addressed these findings in children [34,35•]. Common MRI findings in children may include one of several patterns (Fig. 2).

Grey matter nuclei involvement may be a predominant finding. These tend to be symmetric, but may appear partial or patchy. In acute phases, they may appear swollen, with a high signal appearance on T2-weighted images. They become shrunken in chronic cases. In the brainstem, the periaqueductal grey matter, pons, and mesencephalon are common sites of involvement. The cerebellum, particularly the dentate nuclei, may be affected. Many patients will show progressive grey matter nuclei involvement, and hence, MRI may be used to monitor disease progression because it correlates with clinical impairment.

Typical imaging findings are the diagnostic hallmark of Leigh syndrome, which explains the uniformity of the MRI findings. The diagnosis of Leigh syndrome, which earlier could be made only by postmortem examination, is characterized by vascular proliferation and demyelination, which leads to necrosis and cavitation in the basal ganglia, midbrain, pons, and posterior column of the spinal cord. MR lesions in corresponding locations, therefore, strongly suggest the presence of a defect in the energy-producing pathway. Putaminal involvement is reported to be a consistent feature in Leigh syndrome.

A frequent finding on pediatric MRI in patients with mitochondrial cytopathies is abnormal myelination [36]. Abnormalities of myelin, including delayed myelination, leukodystrophic pattern, and demyelination are common. Extensive areas of demyelination may be demonstrated in the cerebral hemispheres near the corpus callosum and adjacent white matter. This type of finding may mimic a leukodystrophy, and has recently been recognized as a finding consistent with a mitochondrial presentation [37].

Infarct-like, often transient lesions not confined to the vascular territories are the imaging hallmark of MELAS. Focal necrosis and laminar cortical necrotic changes are the histopathologic correlates of this disease, together with neuronal degeneration and mineral deposits within the basal ganglia. The pathogenesis of the lesions in MELAS is presumably due to deficient oxidative phosphorylation, and also dysfunction of the endothelium of small pial arterioles and capillaries due to accumulation of abnormal mitochondria.

Magnetic resonance spectroscopy (MRS) is a noninvasive, nonquantitative method used to assess CSF lactate levels and tissue metabolism in vivo. 31P studies may be used to study energy metabolism in muscle at rest, during exercise, and during recovery, as well as monitor cerebral energy metabolism. 1H MRS can be used to assess elevations of lactate in the CNS.

Muscle biopsy

Muscle biopsy is often diagnostic, although patients with mitochondrial myopathy due to mtDNA mutations and those with LHON may have normal biopsies. The hallmark of mitochondrial dysfunction is abnormal mitochondrial proliferation, seen as RRF with modified Gomori trichrome staining (Fig. 3). These fibers also stain strongly for succinate dehydrogenase (SDH, complex II, ragged blue fibers), and negatively for cytochrome oxidase (COX, complex IV). These fibers represent the accumulation of mitochondria in response to a defect in OXPHOS.

The COX staining reaction can show foci of scattered cytochrome-*c*-oxidase–negative fibers that may correspond to RRFs. This is suggestive of impaired mitochondrial protein synthesis. Severely decreased COX staining

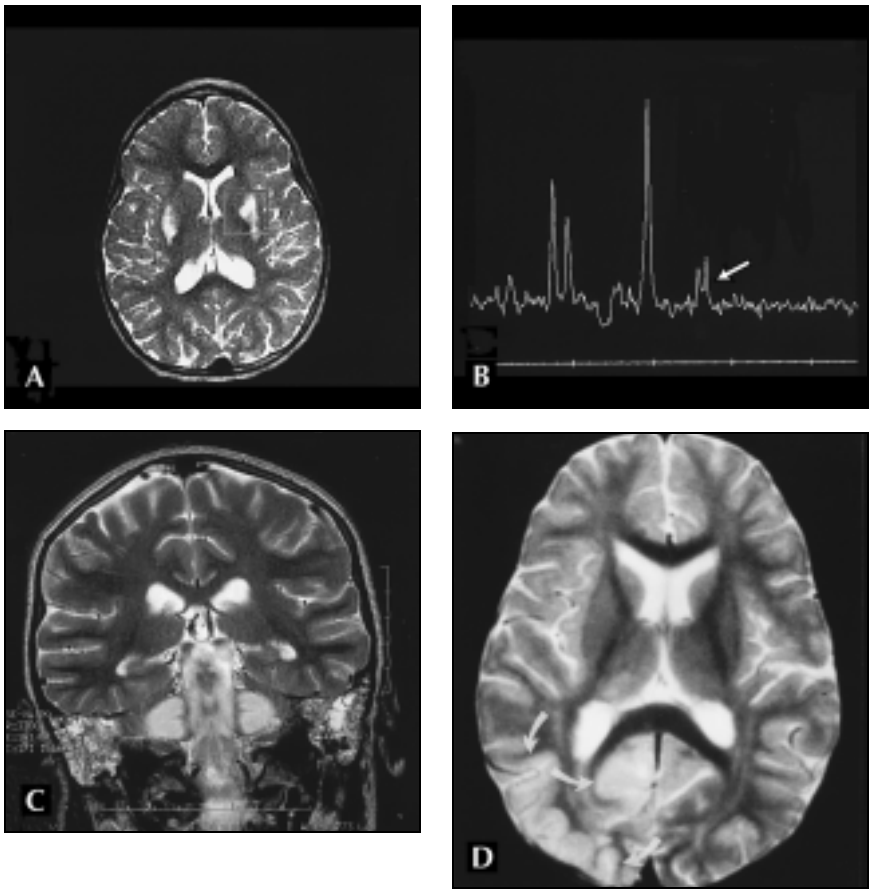

Figure 2. Grey matter nucleus involvement in a 2-year-old girl with mutation at np14459. **A**, Axial T2-weighted magnetic resonance imaging (MRI) at the level of basal ganglia shows symmetrical involvement of the putamen. **B**, Magnetic resonance spectroscopy on the same patient demonstrates elevated lactate shown as a doublet at 1.3 ppm. **C**, Extensive brainstem involvement in this 8-year-old girl with Leigh syndrome. Axial T2-weighted scan shows white matter hyperintensity involving the pons, medulla, cerebellum, and spinal cord. **D**, Right occipital stroke in a patient with mitochondrial encephalopathy with lactic acidosis and stroke-like episodes (MELAS). T2-weighted MRI at the level of the basal ganglia shows increased signal consistent with infarct (*arrow*).

may be consistent with fatal infantile myopathies. An unusually low level of COX staining may suggest a nuclear-encoded gene defect [38].

Biochemical analysis of respiratory chain enzymes can be performed in lymphocytes, cultured skin fibroblasts, or muscle biopsies. Fresh muscle allows for the isolation of intact mitochondria that can be used for polarographic analysis. However, biopsies that have been snap frozen can be stored and shipped to specialized laboratories that can measure the activities of the individual respiratory chain complexes I to IV, and the combined activities of complexes I + III and II + III. In order to correct for mitochondrial proliferation, the enzyme activities should be normalized against the activity of citrate synthase, a mitochondrial matrix enzyme that serves as a marker of mitochondrial mass.

Electron microscopy is used to some degree in the diagnosis of mitochondrial myopathies. Ultrastructural analysis with electron microscopy may reveal intramitochondrial paracrystalline inclusions or disrupted cristae.

Mitochondrial DNA analysis

Genetic analysis is needed for genetic counseling. Single mtDNA deletion is common in patients presenting in adolescence or adulthood, whereas single point mutations are common in infancy and childhood. If the patient fits a specific phenotype (*ie*, LHON, MERRF, MELAS), a blood/muscle test for a point mutation may be positive. Testing may be expensive and potentially affect the insurability of the family.

Mitochondrial DNA analysis is now offered in many molecular laboratories. It is important to know which mutations are screened, how many, and the method to best interpret the results for the patient.

In some patients the studies are negative, despite high clinical suspicion. In these cases, repeat biochemical analysis with collection of blood and urine studies under stressed conditions such as fasting, post exercise, after glucose infusion, or illness may clarify diagnosis. In those patients who remain undiagnosed, screening of entire tRNA genes is recommended. This may be performed in specialized laboratories utilizing techniques such as single stranded conformational polymorphism (SSCP) analysis, which allows multiple tRNA mutations to be screened simultaneously, or temporal temperature gradient gel electrophoresis (TTGE), which relies on heteroduplex formation as a function of temperature. These methods have high sensitivity and allow detection of new mutations even at levels of low heteroplasmy [39,40].

Mitochondrial DNA length mutations (common deletion) are best detected by Southern blot analysis in total mtDNA extracts from blood lymphocytes. In certain cases of sporadic disorders, muscle is the tissue of choice. Even though precise diagnosis may be possible by direct DNA analysis from blood cells, in most cases a muscle biopsy will be required.

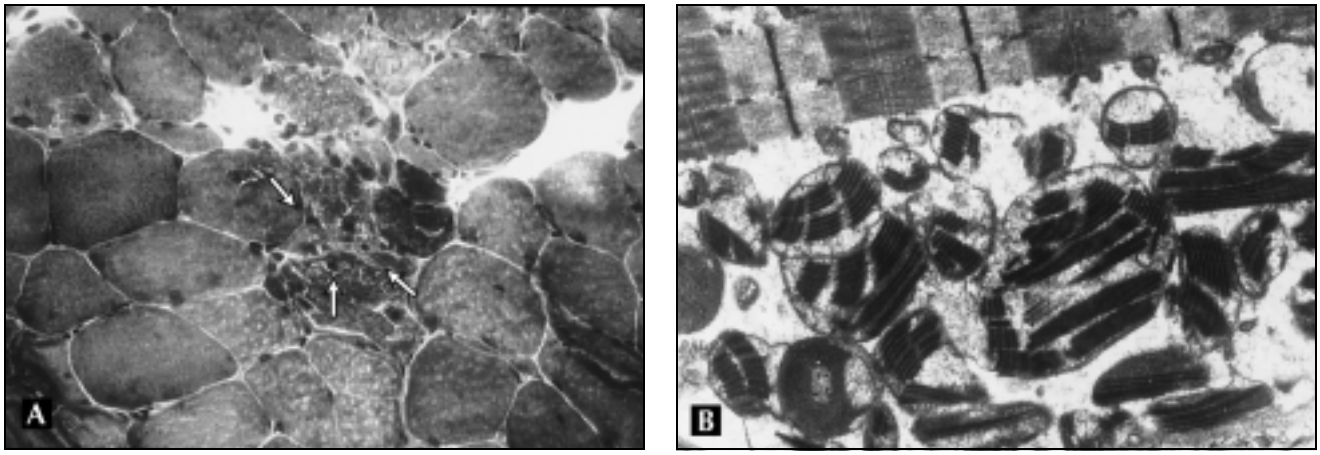

Figure 3. Histochemical staining of the muscle showing ragged-red fibers in this patient with A8344G (myoclonic epilepsy with ragged red fibers [MERFF]) mutation when stained with modified Gomori trichrome. **B**, Electron micrograph demonstrating "parking lot inclusions" representing clusters of abnormal mitochondria.

Treatment of Childhood Mitochondrial Disease

Once the diagnosis is made, the family and treating physicians should become educated about mitochondrial disorders and avoid stressors that can exacerbate disease. Although difficult, genetic counseling to identify at-risk individuals should be undertaken. Yearly evaluation (or more frequent if affected) of organ systems at risk or involved in mitochondrial disorders should occur by appropriate clinicians. Adequate nutrition is essential in coping with mitochondrial disease, and should be evaluated to ensure caloric sufficiency.

Genetic counseling

In cases of mitochondrial cytopathies due to nuclear-encoded gene defects, genetic counseling is based on the rules for autosomal recessive, autosomal dominant, or X-linked inheritance. However, for maternally inherited disease the situation is more complex. At the present time, there is no sure way to predict to what extent a heteroplasmic mtDNA mutation is transmitted from a mother to her offspring. If a mutation has been transmitted to offspring, it cannot be predicted whether or not a clinical phenotype will occur. Males with mtDNA mutations will not transmit disease.

Therapies

At present, there are no cures for these disorders. The goals of treatment are to improve symptoms and halt progression of disease. The effectiveness of treatment varies with each patient. Treatment will not reverse damage already incurred.

Many patients self adjust their diets to meet their metabolic demands; therefore, the clinician should ask about diet history. Frequent recommendations given to patients with mitochondrial cytopathies are to avoid fasting and encourage bedtime snacks (complex carbohydrates) and regular meals. Dehydration due to vomiting or illness should be treated with intravenous fluid if the patient is unable to take fluids orally. Dietary manipulations have been suggested on the basis of specific metabolic defects, including increased fat intake in complex I defects and provision of complex carbohydrates relative to fat in complex V defects. Patients with pyruvate dehydrogenase deficiency are treated with the ketogenic diet [41].

Seizures should be controlled with anticonvulsants and intractable seizures may require careful polypharmacy. Agents such as phenobarbital and sodium valproate should be used with caution in these patients, as they inhibit various pathways of intermediary metabolism or inhibit OXPHOS. Some patients will benefit from physical, occupational, and speech therapies that are specifically tailored to their needs. Patients with mitochondrial disease may respond to aerobic training [42].

There are anecdotal reports of benefit from a variety of dietary agents, including ubiquinone (coenzyme Q10), ascorbic acid, riboflavin, thiamine, vitamin E, creatine, and succinate [43–45]. Studies evaluating different supplements have been complicated by small sample size and widely diverse clinical manifestations. Not all vitamins are benign (*eg*, iron can increase free radical production). The B vitamins are often recommended due to their role as cofactors for ETC enzymes (thiamine as a cofactor for decarboxylases, biotin as a cofactor for carboxylases, and riboflavin as a cofactor for ETC). These supplements may possibly boost enzyme function and act as antioxidants to slow disease progression. Ubiquinone is found in human tissues and occurs in cell membranes [46]. Efficient electron transport depends upon high levels of this substance. The reduced form of coenzyme Q10 has antioxidant properties. Carnitine replacement is used if a coexisting deficiency exists. It should be noted that use of these agents is still somewhat controversial.

Dichloroacetate (less than 25 mg/kg/d), an investigational drug with a wide pharmacologic spectrum, has been used to reduce serum lactate levels through activation of pyruvate

dehydrogenase complex, and has been shown to decrease cerebral lactic acidosis in patients [47–49]. Dichloroacetate is potentially toxic. In humans, adverse effects include reversible elevation of serum transaminases and peripheral neuropathy. There are several clinical trials being conducted to assess efficacy and safety of this agent in treatment of congenital lactic acidosis due to mitochondrial disorders.

Idebenone, a synthetic ubiquinone, has been used to treat some patients with mitochondrial disease. This agent is not available in the United States. A recent study showed some improvement in cardiomyopathy in patients with Friedreich's ataxia [50], a mitochondrial disorder caused by a nuclear-encoded protein involved in mitochondrial iron homeostasis. Results of this agent in the United States have not been reported.

Conclusions

The mitochondrial cytopathies represent a diverse group of disorders with complex genetics and limited treatment options. Physicians in all specialties are becoming increasingly familiar with these disorders given the potential for any organ system to be involved. Many patients will first present to the neurologist, given the multitude of CNS, peripheral nervous system, and autonomic presentations. A basic understanding of the phenotypes and work-up of these patients is important for diagnosis, genetic counseling, and management. Advances in our understanding will likely lead to improved diagnosis and therapies.

References and Recommended Reading

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- • Of importance
- •• Of major importance

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