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Presentation and Diagnosis of Mitochondrial Disorders in Children

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Abstract

The first disorder of mitochondrial function was described by Luft in 1959. Over the ensuing decades, multiple cases of mitochondrial dysfunction were reported, and the term “mitochondrial disorder” arose to describe any defect in the mitochondrial electron transport chain. The sequence of the mitochondrial genome was elucidated in 1981 by Anderson et al., and during the next 20 years, >200 pathogenic point mutations, deletions, insertions, and rearrangements were described. Most of the original cases were adults, and the diagnosis of a mitochondrial disorder in an adult patient became relatively straightforward. Adults present with well-defined “mitochondrial syndromes” and generally carry mitochondrial DNA mutations that are easily identified. Children with mitochondrial disorders are much harder to define. Children are more likely to have a nuclear DNA mutation, whereas the “classic” syndromic findings tend to be absent. This review describes both the varying presentations of mitochondrial disorders and the common laboratory, imaging, and pathologic findings related to children.

Introduction

Mitochondria are evolutionary parasites thought to have entered into a symbiotic relationship with eukaryotic cells over one billion years ago [1]. Mitochondria provide cells with an advanced system for energy production, and cells provide mitochondria with the nutrients and proteins needed to function. Mitochondria contain their own DNA [2,3] that codes for two ribosomal RNAs, 22 transfer RNAs, and 13 polypeptides involved in the electron transport chain. The remainder of the estimated 1000 proteins involved in normal functioning of the mitochondria are encoded by nuclear DNA [4].

History

The first disorder of mitochondrial function was described in 1959 by Luft [5]. He evaluated a young woman with generalized weakness, an inability to gain weight, and extensive perspiration. Muscle pathology demonstrated large accumulations of variably sized mitochondria containing paracrystalline inclusions. Over the next two decades, many cases of mitochondrial dysfunction were described, and although there are multiple metabolic pathways in the mitochondria (the pyruvate dehydrogenase complex, the carnitine cycle, beta-oxidation, and the Krebs cycle), the term “mitochondrial disorders” arose to describe only defects in the mitochondrial electron transport chain. In 1981, the sequence and organization of the human mitochondrial genome were elucidated by Anderson et al. [6]. By 1989, Holt et al. had described the first deletion in the mitochondrial genome associated

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with clinical pathology [7]. Currently, >200 pathogenic point mutations, deletions, insertions, and rearrangements of the mitochondrial genome are known [4].

Epidemiology

The true incidence and prevalence of mitochondrial disorders remain unclear. Chinnery et al. [8] and Majamaa et al. [9] demonstrated the prevalence of pathogenic mitochondrial DNA mutations to be at least 1 in 8000 people, but with advancing technology and understanding, it has become apparent that the majority of cases of mitochondrial disorders in children result not from mitochondrial DNA mutations, but from nuclear DNA mutations. In 1998, Lamont et al. demonstrated that mitochondrial DNA mutations account for <10% of all mitochondrial disorders in children [10]. With the inclusion of mutations of the nuclear genome in epidemiologic evaluations, the incidence and prevalence of mitochondrial disorders rise dramatically. It is now clear that mitochondrial disorders are more prevalent than other, more recognized inherited myopathies such as Duchenne or myotonic dystrophies [8]. In 2003, Skladal et al. estimated that the minimum birth prevalence of inherited mitochondrial disorders (via mitochondrial DNA and nuclear DNA) is >1 in 5000 births [11].

Presentation

Defects in the electron transport chain can affect any tissue. Tissues requiring the highest levels of energy production are the most severely affected. Despite our increasing recognition of these disorders, common presentations in children remain difficult to define. However, most authors agree that whereas adults present with recognizable mitochondrial syndromes (Table 1), presentations in children are more nonspecific.

The nervous system is the most commonly affected system in mitochondrial disorders. Approximately 45% of children present with neurologic signs [12] (Table 2). Most patients with neuromuscular symptoms will have either normal or only slightly elevated (<2 × normal) serum creatine kinase [12–15]. The results of electromyography and nerve conduction studies are also usually normal [12]. The unexpected findings of a normal creatine kinase level or normal electromyography in a patient with significant muscle weakness should therefore be a clue to search for a mitochondrial disorder. Approximately 20% of patients demonstrate intellectual dysfunction [13] or psychiatric disturbances [16,17]. Ten percent demonstrate hepatic signs [4,12]. Mitochondrial patients are particularly sensitive to valproate-induced liver failure, and a mitochondrial disorder should be considered in any child presenting with valproate-induced hepatic signs [18,19]. Cardiac presentations include arrhythmia, cardiomyopathy, cardiac murmur, or sudden death [12]. Up to 10% of children demonstrate hematologic symptoms, including pancytopenia [12]. Renal symptoms are uncommon (<5%) [12], but the most commonly found abnormality is proximal tubulopathy. Nephritic syndrome, tubulo-interstitial nephritis, and nonspecific renal failure are also found [12,20]. Growth failure presents as short stature in approximately 20% of mitochondrial patients [13], and endocrine problems include hypoparathyroidism, hypothyroidism, diabetes insipidus, diabetes mellitus, hypogonadism, and adrenocorticotropin hormone deficiency [12,17,21]. There are even several case reports of children with mitochondrial disorders with ketotic hypoglycemia as their only presenting sign [4,22]. Although such a presentation is uncommon, children may be dysmorphic, demonstrating features similar to those of fetal alcohol syndrome (microcephaly, round face, high forehead, featureless filtrum, low-set ears, and a short neck) [12]. Skin findings include multiple lipomatosis, scaly pruritic erythema, reticular pigmentation, hypertrichosis, eczema, or vitiligo [17]. Patients may exhibit ophthalmologic abnormalities such as retinitis pigmentosa [13], but vision is usually preserved in childhood [12]. Reports of sensorineural

deafness range from 7–26% in children; deafness is more common with increasing age [13,23]. Gastrointestinal symptoms include anorexia, frequent emesis, abdominal pain, diarrhea, and constipation that can be so severe as to cause chronic intestinal pseudo-obstruction [4].

The suspicion of a mitochondrial disorder in a child should be based not only on an awareness of the presenting signs, but on recognizing that mitochondrial disorders are prevalent in the population. There are certain “red flags” which should immediately increase the suspicion of a mitochondrial disorder. These include short stature, neurosensory hearing loss, progressive external ophthalmoplegia, axonal neuropathy, diabetes mellitus, hypertrophic cardiomyopathy, and renal tubular acidosis [15]. Physicians should have a high index of suspicion for mitochondrial disorders, and should consider such a diagnosis in any child presenting with multisystem involvement or with single-system involvement without a clear explanation.

Diagnosis

If the recognition of mitochondrial disorders in children is challenging, their diagnosis in childhood is even more so. Studies attempting to determine the value of laboratory results are limited by: small patient samples; bias in patient selection, allowing only those well-recognized mitochondrial syndromes to be studied; combination studies including both children and adults; and a lack of consensus on what constitutes an abnormal result. Probably the single largest bias introduced into studies of mitochondrial patients results from the underdiagnosis of children who present with “nonclassic” symptoms.

Over the last few decades, several sets of diagnostic criteria were developed to assist in the recognition and diagnosis of mitochondrial disorders. Walker et al. were the first to propose a set of major and minor criteria for the identification of mitochondrial disorders [24]. Several years later, Bernier et al. evaluated the criteria of Walker et al. in the pediatric population, and proposed a set of modifications for children that have come to be known as the “modified Walker criteria” [25]. Other attempts to define diagnostic criteria include the Nijmegen Center for Mitochondrial Disorders scoring system [26] and the Mitochondrial Disease Criteria [27]. All are based on some combination of clinical, laboratory, pathologic, biochemical, and genetic findings.

Lactic Acidosis

The most recognized laboratory abnormality in patients with mitochondrial disorders is lactic acidosis. Dysfunction in the electron transport chain causes decreased production of adenosine triphosphate. Low adenosine triphosphate levels result in an up-regulation of glycolysis, leading to an overproduction of pyruvate. Excess pyruvate is either transaminated to alanine, or reduced to form lactate. Jackson et al. studied 51 adults and children with mitochondrial disorders, and found lactic acidosis in 50% [14]. However, lactic acidosis is found more commonly in patients with mitochondrial DNA mutations [12], and mitochondrial DNA mutations account for <10% of all mitochondrial disorders in children [10]. No comments were offered on the prevalence of mitochondrial DNA mutations in the population studied, and the inclusion of adults makes it difficult to extrapolate the results to children. Hutchesson et al. evaluated the prevalence of lactic acidosis in a group of 11 children with known mitochondrial disorders [28]. They found elevated plasma lactate levels of >2.4 mmol/L in 8 (73%), and >3.0 mmol/L in 7 (64%). Three of their patients (30%) with significantly elevated lactate had known mitochondrial DNA point mutations, thus greatly over-representing the incidence of mitochondrial DNA mutations found in children, and introducing bias into the results. The largest study to examine lactic acidosis exclusively in children with mitochondrial disorders was performed

by Munnich et al. [12]. They examined 235 children with mitochondrial disorders, and found an elevated venous lactate level in only 30%. This larger study is likely more representative of the true prevalence of lactic acidosis in children with mitochondrial disorders, and indicates that up to 70% of children with a mitochondrial disorder will have a normal venous lactate value. When lactic acidosis is present, consideration must be given to the fact that it is not specific to mitochondrial disorders. Elevated venous lactate can be seen with venous stasis, hypoxia, hypoperfusion, hepatic dysfunction, renal failure, drug toxicity, neurodegenerative diseases, sepsis, spasticity, hyperinsulinism, chronic thiamine deficiency, seizures, and many other metabolic disorders [12,15,28,29].

Metabolic Evaluation

Investigations for metabolic disorders that may mimic mitochondrial disorders should be undertaken during the initial evaluation. The electron transport chain is part of a complex energy-generating system inside cells, and involves multiple levels of control. Defects in other systems may produce an overall decrease in mitochondrial function. Metabolic studies generally include plasma amino acids, urine amino and organic acids, pyruvate, and acylcarnitine profiles. Urine organic acids may demonstrate elevated lactate levels secondary to renal tubular leakage. If enough lactate leaks into the urine, the level of plasma lactate can even be normalized [20]. A generalized increase in metabolites in the urine is indicative of generalized renal dysfunction [30]. An elevated level of pyruvate may be present secondary to overproduction [31]. If pyruvate is metabolized to alanine, amino-acid studies may demonstrate an elevation in alanine [30]. Specific abnormalities may be informative, but it is important to remember that normal values for metabolic tests do not rule out the diagnosis of a mitochondrial disorder [31].

Cerebrospinal Fluid

The evaluation of cerebrospinal fluid usually includes cell count, protein, glucose, lactate, pyruvate, and amino acids. Cerebrospinal fluid, including cerebrospinal fluid lactate, may be normal or may demonstrate a wide range of abnormalities. Elevations of protein, lactate, pyruvate, and even white blood cells were demonstrated in the cerebrospinal fluid of patients with mitochondrial disorders [17]. Several studies demonstrated that lactate may be elevated in cerebrospinal fluid, even in the presence of normal venous values, and cerebrospinal fluid lactate may be more reliable than venous lactate in patients with neurologic symptoms [12,30]. As is the case in venous lactate, other conditions may result in an elevation of cerebrospinal fluid lactate, including other metabolic disorders, seizures, subarachnoid hemorrhage, meningitis, encephalitis, and ischemia [12,28].

Spectroscopy

Normal brain parenchyma does not demonstrate a detectable lactate peak on proton magnetic resonance spectroscopy. In the setting of a mitochondrial disorder, lactate can sometimes be detected in the brain via proton magnetic resonance spectroscopy, even without evidence of systemic lactic acidosis [17,32]. Dinopoulos et al. [33] evaluated 49 children with suspected mitochondrial disease. Seventeen of these children demonstrated an elevated lactate level on proton magnetic resonance spectroscopy. In 7 cases, the abnormality was detected in normal-appearing parenchyma, and in another 7 cases, the plasma lactate level was normal [33]. If abnormal parenchyma is present, other causes of increased cerebral lactate should be considered, such as infection or ischemic injury. However, the persistence of detectable lactate on proton magnetic resonance spectroscopy after the acute phase of an illness is suggestive of a metabolic etiology [33].

Reports also describe the use of near-infrared spectroscopy in the diagnosis of mitochondrial disorders. Oxygenated and deoxygenated hemoglobin emit at different wavelengths,

permitting an evaluation of the degree of oxygen extraction in living tissue. Oxygen extraction by muscle tissue can be measured and compared with standards. Healthy control subjects demonstrate prompt deoxygenation during exercise, followed by prompt reoxygenation once the exercise ends. Patients with mitochondrial disorders have an impaired ability to utilize oxygen and demonstrate increased oxygenation during exercise, followed by a rapid return to baseline. This paradoxic change reflects the increased delivery of oxygen to tissues expected during exercise in conjunction with a decreased ability of the tissue to utilize the oxygen [34].

Phosphorous magnetic resonance spectroscopy was also evaluated as a noninvasive measure of energy utilization in exercising muscle. Phosphorous magnetic resonance spectroscopy demonstrated five major peaks associated with energy metabolism: one for phosphocreatine, one for inorganic phosphate, and one representing each of the three phosphate atoms of adenosine triphosphate. The muscle energetic state is characterized by the balance between adenosine triphosphate utilization and adenosine triphosphate production, and this ratio is directly related to the ratio of phosphocreatine/inorganic phosphate at a given pH. At rest, normal muscle has a low metabolic rate and a high energy capacity, resulting in a high phosphocreatine/inorganic phosphate ratio. Impaired mitochondrial function reduces the resting energy state, and decreases the resting phosphocreatine/inorganic phosphate ratio. Patients with mitochondrial disorders also demonstrate a rapid decline in phosphocreatine/inorganic phosphate ratio with exercise. The repletion of phosphocreatine after exercise is primarily controlled by mitochondrial oxidative phosphorylation, and the rate of recovery in patients with mitochondrial disorders is slower than in healthy control subjects. The more severe the muscle involvement, the more likely will a patient demonstrate abnormalities on phosphorous magnetic resonance spectroscopy [35,36].

Pathology

Muscle Pathology

A muscle biopsy in patients with suspected mitochondrial disorders can be very helpful. Biopsy specimens should be examined with routine light microscopy for structural changes, evaluated for specific enzymes using histochemical stains, and examined with electron microscopy to visualize ultrastructure.

Light Microscopy

Similar to the finding of lactic acidosis in the blood, the sub-sarcolemmal accumulation of mitochondria on muscle pathology is considered the hallmark of mitochondrial disorders. This accumulation can be visualized with either a modified Gomori trichrome stain (raggedred fibers) [37] or with a stain for succinate dehydrogenase (ragged-blue fibers) [31]. In reality, 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 [38]. Cytochrome oxidase-negative fibers are not evident in healthy individuals aged <30 years, and their presence is always pathologic [14]. Although they are helpful, abnormalities on light microscopy are not invariably present [31,39]. In 2004, Patterson demonstrated that 50% of patients with mitochondrial disorders demonstrated normal light microscopy [40]. Rollins et al. evaluated 118 muscle-biopsy specimens from mitochondrial patients of all ages, and found normal light microscopy in 45% [41]. Ragged-red fibers were found in only 2.5%, and cytochrome oxidase-negative fibers were found in 7% [41]. Lamont et al. [10] evaluated 177 children with mitochondrial disorders. They demonstrated ragged-red fibers or cytochrome oxidase-negative fibers in 89% of children with mitochondrial DNA mutations, but these changes were only present in 17% of patients without detectable mutations in their mitochondrial DNA [10]. These findings suggest that

light microscopy abnormalities are more commonly present in the setting of a mitochondrial DNA mutation. Kyriacou and Mikellidou examined 20 patients (adults and children) with mitochondrial disorders, and found light microscopy abnormalities in 78%, but none of the children demonstrated ragged-red fibers [38]. Gire et al. studied six neonates with mitochondrial disorders, and found only a single patient with any detectable abnormality on light microscopy [42]. Jackson et al. described two patients with confirmed mitochondrial disorders who had an initial normal muscle biopsy that demonstrated a sub-sarcolemmal accumulation of mitochondria 3 years later [14]. These findings suggest that detectable abnormalities develop with disease progression, and therefore should be much less common in children than in adults with mitochondrial disorders. As with other findings in mitochondrial patients, light microscopy changes are generally nonspecific, and although commonly associated with mitochondrial disorders, ragged-red fibers may be evident in normal aging, antiretroviral therapy, muscular dystrophies, myositis, and inflammatory myopathies [31]. Other findings in the muscle biopsies of patients with mitochondrial disorders include internal nuclei, atrophic fibers, increased lipid droplets, fiber-type grouping, type I or type II fiber atrophy, fiber hypertrophy, glycogen, and inflammation [14,31,41,43]. In summary, the histologic evaluation of muscle may provide evidence to support a diagnosis of a mitochondrial disorder, but muscle pathology is more common with mitochondrial DNA mutations and with advancing age. Therefore, normal muscle pathology does not exclude a mitochondrial disorder, especially in children [40].

Ultrastructure/Electron Microscopy

Electron microscopy is important in the evaluation of children with suspected mitochondrial disorders. Thirty to 44% will demonstrate abnormalities on electron microscopy [15,38]. A study by Kyriacou and Mikellidou of patients demonstrated ultrastructural findings in 33% who had no abnormality visualized on light microscopy [38]. Ultrastructural findings include an increase in mitochondrial number (48%) or size (7%), increased lipid (8%), glycogen droplets (12%), increased mitochondrial matrix, abnormal cristae, and paracrystalline inclusions (3%) [15,38,44]. Similar ultrastructural changes to the mitochondria can be seen in a variety of other conditions, including muscular dystrophies, neurogenic atrophy, inflammatory myopathies, antiretroviral therapy treatment, chronic steroid use, aging, or other metabolic disorders [14,43]. As with normal light microscopy, normal electron microscopy does not eliminate the possibility of a mitochondrial disorder.

Pathology of Other Tissues

A liver biopsy (open or needle) in the setting of a mitochondrial disorder can demonstrate a variety of non-specific findings, including steatosis, micronodular cirrhosis, cholestasis, bile-duct proliferation, fibrosis, apoptosis, and mitochondrial ultrastructural abnormalities [4,12]. A study of 118 children by Bernier et al. demonstrated that 61% of liver biopsies demonstrated some nonspecific pathology, but only 9% had mitochondrial abnormalities on electron microscopy [25]. Panetta et al. reported four patients with liver involvement confirmed by abnormal electron transport chain activity in the liver. Three of the four manifested no ultrastructural mitochondrial abnormalities on liver biopsy [45]. Cardiac tissue also indicates nonspecific pathologic changes. Bernier et al. [25] demonstrated ultrastructural cardiac abnormalities in only 3% of their patients studied. Fibroblasts can sometimes demonstrate ultrastructural mitochondrial abnormalities, especially in patients with mitochondrial DNA defects [46]. Patients with significant central nervous system involvement may receive a brain biopsy, but brain pathology is also nonspecific, demonstrating focal necrosis, a loss of cortical neurons, reactive astrogliosis, astrocytic inclusions, demyelination, spongiosis, endothelial proliferation, or capillary hyperplasia [17].

Brain Imaging

Brain imaging of patients with mitochondrial disorders can be normal, or can demonstrate a wide array of abnormalities. Computed tomography scans may demonstrate punctate calcifications, particularly in the basal ganglia or cerebellum [17,47]. Magnetic resonance imaging may demonstrate a nonspecific high T_2 signal in the basal ganglia, brainstem, or cerebellum. Magnetic resonance imaging may also demonstrate a high cortical T_2 signal that does not correspond to a vascular territory, representing “stroke-like” lesions (most commonly associated with mitochondrial encephalopathy, lactic acidosis, and stroke). Other findings include edema, atrophy, or abnormal myelination [15,17,32,33,42,47,48]. Similar brain imaging changes can be seen in a variety of conditions, including anoxic injury, cyanide poisoning, Wilson’s disease, gangliosidosis, carbon monoxide poisoning, radiation injury, and other metabolic conditions [47]. Interestingly, the severity of changes observed on magnetic resonance imaging in mitochondrial patients is often not reflected in the clinical picture [17]. Thus, severe abnormalities on magnetic resonance imaging with a relatively normal clinical picture may be a clue to assess patients for a mitochondrial disorder. Conversely, patients with clinically severe mitochondrial disorders may have completely normal brain imaging, and normal imaging cannot rule out the diagnosis of a mitochondrial disorder.

Testing Enzymes of the Electron Transport Chain

Finding a mutation is difficult in most children with a mitochondrial disorder. Ninety percent carry a nuclear DNA mutation in one of the 1000 proteins involved in normal functioning of the mitochondria, and most of these mutations have yet to be elucidated [10]. The presentation, imaging, and metabolic investigations may be suggestive of a mitochondrial disorder, but the lack of a genetic confirmation makes enzyme analysis important in the diagnosis of mitochondrial disorders [49,50].

Polarographic and Spectrophotometric Assays

Two well-described procedures are available to evaluate the functionality of the electron transport chain [51,52]. Polarographic assays measure mitochondrial substrate oxidation in fresh muscle tissue, yielding an overall estimate of electron transport chain activity [52,53]. Polarographic studies cannot be performed on frozen tissue because the freezing of samples disrupts the mitochondrial membrane potential upon which these assays rely [50,52]. The major limitation of polarographic assays is the propensity of mitochondria to increase in number in patients with mitochondrial disorders. The overpopulation of mitochondria may result in an increased overall activity of the electron transport chain, masking a deficit [53]. Spectrophotometric assays, performed on either fresh or frozen muscle tissue, measure the activity of each individual complex in the electron transport chain. An electron donor (or acceptor) is added to the muscle homogenate, and the rate of oxidation of the donor (or reduction of the acceptor) is measured [52,53]. There is considerable variation among tissues and even among individuals regarding the absolute activity of each complex, but there is a constant ratio of electron transport chain enzymes to each other in all human tissues [52,53]. This constant allows the development of standards, and therefore, in spectrophotometric assays, each enzyme is compared with the activity of another enzyme in the electron transport chain [15,33]. Spectrophotometric assays are limited insofar as the ratios of complexes to each other can miss a generalized enzyme deficiency; therefore, complex activity should also be compared with an enzyme not involved directly in the electron transport chain. Most centers use citrate synthase, a Krebs-cycle intermediate, as the enzyme for comparison [52,53]. Care must still be taken in interpreting the results of enzymatic testing, because a general limitation that remains is the inability to distinguish patients with a primary electron transport chain deficiency from those with secondary

deficiencies due to down-regulation of the electron transport chain enzymes from other metabolic disorders [54].

Sample Preparation

Biopsy samples must be frozen immediately to a temperature of −80°C, to prevent the loss of enzyme activity and the generation of false-positive results [31,49,52,53]. If samples are frozen rapidly and stored at −80°C, the activities of electron transport chain complexes and of citrate synthase are stable for >10 years [15,50]. Open biopsies are preferable because needle biopsies do not provide sufficient tissue for functional electron transport chain analysis. Purified mitochondrial fractions should be avoided. Mitochondrial purification risks the undesirable loss of dysfunctional mitochondria through selection of the healthy ones [49,52,55]. Mutant mitochondria tend to be abnormal in size and shape, and standard procedures for mitochondrial isolation include steps of sequential centrifugation and washing that selectively remove any odd-shaped or variably sized mitochondria. When muscle homogenates are studied, no mitochondrial selection occurs, and false-negative results are less likely [49]. If samples are prepared correctly, spectrophotometric assays of frozen muscle should be adequate to detect functioning of the individual enzyme complexes, and polarographic testing is usually not necessary [48].

Use of Other Tissues

Fibroblasts are occasionally used to study electron transport chain enzyme activity, but abnormalities in fibroblast enzyme activity are usually evident only in the presence of persistent lactic acidosis. Half of all children with deficits in their muscles have normal enzyme activity in cultured fibroblasts [50]. Care must be exercised in culturing fibroblasts for study, because cells with electron transport chain deficiencies are fragile. They may die in culture, leaving only the healthy cells to grow [53]. The addition of uridine and pyruvate to the culture medium allows the survival of abnormal mitochondria, and prevents false-negative results [52,53]. Hodges and Snyder developed a novel technique using fibroblast cultures to assay for global mitochondrial function [56]. A medium was developed that relied on a functioning electron transport chain for growth. A series of fibroblast lines with known mitochondrial defects were placed in this “mitochondrial-dependent” medium, and the cells were unable to proliferate. The same cells grew to confluence in a medium which was “mitochondrial-sparing” or independent of a functional electron transport chain for energy [56]. This technique could be used as a screening tool to evaluate global fibroblast mitochondrial function. If cells are unable to grow, or grow more slowly than healthy control cells in the “mitochondrial-dependent” medium, such results indicate a complete or partial defect of the electron transport chain [56]. Enzyme activity in other tissues can be studied, such as the liver or heart. There are multiple case reports of patients with normal skeletal muscle presenting enzymatic defects in the liver or heart [12,45]. Because mitochondrial disorders are known to exhibit clinical and biochemical tissue specificity, in situations of single-organ involvement, it may be preferable to study the tissue involved [45,50]. Care must be taken in interpreting results from nonmuscle samples, because tissue standards are not well-defined.

DNA Studies

Mitochondrial DNA codes for 37 proteins, including specific subunits for several complexes of the electron transport chain, ribosomal RNA, and transfer RNA. A defect in any of these genes can produce clinical disease. Nuclear DNA contains genes involved in mitochondrial function that code for the remainder of the specific complex subunits, those involved in mitochondrial signaling, mitochondrial DNA replication, mitochondrial DNA maintenance, and mitochondrial protein synthesis.

Mutations in either mitochondrial DNA or nuclear DNA can result in a deficit of a specific electron transport chain complex, or can give rise to global mitochondrial dysfunction via signaling, transcription, or translation [15]. Mutations can result in an amino-acid change in a subunit of the electron transport chain, thus altering the function of a single complex. Mutations affecting either a transfer RNA or a ribosomal RNA can result in global mitochondrial dysfunction secondary to defective mitochondrial protein synthesis [31,48]. Mutations in genes integral to mitochondrial DNA maintenance or replication can cause multiple deletions or rearrangements in the mitochondrial DNA, again resulting in global mitochondrial dysfunction [4,32]. Mitochondrial DNA depletion syndrome is characterized by a quantitative reduction in the mitochondrial DNA copy number within a cell, and generally results from a nuclear mutation of a gene integral to mitochondrial DNA maintenance or replication [4,31,32].

Clinical or family histories can sometimes localize a mutation to either mitochondrial DNA or nuclear DNA. Point mutations in mitochondrial DNA are usually maternally inherited [4,17,48]. Classic Mendelian inheritance (autosomal-dominant, autosomal-recessive, or X-linked) is usually indicative of a nuclear mutation [48]. The age of onset is generally later with mitochondrial DNA mutations [10], and lactic acidosis is more commonly associated with mitochondrial DNA mutations. Studies demonstrated that a plasma lactate level of <2 mmol/L carries a 97% negative predictive value for a mitochondrial DNA mutation [19]. When searching for clues to suggest a mitochondrial DNA etiology, three features are consistently associated with these mutations: progressive external ophthalmoplegia, myopathy, and pigmentary retinopathy [10].

Currently, >100 pathogenic mutations have been identified in the mitochondrial DNA genome [1], and polymerase chain reaction can be used to screen for many of these. Mitochondrial DNA can also be sequenced in a proband to seek a novel mutation [31]. In many situations, sequencing is more efficient and more cost-effective than screening for rare mutations on an individual basis [39]. When sequencing the mitochondrial genome, care must be exercised in defining a pathogenic mutation. There is extensive polymorphic variation among the population, and the majority of mitochondrial DNA sequence variants are neutral polymorphisms without pathogenic significance [1,39]. The detection of depletion is performed through Southern blotting and through the quantitation of the amount of mitochondrial DNA compared with total DNA [31,32,39,48].

Mutations of mitochondrial DNA can be detected in a variety of tissues. Point mutations can often be detected in blood leukocytes [10,15], but large-scale mitochondrial DNA deletions and rearrangements are more likely found in muscle. Urinary epithelial cells, hair follicles, buccal mucosa, and skin fibroblasts may also be used to detect mitochondrial DNA mutations in some situations [39,48]. These samples are particularly useful in screening children or asymptomatic family members. If possible, the most easily accessed, symptomatic tissue should be tested, bearing in mind that a normal result does not eliminate the possibility of a heteroplasmic mitochondrial DNA mutation.

In children, > 90% of mitochondrial disorders are the result of nuclear mutations [25,39], and nuclear DNA testing remains difficult. Unless the patient fits a particular clinical syndrome, shotgun testing of all known nuclear DNA mutations involved in mitochondrial disorders will produce a very low yield. A pragmatic approach involves sequencing the mitochondrial DNA genome, evaluating for pathogenic mutations. The lack of pathogenic mitochondrial DNA mutations can thus assign a disorder to a nuclear DNA category, providing information for genetic counseling.

Conclusions

The diagnosis of mitochondrial disorders in adults has become relatively straightforward. Adults are likely to present with a well-defined “mitochondrial syndrome,” and generally carry mitochondrial DNA mutations that are easily identified [26]. Children with mitochondrial disorders are much harder to define. Children are more likely to have a nuclear DNA mutation, and the “classic” findings tend to be absent. In the study by Bernier et al. of 118 children, a genetic defect of any type was only identified in 5% [25] of patients with a known mitochondrial disorder, and without the identification of a mutation, there is no single test that will exclude or confirm the diagnosis of a mitochondrial disorder [32].

Over the last few decades, several sets of diagnostic criteria have been developed (Walker, modified Walker, Nijmegen Center for Mitochondrial Disorders, and Mitochondrial Disease Criteria). These criteria are based on combinations of clinical, laboratory, pathologic, biochemical, and genetic findings. Such criteria may be important in defining patient populations for research studies, but they are not very practical in the clinical setting. In the words of DiMauro et al., “the diagnostic approach is no different from that employed for other diseases” [48]. Physicians have been diagnosing medical conditions for centuries, and in the majority of situations, the diagnosis of a mitochondrial disorder remains a clinical diagnosis. It is based on the acumen of the examining physician, keeping some key points in mind. Ninety percent of mitochondrial disorders in children are the result of a nuclear mutation [10], and routine mitochondrial DNA screening may not be appropriate in this population. Although it is not specific, an unexplained elevation of lactate in any tissue (blood, cerebrospinal fluid, brain, or urine) should raise suspicions for a mitochondrial disorder and warrants evaluation, whereas a normal lactate level in any or all tissues does not eliminate the possibility of a mitochondrial disorder. Mitochondrial disorders are progressive, and especially in children, normal muscle pathology is likely to be present in early stages of the disease. Repeated sampling of muscle tissue may be necessary to detect abnormalities with disease progression.

Hopefully the future will bring improved methods for diagnosing mitochondrial disorders in children, such as genomic microarray screening for nuclear mutations. Currently the most important step in diagnosis consists of suspecting the disorder. Mitochondrial disorders can present in any organ, in any manner, and often mimic other, more recognizable disorders. A mitochondrial disorder should be considered in any child presenting with nonspecific signs, such as mental retardation, learning disorders, epilepsy, or multisystem failure. The more adept that clinicians become at recognizing mitochondrial disorders, the more opportunity there will be for improving diagnosis, and ultimately treatment.

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Table 1

Classic mitochondrial syndromes*

CPEO	Chronic progressive external ophthalmoplegia
LHON	Leber hereditary optic neuropathy
MELAS	Mitochondrial encephalopathy, lactic acidosis, and stroke
MERRF	Myoclonic epilepsy with ragged red fibers
MNGIE	Mitochondrial myopathy, peripheral neuropathy, and gastrointestinal encephalopathy
NARP	Neuropathy, ataxia, and retinitis pigmentosa
KSS	Kearns-Sayre syndrome

*This is not a complete list of known clinical syndromes.

Table 2

Neurologic signs

Muscle weakness (proximal > distal and upper > lower extremities)
Hypotonia
Peripheral neuropathy
Ataxia
Ptosis
Ophthalmoplegia
Bulbar signs
Spasticity
Stroke-like episodes
Migraine headaches
Tremor, chorea, and ballismus
Dystonia
Seizures
Myoclonus