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Neurodevelopmental Manifestations of Mitochondrial Disease

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Abstract

Mitochondrial disease is an increasingly recognized but widely heterogeneous group of multi-systemic disorders that commonly involve severe neurodevelopmental manifestations in childhood. This article will review the presentation, genetic basis, and diagnostic evaluation of primary mitochondrial disease. Emphasis will be placed on neurodevelopmental findings that may be encountered by a Developmental Pediatrician that should provoke consideration of a mitochondrial disorder. The inheritance patterns and mechanisms by which mutations in genes located in either the nuclear or mitochondrial genomes can cause mitochondrial diseases will be discussed. A general overview of the current diagnostic evaluation that can be readily initiated by the Developmental Pediatrician will be provided, along with a summary of currently available treatment options.

Overview of human mitochondrial disease

A recent development in the world of inherited disease has been the realization that dysfunction of the mitochondrial respiratory chain constitutes a frequent group of disorders that afflict all ages and ethnicities (1, 2). Indeed, mutations in hundreds of discrete genes – originating in either nuclear (nDNA) or mitochondrial (mtDNA) genomes – culminate in energy deficiency and organ failure. Also known as the electron transport chain, the 5-complex respiratory chain (RC) is the site of oxidative phosphorylation (OXPHOS) within the inner mitochondrial membrane in which the end products of intermediary metabolism are oxidized to generate energy in the form of adenosine triphosphate (ATP). *Primary* mitochondrial disease refers to disorders whose underlying genetic cause directly impairs the composition or function of RC. *Secondary* OXPHOS dysfunction, by contrast, has been described in a host of other genetic or environmental disorders, including other genetic disorders (i.e., Rett syndrome (3), other metabolic defects (4-7), chromosomal aneuploidies (8, 9), etc.) or toxicities from drugs (i.e., valproate (10), statins (11), pesticides (12, 13)). The minimal prevalence of primary mitochondrial disease is 1 in 5,000 (1, 2), although pathogenic mutations in mitochondrial DNA (mtDNA) may occur as frequently as 1 in 200 births (14).

Primary RC (OXPHOS) dysfunction is commonly characterized by progressive, multi-systemic involvement of high-energy demand tissues (15). Indeed, clinical problems involving three or more systems should invoke consideration of mitochondrial disease into the differential diagnosis. Typical sequelae of RC dysfunction may involve almost any system: neurologic (central, peripheral, or autonomic nervous systems), heart (arrhythmias, cardiomyopathy), skeletal muscle (myopathy), eyes (eye movement abnormalities, vision loss due to retinal or optic nerve disease, ptosis), endocrine organs (diabetes mellitus,

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hypothyroidism, hypoparathyroidism, growth hormone deficiency, adrenal insufficiency), kidneys (renal tubular acidosis, aminoaciduria, nephropathy), gastrointestinal tract (liver failure, dysmotility), hearing (sensorineural hearing loss, vestibular dysfunction), and/or may cause global metabolic instability with susceptibility to infections and other catabolic stressors (15). Each gene, and even mutation, implies a different disorder, hence the extensive variability in manifestations, prognosis, and inheritance characteristic of “mitochondrial disease” (16, 17).

Neurodevelopmental manifestations of mitochondrial disease

Neurologic “red-flag” and “nonspecific” findings in mitochondrial disease

Neurodevelopmental abnormalities are common in primary mitochondrial disease and may present at any age. **Table 1** lists both “Red Flag” neurologic findings that may present in infants and children with primary mitochondrial disease (15), as well as a wide range of “nonspecific” neurologic findings that often occur in combination in mitochondrial disease. Nonspecific presentations, by definition, also lead to a wide range of other primary diagnoses. Axonal neuropathy and/or autonomic nervous system involvement may occur in mitochondrial disease, although these presentations are more commonly diagnosed in older children and adults. Sensorineural hearing loss is a very common finding that occurs in a wide range of mitochondrial diseases, although ototoxicity to certain medications can be indicative of a specific mitochondrial disorder (i.e., aminoglycoside-induced hearing loss in individuals with the 1555G mtDNA mutation) (18). A newborn, infant, or young child with unexplained hypotonia, weakness, failure to thrive, and a significant anion-gap metabolic acidosis (with elevated lactate) is a classic presentation for often severe primary mitochondrial RC disease.

Hypotonia or myopathy may be associated with gross and/or fine motor delay. Speech delay is not typical in the absence of severe neurologic and/or neuroimaging abnormalities, such as encephalopathy, hearing loss, or stroke. Behavior problems in the pervasive developmental delay or autism-spectrum range may be seen among other multi-system manifestations in primary mitochondrial disease, although recent debate has erupted as to whether autism can be an isolated manifestation of mitochondrial disease (19-21). Such speculation has focused in large part on typically developing children who experience developmental regression with resulting autism spectrum behaviors following an immune stressor, such as infection or immunization often in association with fever, particularly during a “susceptible” period between 9 and 18 months of life (22). While no clear incidence figures are known specific to the presentation of autism, concern for primary mitochondrial dysfunction in any individual should be followed by the same clinical diagnostic inquiries as outlined below (**Figure 1**).

Psychiatric manifestations are common but often under-diagnosed in primary mitochondrial diseases, including generalized anxiety, depression, and perhaps, obsessive compulsive spectrum disorder (23, 24). Dementia has been described in a wide-range of mtDNA and nuclear-encoded mitochondrial diseases, often without general intellectual deterioration but starting with impaired visual construction, attention, abstraction, and/or flexibility (25, 26). Recent literature has further suggested that mitochondrial dysfunction may also be an underlying factor in the etiology of common psychiatric disorders such as depression, bipolar disorder, and schizophrenia (23, 27). Multiple aging and late-onset neurodegenerative disorders have also been associated with secondary mitochondrial dysfunction (23), including Parkinson's disease, Alzheimer's disease, and Huntington's disease (28).

Neuroimaging hallmarks of mitochondrial disease

Brain imaging (MRI) findings that are highly concerning for primary mitochondrial disease include cerebral stroke-like lesions in a non-vascular pattern that may be transient (with increased diffusion on diffusion-weighted images), cerebral or cerebellar atrophy, and bilateral T2 focal hyperintensities of any deep gray structure - particularly the basal ganglia as is characteristic of "Leigh disease". However, classic MRI findings of Leigh disease are present in only approximately 18 percent of children with primary mitochondrial disease (29). Magnetic resonance spectroscopy (MRS) is increasingly used in the non-invasive neuroimaging evaluation of suspected mitochondrial disease, as it may reveal characteristic peaks of lactate (at 1.3 ppm at 35 and 135 time to echo) or succinate (at 2.4 ppm). Since detection of some lactate is not uncommon in normal children with stronger (3 Tesla) imaging magnets, it is particularly helpful if quantification of these peaks is performed in voxels placed over both deep gray structures and the CSF, as well as reported relative to machine-specific normal ranges (16). Lactate and pyruvate (with lactate:pyruvate ratio > 20) may also be elevated upon CSF analysis by lumbar puncture. However, such elevations may only be episodic in mitochondrial disease patients at the time of clinical exacerbations and/or may be elevated in a host of other non-mitochondrial diseases (15). A more complete discussion of the neuroimaging abnormalities that may be seen in mitochondrial disease is beyond the scope of this review, but has been recently reviewed in great detail (16).

Clinical manifestations that lessen the likelihood of primary mitochondrial disease

Cognitive function in many individuals with primary mitochondrial disease is commonly in the normal range, such that substantial intellectual disability should raise consideration of other primary etiologies, such as chromosomal aneuploidies or other genetic syndromes. Similarly, while dysmorphic features and/or structural brain anomalies may be seen in a subset of primary mitochondrial diseases (such as pyruvate dehydrogenase deficiency), their presence warrants Clinical Genetics evaluation and high resolution chromosomal microarray analysis for copy number abnormalities. Neurodevelopmental regression may be seen in mitochondrial disease, particularly at the time of intercurrent infection or other catabolic stressor (such as prolonged fasting or dehydration). However, true regression in terms of loss of previously acquired developmental milestones or neurologic function should also warrant consideration for a host of other inborn errors of metabolism and referral to a Metabolic disease specialist. Although rhabdomyolysis may be seen in some individuals with mitochondrial disease, creatine kinase is not typically elevated at baseline in primary mitochondrial disease. Thus, markedly elevated creatine kinase in individuals with myopathy should warrant diagnostic consideration for other dystrophic muscle problems rather than primary mitochondrial disease.

Diagnostic evaluation of mitochondrial disease

Identifying the definitive etiology in suspected mitochondrial dysfunction is not a simple task, as no single biomarker indicates all, or even most, cases with sufficient sensitivity or specificity (30). The current diagnostic paradigm therefore involves a tiered approach based on clinical clues, analysis of complex biological parameters such as blood lactate (30) and *in vitro* studies of immunohistopathology, OXPHOS capacity and/or RC enzyme activities, as well as targeted genetic testing (31, 32). Genetic diagnostic testing presents a significant clinical challenge, as an estimated 800 to 1,160 proteins are present in human mitochondria (33-35). Pathogenic mutations have already been identified in over 60 nuclear genes (16) and all 37 mtDNA-encoded genes (36, 37). However, no clear etiology is identified in up to 60% of individuals suffering from presumed RC disease (38), leaving families feeling abandoned and desperate as they confront ambiguity in prognosis, recurrence risk, and management. Several complex diagnostic algorithms have been proposed to assist the

metabolic specialist in categorizing the likelihood of mitochondrial disease in an individual patient as ‘definite’, ‘probable’, ‘possible’ or ‘unlikely’ (39, 40). Overviews of these diagnostic criteria and related considerations are available online from The Mitochondrial Medicine Society (www.mitosoc.org under “Diagnostic Toolkit”). **Figure 1** depicts a simplified overview of the diagnostic evaluation for mitochondrial disease that may be properly initiated by the Developmental Pediatrician.

Screening investigations for mitochondrial disease

Clinical investigations

Individuals with suspected mitochondrial disease should receive a careful clinical evaluation, including complete review of systems and three-generation family history. A multi-disciplinary approach is typically warranted to complete clinical evaluations necessary to accurately establish the extent of systemic involvement in individuals with suspected mitochondrial disease. All suspected individuals should undergo dilated eye examination and visual acuity assessment by an Ophthalmologist, formal hearing evaluation by an Audiologist, and cardiac evaluation with electrocardiogram and echocardiogram by a Cardiologist. Depending on individual manifestations, it may also be prudent to refer for a detailed dysmorphic examination by a Clinical Geneticist, as well as a complete neurologic evaluation with determination of appropriate brain and/or spine imaging studies by a Neurologist. Gastroenterology evaluation should be considered in individuals with failure to thrive, liver disease, chronic diarrhea, constipation or vomiting. Nephrology evaluation for renal tubular acidosis or other renal disease should be considered in individuals with chronic acidemia, generalized aminoaciduria, or unexplained failure to thrive. Endocrinology evaluation should be considered in individuals with growth failure or manifestations of hypothyroidism, hypoparathyroidism, diabetes mellitus, or adrenal insufficiency.

Laboratory investigations

Comprehensive metabolic screening studies should be obtained on individuals with suspected mitochondrial disease to investigate their “metabolic stability”, as well as the presence of analyte abnormalities that may either suggest alternative metabolic disorders or potentially support the diagnosis of primary mitochondrial disease. Initial laboratory studies to obtain are outlined in **Figure 1**. These studies should be obtained approximately 1 to 4 hours after eating, as prolonged fasting or recent food intake can significantly alter results. Care should be taken in interpretation of elevated blood lactate and pyruvate, which are more often the result of erroneous specimen collection (as they need to be drawn in the correct tubes from a free-flowing sample in a non-struggling individual) than true lactic acidemia (15, 30). Consideration may also be given to obtaining hormone screening studies, depending on individual clinical manifestations. These tests are best initiated by the primary care or other specialist physician, such as the Developmental Pediatrician, given the often prolonged analytic times and limited metabolic specialist population (15). However, results in individual cases as well as any further concerns should be discussed with a Metabolic disease specialist. A detailed review discussing the interpretation of this sophisticated panel of metabolic screening laboratory studies specific to the evaluation of mitochondrial disease is available from the Mitochondrial Medicine Society (16).

Tissue investigations

Based on clinical manifestations and results of blood and urine-based laboratory screening investigations, consideration may be given to obtain a tissue specimen for purposes of performing further histopathologic, biochemical, and/or genetic analyses. Skeletal muscle (quadriceps) is the gold-standard for disease diagnosis, although liver may be warranted if that is the site of primary clinical involvement. The tissue biopsy can be of value to evaluate

for a host of metabolic or other disorders that may have muscle (or liver) manifestations, such as storage disorders or muscular dystrophies. Freshly analyzed tissue allows for assay of integrated RC function, using polarography to quantify how isolated mitochondria consume oxygen in the presence of various metabolic substrates and inhibitors (41). Assessment of enzymatic activity of RC complexes I through IV can also be performed in either fresh or frozen tissue using spectrophotometric techniques (42). However, these assays commonly do not show any definite abnormality of mitochondrial RC function – with as many as 60-90% of individuals sent for tissue biopsy for the indication of suspected mitochondrial disease having no discernible biochemical abnormalities (43). Similarly, tissue pathology and immunohistochemical studies are commonly normal in children with mitochondrial disease. Tissue biopsy is more often helpful in adults who may manifest mitochondrial proliferation (“ragged red fibers”), lipid storage, or immunohistochemical abnormalities (i.e., cytochrome oxidase (COX) deficient fibers) (44). Tissue may further be useful as providing an optimal source to confirm or refute a mitochondrial DNA cytopathy (by whole mitochondrial DNA genome sequencing), a mitochondrial DNA deletion disorder (such as Kearns Sayre syndrome), a mitochondrial DNA depletion disorder (which result from nuclear gene defects), or primary Coenzyme Q10 deficiency (45). Detailed algorithms have been proposed to aid the Metabolic disease specialist in the determination of when to proceed with muscle biopsy in certain individuals (16). Needle-muscle biopsies are performed under local anesthetic in adults at some centers. In contrast, it is often a challenging decision to proceed with muscle biopsy in children given the need for general anesthesia. Some mitochondrial RC diseases, particularly involving dysfunction of complex I, cause hypersensitivity to volatile anesthetics (46, 47). Practically speaking, however, it is not uncommon to obtain a skeletal muscle biopsy (most often quadriceps) in children as a second procedure at the time of another scheduled surgical procedure (i.e., gastrostomy tube or pneumatic tube placement, strabismus repair, etc.). A more recent clinical diagnostic alternative has involved evaluation of mitochondrial RC enzyme activity in skin fibroblast cells obtained by skin biopsy under local anesthetic in the office setting (48). Fibroblast cell lines offer the further advantage of providing an ongoing source of cells for enzymatic assays and/or genetic testing, as they emerge. Although finding a specific biochemical abnormality in fibroblasts may suggest a particular mitochondrial disorder or genetic etiology, failure to identify a definitive biochemical problem in skin does not eliminate the diagnosis of mitochondrial dysfunction, which might only be discernible in higher energy-demand tissues.

Genetic basis of mitochondrial diseases

Indications for genetics evaluation and/or genetic counseling

Clinical Genetics evaluation and formal genetic counseling is important for individuals with mitochondrial disease in order to: (i) interpret genetic diagnostic tests performed in affected individuals and their relatives; (ii) review recurrence risk estimations for the siblings and offspring of affected individuals; and (iii) discuss prognosis. Another important aspect of genetic counseling is to address the psychosocial concerns that commonly accompany the diagnosis of a mitochondrial disorder. Concerns often include grief over the loss of a presumed healthy infant or child, bonding difficulties when symptoms are present in early infancy, parental guilt for perceived failure to provide adequate assistance to an ailing child given the lack of definitive therapies, uncertainty about whether to pursue unproven and often costly therapies, financial uncertainty related to long-term medical management, and possibly, lifelong care. An appropriate question to be addressed is whether other family members may themselves be affected or at risk. Ethical issues may arise regarding whether to proceed with long-term support or surgical interventions, such as gastrostomy tube placement, in the face of a poor prognosis. It is also important to recognize that families may be unable to focus their full energies on optimizing their child's symptomatic care until a

clear diagnosis has been established. Genetic counseling can help address many of these areas of concern (17).

Inheritance patterns

Primary mitochondrial diseases by definition have a genetic etiology, although widely different inheritance patterns characterize particular disorders. Not all genetic problems are necessarily “running through” one’s family, but may result from a new (*de novo*) genetic alteration arising in the affected individual. An overview of the major genetic disease patterns associated with primary mitochondrial disease (maternal, autosomal recessive, autosomal dominant, and X-linked) are highlighted in **Table 2**, detailing their specific implications for recurrence risk to an affected individual’s siblings and own future children. Most primary mitochondrial diseases diagnosed in childhood (67% to 90%) are inherited in an autosomal recessive fashion, although the increasing pursuit in recent years of more comprehensive whole mitochondrial DNA genome analysis has increasingly identified a range of mtDNA disorders that may manifest in both children and adults.

mtDNA-based mitochondrial diseases

Genetic defects of mtDNA that are inherited in a maternal fashion include mtDNA point mutations, small deletions (several basepairs), and, rarely, large deletions with or without duplications. Maternally-inherited mtDNA disorders can variably affect both genders, but are passed down only through females. Sensitive diagnostic testing for these classes of genetic alterations is clinically available. However, it is important to test an informative tissue based on a particular individual’s symptoms (most commonly muscle). Furthermore, the extent of disease may be dependent not simply on the presence of a mtDNA mutation, but on the percent of the mutation relative to the wild-type sequence (mutant heteroplasmy loads greater than 80% are typically associated with greater disease incidence and/or severity). Identification of a mtDNA mutation as pathogenic in a child without a maternal family history of disease likely represents a new (*de novo*) mutation. *De novo* mtDNA mutations have a low recurrence risk for the mother, on the order of 1% to 4% with each subsequent pregnancy, based on the theoretical possibility that even if she is asymptomatic, some of her eggs might carry the mtDNA mutation (germline mosaicism). Assaying mutant load (percent heteroplasmy) in other tissues in the affected individual or their maternal family members may help further refine the recurrence risk estimation. For mutations that occur in one of the 22 mtDNA transfer RNA genes, however, it is often not possible to predict the severity of symptoms should the mutation recur in other family members (17).

Many mtDNA cytopathies commonly involve multi-generational lineages related through the maternal line where adults or children present with any combination of headache (often migraine variant), stroke, mood disorders (depression, bipolar, anxiety, chemical dependence), optic nerve or retinal problems, fibromyalgia or chronic fatigue syndrome, irritable bowel syndrome, peripheral neuropathy, autonomic dysfunction, and/or gastric dysmotility. Such pedigrees should provoke consideration for obtaining mtDNA sequence analysis. Panel testing of approximately a dozen “common” mtDNA point mutations that cause well-characterized disorders such as mitochondrial encephalopathy, lactic acidosis and stroke (MELAS), mitochondrial encephalomyopathy, ragged red fiber disease (MERRF), or neurogenic ataxia and retinitis pigmentosa (NARP) have historical significance but current utility primarily in adults. Indeed, the high cost of such limited point mutation panel testing currently approximates that of sequencing the entire 16,569 basepair mitochondrial DNA genome. Furthermore, over 200 mutations occurring in all 37 mtDNA-encoded genes have now been implicated as causative of primary mitochondrial cytopathies. Thus, it is typically more cost-efficient and ultimately informative to complete whole mitochondrial DNA genome sequence analysis to evaluate individuals (particularly children) with strong

maternal pedigrees. The Developmental Pediatrician may properly initiate whole mitochondrial DNA genome sequence analysis in blood, when a strong suspicion exists based on the symptom constellation and family history.

Should blood-based testing fail to identify a clearly pathogenic mutation, it may be necessary to perform whole mitochondrial genome sequencing on an affected tissue (such as muscle, as above) to definitively rule-out a mitochondrial DNA point mutation as the underlying pathogenic etiology. It is important to realize that mtDNA may harbor other genetic abnormalities that are not detectable by whole genome sequence analysis. These include defects in mitochondrial DNA composition (deletions or duplications) or content (depletion or proliferation), which are inherited sporadically or in a classic Mendelian fashion, respectively (**Table 2**). If mitochondrial DNA depletion in a tissue biopsy specimen is detected, subsequent genetic testing should include sequencing of the known nuclear genes required for mitochondrial DNA nucleotide pool maintenance and turnover (16).

nDNA-based mitochondrial diseases

If mtDNA is extensively analyzed and no pathogenic mutations are found, nuclear inheritance becomes an even more likely cause for mitochondrial disease. In this situation, the recurrence risk of mitochondrial disease for siblings of affected individuals does not exceed 50% (**Table 2**). As affected children are most likely to have an autosomal recessive form of mitochondrial disease, the likely recurrence risk for parents of an affected child would fall in the range of less than 1% up to 25%. A recessive scenario implies that both parents are asymptomatic carriers for a causative gene mutation, with a 25% (1 in 4) chance for each subsequent pregnancy together to be similarly affected. Unaffected siblings of affected individuals have a 67% (2 in 3) chance they are themselves asymptomatic carriers in autosomal recessive conditions. Autosomal dominant inheritance remains a possibility, but the absence of clinical findings in a parent is suggestive of either reduced penetrance or a *de novo* mutation, the latter having a low recurrence risk (under 1% considering the possibility of germline mosaicism). Of course, the specific genetic cause has to be identified to provide the most accurate recurrence risk estimate. In the event that a causative nuclear gene mutation cannot be identified, it is not possible to determine the expected severity for another affected individual regardless of the inheritance pattern. To date, pathogenic mutations causing a wide spectrum of primary mitochondrial diseases have been identified in over 60 nuclear genes (16). As the number of genes implicated in mitochondrial disease continues to rise, testing methodologies improve, and additional diagnostic testing options become available, the potential to further pursue a genetic-based diagnosis of mitochondrial disease should be revisited with time.

POLG1-related mitochondrial diseases

The nuclear gene that has become most commonly implicated in mitochondrial disease is *POLG1*, which encodes the only mitochondrial DNA polymerase that functions in mtDNA replication and repair (49). Thus, individuals with defective *POLG1* accumulate mtDNA mutations and deletions, and may have depleted mtDNA content in tissues over time. However, such mtDNA abnormalities may not be discernible if tissue biopsy is performed at a very early age. Indeed, mutations in this gene are estimated to cause up to 8% of all mitochondrial disease (personal communication with Robert K. Naviaux, M.D., Ph.D.). *POLG1* may cause disease in either an autosomal dominant or autosomal recessive fashion (**Table 2**), depending on the site and nature of individual mutations within the gene. Multiple manifestations along a continuum of severity have been described for *POLG1*-related diseases, primarily involving neurologic and/or hepatic dysfunction. *Autosomal dominant*-related *POLG1* manifestations range from progressive external ophthalmoplegia (adPEO, where PEO is characterized by an inability to abduct the eyes) to idiopathic

Parkinson symptoms, male infertility, or testicular cancer. *Autosomal recessive* mutations in *POLG1* are the cause of many distinct neurologic disorders that may present with developmental variations across the age spectrum (personal communication with Robert K. Naviaux, M.D., Ph.D.): (1) autosomal recessive progressive external ophthalmoplegia (arPEO) with or without premature menopause, idiopathic dementia, and parkinson-like symptoms; (2) Ataxia-neuropathy spectrum (ANS), which includes sensory-ataxia with neuropathy, dysarthria and ophthalmoplegia (SANDO), mitochondrial recessive ataxia syndrome (MIRAS), or juvenile spino-cerebellar ataxia-epilepsy syndrome (SCAE) (50); (3) myoclonus, epilepsy, myopathy, sensory ataxia (MEMSA) with or without PEO; (4) a Charcot-Marie Tooth-like disease in adults with ataxia and dysarthria; (5) Leigh syndrome in pre-school age children (6) Alpers-Huttenlocher syndrome, a liver-brain disorder that may manifest as valproate-induced liver failure and encephalopathy in infants, children, or young adults; or (7) a severe infantile myocerebrohepatopathy spectrum (MCHS) with multi-systemic organ disease (51). Thus, *POLG1* gene sequencing can be initiated by the Developmental Pediatrician in any individual with suspected mitochondrial disease manifesting hepatic and encephalopathic manifestations in infancy or early childhood (Alpers syndrome), valproate-induced liver failure or encephalopathy, Leigh syndrome, ataxia-neuropathy syndrome, PEO, epilepsy with intellectual disabilities of unknown origin, and/or parkinsonism (49).

Common neurodevelopmental sequelae of mutations in known nDNA genes

As above, more than 60 nuclear genes have now been implicated in the pathogenesis of mitochondrial disease. While a full discussion of all of these is beyond the scope of this review, their major presenting features has been previously summarized (16). A subset of these genes should be considered for specific neurodevelopmental presentations (although mtDNA mutations may also cause all of these phenotypes). **Table 3** provides a detailed overview of the major functional classes and specific mitochondrial disease genes currently known to cause leukoencephalopathy, basal ganglia abnormalities, ataxia, seizures, peripheral neuropathy, and dystonia in the pediatric population. Although all of these genes cause neurologic manifestations, this typically occurs in the setting of multi-systemic disease. Thus, individual genetic testing is best pursued by a Clinical Geneticist and/or Metabolic disease specialist based on the overall pattern of an individual's presentation, together with the detailed findings from specialist clinical evaluations, laboratory screening studies, and when available, tissue biopsy biochemical analyses (16). Next generation sequencing technologies may soon permit comprehensive screening in a single assay for all potential mitochondrial disease candidate genes in both genomes, although this testing currently remains an area of active research investigation (52).

Current therapeutic options in mitochondrial disease

No known “cure” or drug therapies are proven widely effective for most primary mitochondrial RC diseases. Exercise training has been shown to be beneficial for primary mitochondrial cytopathies manifesting myopathic symptoms (53). In general, however, application of effective therapies for RC disease manifestations is complicated by limited understanding of predominant cellular mechanisms mediating widely variable phenotypic findings (54). Indeed, many known pathogenic genes do not detectably impair *in vitro* mitochondrial respiratory capacity, which is the current “gold standard” diagnostic assay (16, 55). Rather, recent research suggests that mitochondrial disease manifestations may result from impairment of a plethora of other mitochondrial functions, including intermediary metabolic regulation (56), apoptosis (57), reactive species generation and scavenging (58), mitochondrial dynamics (such as fission and fusion) (59), nucleotide

metabolism (37), or calcium signaling (60). This inherent complexity often leaves clinicians perplexed, unable to effectively apply or monitor targeted therapies (61, 62).

Mitochondrial cocktails

Empiric “mitochondrial cocktails” are often prescribed at great expense to families but without ability to objectively monitor clinical response or adverse effects (62). No standard cocktail is universally used, although several vitamin and antioxidant components are commonly employed (63). Common vitamin supplements include thiamine (B1), riboflavin (B2), ascorbate (C), or B complexes (B50 or B100). Antioxidant supplements may include a wide range of CoQ10 formulations and doses, lipoic acid, and α -tocopherol (vitamin E). Although CoQ10 has clear benefit for the small subset of individuals with primary Coenzyme Q deficiency (64), little evidence exists to suggest global benefit in all mitochondrial RC diseases (65). Indeed, CoQ10 has both pro- and anti-oxidant properties (66). Intermediary metabolic modifiers inconsistently recommended for RC disease patients include L-creatine for individuals with myopathy, L-carnitine for individuals with carnitine deficiency or some mitochondrial DNA cytopathies, and folinic acid for individuals with secondary folate deficiency (54). L-arginine is worthy of special mention for its apparent utility in patients with MELAS (and other mtDNA cytopathies in the author's experience) to mitigate the neurologic sequelae of metabolic stroke if administered intravenously within 30 minutes (and perhaps up to 24 hours) of acute neurologic manifestations, as well as to reduce stroke frequency and severity upon chronic oral administration (67-69). Additionally, secondary CSF folate deficiency in mitochondrial disease may present with a progressive leukoencephalopathy and white matter T2 hyperintensities evident on brain and spinal MRI; this finding can be highly responsive to folinic acid, which crosses the blood-brain barrier to replenish CSF folate (70-72). The ketogenic diet is a matter of active controversy for the treatment of intractable epilepsy in RC disease (73) because although it provides an alternative fuel source (ketones) to bypass glycolysis, increases complex II-dependent respiration (74-76), and has been shown to delay progression in a mouse model of mitochondrial myopathy (77), it has been associated with low adherence, occasional lethality, as well as increased morbidity and mortality in a *MTERF2* mouse model having impaired mtDNA transcription (78). Therapeutic monitoring of many “mitochondrial cocktails” is largely relegated to subjective observations of clinical benefit and tolerance, commonly with chronic use of initial doses despite obvious symptomatic progression. A recent review of mitochondrial cocktail components' indications, contraindications, adverse effects, and dosing regimens for pediatric and adult patients with mitochondrial disease has been published by the Mitochondrial Medicine Society (54). Emerging therapeutic agents for which there is promising research in animal models and/or early-stage clinical trials in humans include mitochondrial-targeted drugs to increase their bioavailability at the site where they are needed (79), lipophilic antioxidants such as probucol (80), transcriptional modulators (81), and gene-therapy aimed at mitochondrial delivery of restriction endonucleases to selectively degrade mutant mtDNA and allow cell repopulation with normal mtDNA (82).

SUMMARY

Primary mitochondrial RC disease represents a highly heterogeneous but identifiable group of genetic-based disorders whose presentation in the pediatric population commonly involves severe neurodevelopmental problems. The first step toward diagnosis requires consideration of the potential for mitochondrial dysfunction in one's differential. While specific (“Red Flag”) neurologic manifestations may raise particular concern, it is the overall gestalt of often nonspecific neurodevelopmental and other systemic disease manifestations that warrants further diagnostic evaluation. Preliminary evaluations that may be initiated by the

Developmental Pediatrician include performing a careful review of systems and family history, initiating appropriate specialty evaluations (to evaluate the eyes, hearing, heart, and other systems based on individual presentation), obtaining initial blood and urine-based metabolic screening studies, as well as facilitating appropriate neuroimaging studies (brain and/or spine MRI, brain MRS). Targeted genetic diagnostic tests in blood may be considered depending on disease presentation and family history (particularly including whole mtDNA genome analysis or *POLG1* sequencing). Referral to a Metabolic disease specialist with expertise in mitochondrial disease may subsequently be helpful in interpreting complex laboratory results, providing genetic counseling, and/or assisting in the coordination of invasive tissue and genetic diagnostic testing. Careful neurodevelopmental assessment is warranted, as sometimes subclinical but often progressive neurodevelopmental abnormalities and mood disorders are common at all ages among the heterogeneous group of primary mitochondrial diseases.

Abbreviations

RC	respiratory chain
OXPHOS	oxidative phosphorylation
mtDNA	mitochondrial DNA
nDNA	nuclear DNA

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FIGURE 1. Initiating the diagnostic evaluation for suspected mitochondrial disease

The Developmental Pediatrician can initiate a multi-faceted clinical and baseline laboratory evaluation to screen for multi-systemic involvement, including intermediary metabolic findings, which may support the diagnosis of mitochondrial disease. Abnormalities on these screening studies should prompt referral to a Metabolic disease specialist for further consideration of specific genetic and/or tissue studies (see text). *, since metabolic abnormalities may be seen only intermittently at the time of stress in mitochondrial disease, indicated blood and urine studies may need to be performed at baseline and then repeated at time of illness (i.e., fever or dehydration).

TABLE 1
Neurodevelopmental Findings in Primary Mitochondrial Disease

The presence of “Red Flag” neurologic findings warrant diagnostic consideration of primary mitochondrial disease, as do multiple “Common Nonspecific” findings. However, “Uncommon Findings” lessen the likelihood of primary mitochondrial disease in favor of other genetic and/or metabolic conditions.

<u>RED FLAG FINDINGS:</u>	<u>COMMON NONSPECIFIC FINDINGS:</u>	<u>UNCOMMON FINDINGS:</u>
<ul style="list-style-type: none"> • Myoclonus • Encephalopathy • Stroke in Non-Vascular Pattern • Epilepsia Partialis Continua • Symmetric Basal Ganglia Hyperintensities • Cerebral or Cerebellar Atrophy 	<ul style="list-style-type: none"> • Myopathy • Hypotonia • Status Epilepticus • Intractable Epilepsy • Coma • Chorea or Movement Disorder • Pervasive Developmental Delay • Autism Spectrum Behaviors • ADHD • Axonal Neuropathy • Sensorineural Hearing Loss • Autonomic Dysfunction • Psychiatric Disease (i.e., Depression, Anxiety, Obsessive Compulsive Disorder) • Dementia 	<ul style="list-style-type: none"> • Structural Brain Anomalies • Dysmorphic Features • Severe Intellectual Disability Out of Proportion to Neurologic Findings • Isolated Speech Delay • Rhabdomyolysis and/or Baseline Elevated CK

TABLE 2

Inheritance patterns of primary mitochondrial disease.

INHERITANCE PATTERN:	MATERNAL	AUTOSOMAL RECESSIVE	AUTOSOMAL DOMINANT	X-LINKED	SPORADIC
Disease examples:	mtDNA point mutations (e.g., MELAS, MERRF, NARP, some causes of Leigh syndrome)	mtDNA depletion disorders caused by nuclear gene mutations (e.g., <i>POLG1</i> or <i>DGUK</i>) nDNA mutations in OXPHOS subunit or assembly genes	<i>POLG1</i> -related diseases (e.g., progressive external ophthalmoplegia or parkinsonism)	One cause of OXPHOS Complex I deficiency Pyruvate dehydrogenase deficiency (e.g., <i>PDHA1</i>) Barth syndrome	Persistent lactic acidemia of unclear etiology Muscle biopsy abnormality of unclear etiology
Recurrence risk to full siblings:	<ul style="list-style-type: none"> • 1-4% if asymptomatic mother • < 50% risk if symptomatic mother 	25%	50% if affected parent <1% if asymptomatic parent based on risk of gonadal mosaicism	If carrier mother: 50% for brothers to be affected; 50% for sisters to be carriers/ ** affected If <i>de novo</i> : <1% for brothers to be affected or sisters to be carriers **	Uncertain
Recurrence risk to half-maternal sibs:	Same as above	None *			Uncertain
Recurrence risk to half-paternal sibs:	None			<ul style="list-style-type: none"> • None for boys • 50% risk girls will be carriers ** 	Uncertain
Recurrence risk to offspring of affected females:	Up to 50% for both sons and daughters	None * All children will be carriers	50% for both sons and daughters	<ul style="list-style-type: none"> • 50% for sons • 50% for daughters to be carriers/ ** affected 	Uncertain
Recurrence risk to offspring of affected males:	None			<ul style="list-style-type: none"> • None for sons • 50% risk daughters will be carriers/ ** affected 	Uncertain

nDNA, nuclear DNA; mtDNA, mitochondrial DNA; MELAS, mitochondrial encephalomyopathy, lactic acidosis, and stroke; MERRF, myoclonic epilepsy and ragged red fibers; NARP, neurogenic ataxia and retinitis pigmentosa; OXPHOS, oxidative phosphorylation.

* Assuming low carrier frequency in population such that partner is not a carrier for any mutation in causative gene.

** Patterns of x-inactivation determine whether females are asymptomatic carriers or affected with x-linked conditions.

TABLE 3
Common neurodevelopmental sequelae of mutations in nuclear genes

More than 60 nuclear genes have now been implicated in the pathogenesis of mitochondrial disease. This table lists roles and names of specific nuclear genes that cause major neurodevelopmental manifestations in the pediatric population.

Leuko-Encephalopathy:	Basal Ganglia Abnormalities:	Ataxia:	Seizures:	Peripheral Neuropathy:	Dystonia:
12 (of 38) nuclear-encoded complex I subunits or 5 assembly factors (83)	12 (of 38) nuclear-encoded complex I subunits or 5 assembly factors (83)	Incorporate heme into complex IV (<i>COX10</i>) (88)	Complex I structural subunits (52)	Coenzyme Q10 deficiency (<i>APTX</i>) (94)	Mitochondrial transporter gene (<i>DDP1</i>) (84)
Complex III assembly factor (<i>BCS1L</i>) (85, 86)	Complex II subunits (<i>SDHA</i>) (87)	Coenzyme Q10 biosynthetic pathway enzyme (<i>PDSS2</i>)	Incorporate heme into complex IV (<i>COX10</i>) (88)	mtDNA depletion (<i>POLG1</i> , <i>RRM2B</i>) (51, 102)	
Complex IV copper incorporation (<i>SCO1</i>) (89) or matrix formation (<i>ETHE1</i>) (90)	Complex IV assembly factors (<i>SURF1</i> , <i>COX15</i> , or <i>ETHE1</i>) (90-92)	Coenzyme Q10 deficiency (<i>APTX</i> , <i>ADCK3</i>) (93, 94)	Complex V assembly factor (<i>ATP12</i>) (58)	Nucleotide salvage (<i>ECGF1</i>) (109)	
Complex V assembly factor (<i>ATP12</i>) (58)	Complex IV mRNA processing protein (<i>LRPPRC</i>) (95)	mtDNA deletions (<i>POLG1</i> , <i>TWINKLE</i>) (96)	Coenzyme Q10 biosynthetic pathway enzymes (<i>PDSS2</i> , <i>COQ2</i>) (98, 99)	Impaired metalloprotease activity (<i>SPG7</i>) (97)	
Coenzyme Q10 biosynthetic pathway enzymes (<i>PDSS2</i> , <i>COQ2</i>) (98, 99)	Complex V assembly factor (<i>ATP12</i>) (100)	Abnormal iron export (<i>ABC7</i>) (101)	mtDNA depletion (<i>POLG1</i> , <i>RRM2B</i>) (102)		
Multiple RC deficiency due to abnormal mtDNA translation (<i>TSFM</i> , <i>TUFM</i> , <i>EFG1</i> , <i>DARS2</i>) (103)	Multiple RC enzyme deficiency (<i>EFG1</i> , <i>DARS2</i>) (104, 105)	Abnormal iron storage (<i>FXN</i>) (106)			
Pyruvate dehydrogenase enzyme components (<i>PDHA1</i> or other complex components) (61)	Pyruvate dehydrogenase enzyme components (<i>PDHA1</i> or other complex components) (107)				
mtDNA depletion due to abnormal mtDNA replication (<i>POLG1</i>) (51) and/or nucleotide salvage (<i>DGUOK</i> , <i>MPV17</i> , <i>ECGF1</i>) (108-110)					