Neuromuscular and Systemic Presentations in Adults: diagnoses beyond MERRF and MELAS

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Abstract Mitochondrial diseases are a diverse group of inherited and acquired disorders that result in inadequate energy production. They can be caused by inheritable genetic mutations, acquired somatic mutations, and exposure to toxins (including some prescription medications). Normal mitochondrial physiology is responsible, in part, for the aging process itself, as free radical production within the mitochondria results in a lifetime burden of oxidative damage to DNA, especially the mitochondrial DNA that, in turn, replicate the mutational burden in future copies of itself, and lipid membranes. Primary mitochondrial diseases are those caused by mutations in genes that encode for mitochondrial structural and enzymatic proteins, and those proteins required for mitochondrial assembly and maintenance. A number of common adult maladies are associated with defective mitochondrial energy production and function, including diabetes, obesity, hyperthyroidism, hypothyroidism, and hyperlipidemia. Mitochondrial dysfunction has been demonstrated in many neurodegenerative disorders, including Alzheimer's disease, Parkinson disease, amyotrophic lateral sclerosis, and some cancers. Polymorphisms in mitochondrial DNA have been linked to disease susceptibility, including death from sepsis and survival after head injury. There is considerable overlap in symptoms caused by primary mitochondrial diseases and those illnesses that affect mitochondrial function, but are not caused by primary mutations, as well as disorders that mimic mitochondrial diseases, but are caused by other identified mutations. Evaluation of these disorders is complex, expensive, and not without false-negative and false-positive results that can mislead the physician. Most of the common heritable

mitochondrial disorders have been well-described in the literature, but can be overlooked by many clinicians if they are uneducated about these disorders. In general, the evaluation of the classic mitochondrial disorders has become straightforward if the clinician recognized the phenotype and orders appropriate confirmatory testing. However, the majority of patients referred for a mitochondrial evaluation do not have a clear presentation that allows for rapid identification and testing. This article provides introductory comments on mitochondrial structure, physiology, and genetics, but will focus on the presentation and evaluation of adults with mitochondrial symptoms, but who may not have a primary mitochondrial disease.

Keywords Mitochondria · mtDNA · Myopathy · Clinical evaluation · Muscle enzymology · Mitochondrial genetics

Introduction to Mitochondrial Structure, Biochemistry, and Genetics

Mitochondrial diseases are an increasingly recognized cause of human illness. The purpose of this article is to focus on the proper identification of adults that may have mitochondrial diseases, but do not have either classic presentations or identified mutations to account for their illness. In order to achieve this goal, the manuscript will introduce the basic concepts of mitochondrial function, the genetic principles that are necessary to understand these disorders, describe classic mitochondrial symptoms, outline the diagnostic techniques that can separate those with common medical illnesses that mimic mitochondrial diseases, as well as “look-alike” disorders where the pathology can be identified with certainty, but is not due to a primary mitochondrial disease.

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The mitochondria is the organelle in all cells except the mature erythrocyte, and is the source of the majority of energy production and free radical production, as well as being responsible for calcium homeostasis, apoptosis, innate immunity, and inflammation. The physical structure of the mitochondria is dynamic and constantly changing *in vivo*. It is comprised of an outer mitochondrial membrane (OMM) and a heavily infolded inner mitochondrial membrane (IMM). The majority of the mitochondrial volume is within the IMM and called the matrix. Between the IMM and OMM is the intermembrane space. There are contact points between the IMM and OMM that contain the complex group of proteins that form the voltage-dependent anion channel and mitochondrial permeability transition pore, which is responsible for several critical functions that involve adenosine triphosphate (ATP) exchange for adenosine diphosphate (ADP), as well as apoptosis. The mitochondrial structure *in vivo* differs in individual cell types, but generally forms a syncytial network with budding formation (mitochondrial fission) and reorganization of separate mitochondria (mitochondrial fusion). In fixed cells, the mitochondria assume a cylindrical shape, described as a submarine-shaped object about 1 micron in length. Within the matrix of the individual mitochondria are 2–10 copies of mtDNA.

Within the matrix are the hundreds of enzymes and biochemical intermediates that are necessary for much of intermediate energy metabolism, including the tricarboxylic acid cycle, the urea acid cycle, and functional cascade proteins needed for apoptosis. The synthesis of a number of amino acid from tricarboxylic acid cycle intermediates occurs in the mitochondria, as well as the oxidation of 8 oxidizable amino acids. Embedded within the IMM are the 5 multicomplex proteins referred to as the electron transport chain (ETC), also known as the respiratory chain. The intermembrane space has a small volume and functions to store the electrochemical capacitance where the hydrogen ions (protons) are pumped by complexes I, III, and IV of the ETC, before flowing through a pore in complex V, which results in the condensation of phosphate onto a molecule of ADP to form ATP. Reducing equivalents from the tricarboxylic acid cycle and fatty acid beta-oxidation (nicotinamide adenine dinucleotide and flavin adenine dinucleotide) donate electrons into complexes I and II, and supply the electrical force required to pump protons from the matrix into the intermembrane space. The resulting electrochemical charge is the driving force that results in ATP formation at complex V, but also is an effector and effector of calcium regulation, and, ultimately, the most critical factor initiating intrinsic apoptosis if this potential is lost. Coenzyme Q₁₀ is a mobile electron carrier, which shuttles electrons in a redox cycle between complexes I and III and complexes II and III. Cytochrome c shuttles electrons in a redox reaction between complex III and complex IV. In addition to proton pumping,

complex IV is the site in the ETC where molecular oxygen is reduced tetravalently to water. Critical to the function of energy generation is the physical and electrochemical integrity of the IMM, as it must not allow small molecules or electrolytes to penetrate or the proton-motive force will be lost. The process of proton pumping, chemical reduction of molecular oxygen to water, and condensation of phosphate onto ADP is termed oxidative phosphorylation.

Inside the mitochondrion is a small piece of DNA [mitochondrial DNA (mtDNA)] that is distinct from the nuclear DNA (nDNA). The mtDNA is a circular molecule with 16,569 nucleotide pairs, and contains 37 genes that encode for 13 proteins of the ETC, 22 transfer RNAs, and 2 ribosomal RNAs required for mtDNA transcription. The translation components (mt-rRNA and mt-tRNA) differ structurally from those in the nucleus of the cell and are structurally similar to that of the bacteria, the ancestral forerunner to the mitochondria. The rest of the mitochondrial structure and enzymes within are encoded by nDNA, with those genes spread over the 23 pairs of chromosomes. This includes all the other subunits of the ETC; the IMM and OMM components; the proteins responsible for unfolding, chaperoning, and refolding the nuclear gene products into the mitochondria; the assembly proteins that will assemble the ETC; the metal cores (iron-sulfur and copper) of the ETC structures; the matrix enzymes; and—what is the most clinically important finding in the last decade—the system that allows mtDNA to replicate [1–5]. The catalog of these 1013 human (and 1098 mouse) genes has been labeled the MitoCarta [6]. Although the function of most of the gene products of the MitoCarta are known, this is not true for all proteins, and mutations in humans have not yet been found in many candidate genes. The complement of genetic disorders that affects the maintenance of normal amounts of wild mtDNA are referred as mitochondrial depletion disorders. This complement of genes includes those that encode for gamma polymerase and the associated enzymes in the replication process (referred to as the mtDNA replisome), enzymes responsible for maintaining the normal concentrations of the nucleotide pool, and proteins whose function is not yet known. In general, the mtDNA depletion disorders result in reduced amounts of wild mtDNA, infidelity of the mtDNA daughter copy, or both.

Along with the process of mitochondrial fission and fusion, which are controlled by gene products of some of the genes found in the MitoCarta, the mitochondria are undergoing constant expansion or replication, which requires the mtDNA within to also undergo replication. This process occurs in all tissues, including that are postmitotic at birth, such as neurons, muscle cells, and hepatic cells. For example, a neuron formed *in utero* may function for more than 80 years and never undergo cellular division, but the mitochondria within that neuron are undergoing constant replication.

Undoubtedly, the next decade will likely lead to an exponential number of known genes that contribute to human mitochondrial diseases. Primary mitochondrial diseases are those caused by pathogenic mutations in this complement of genes (the mtDNA and nuclear genes encoding mtDNA maintenance, mitochondrial proteins, and other critical primary mitochondrial functions).

General Comments on Diagnostic Criteria

In order to better understand clinical aspects of an illness, that illness must have a set of defining features. Each cycle of new diagnostic testing methods results in improved understanding and, in a reiterative process, better defines the criteria for a correct diagnosis of a mitochondrial disease. In general, the correct diagnosis requires the combination of the proper clinical context along with defined abnormalities of blood, urine or cerebrospinal fluid (CSF) analyte values, microscopic or ultrastructural findings, biochemical abnormalities on ETC enzymology or polarography analysis, or a diagnostic genetic finding [7]. There are established criteria that assign a level of certainty to the diagnosis [8, 9].

Mitochondrial diseases represent hundreds of different disorders that vary considerably in their manifestations. This clinical variability often leads to uncertainty about the correct classification of the illness. Except in situations where the patient has an identified mitochondrial phenotype and an identified disease-causing mutation in a gene linked to that phenotype, it is often difficult, or impossible, to know for sure if the patient has primary mitochondrial disease. The diagnostic criteria available were developed in an era before expansive genetic testing. These diagnostic criteria have served the field well, and when expansive mutational testing has been performed and is negative, still serves a purpose for classifying the certainty of diagnosis. However, medicine is at the cusp of redesigning how mitochondrial disorders will be defined, with likely more emphasis on a genetic diagnosis based on known and accepted pathogenic mutation analysis in the setting of classic mitochondrial phenotypic features. For those patients that do not have a proven pathogenic mutation, the combination of accepted phenotypic features with thoughtful integration of all laboratory testing will be necessary for a working diagnosis of a mitochondrial disease. For those persons with an incomplete mitochondrial phenotype or for those with a single or inadequate sampling of biochemical tests it is probably prudent to consider other potential diagnoses. Most screening tests, such as blood, urine, and CSF analytes, are often useful in identifying patients with ongoing biochemical dysfunction caused by a mitochondrial disease, but are usually not specific or sensitive enough to confirm a diagnosis, and cannot be used in isolation in identifying, with any degree

of certainty, a patient as having a mitochondrial diagnosis [6]. Neuroimaging, especially magnetic resonance imaging (MRI), plays a critical role in identifying patients with possible mitochondrial disease [10].

The microscopic, immunohistochemical tests, and ultrastructural findings are not specific to primary mitochondrial disorders, and findings can be seen in muscular dystrophies, some inflammatory myopathies, and other causes. Biochemical testing of the mitochondria, using enzymatic assays or polarographic assays, will also identify respiratory chain dysfunction, but, again, abnormalities are not specific for a certain primary mitochondrial etiology. These tests continue to have a role in identifying mitochondrial disease but, by themselves, even when grossly abnormal, cannot be used to confirm a certain diagnosis. The exception is when a patient has a specific classic phenotype that, along with the screening tests, neuroimaging, microscopic, or biochemical evidence, provides overwhelming evidence of the certainty of diagnosis [7].

Nomenclature and the Pathological–Biochemical–Genetic Correlates to Disease

Mitochondrial diseases have been cataloged using different nomenclatures over the years, with the nomenclature driven by the technology available to describe the illnesses. Initially, before there was an understanding of mitochondrial dysfunction as a cause of illness, these disorders were given eponyms, such as Alpers syndrome, Leigh syndrome, or Kearns–Sayre syndrome, or acronyms driven by the phenotypic presentation, such as chronic progressive ophthalmoplegia plus (CPEO+) or neuropathy–ataxia–retinitis pigmentosa (NARP). Microscopic changes, such as description of “ragged-red fibers”, which were seen in muscle specimens when stained with the modified Gomori trichrome stain, led to the descriptor of “ragged-red fiber disease”. Although many different muscle disorders led to the finding of ragged-red fibers, this descriptor was often associated with mitochondrial myopathies and often used as a diagnostic label. As biochemical techniques became available, the disease descriptors defined the illness by the biochemical finding, so disease names such as complex I disease or cytochrome c oxidase deficiency or complex IV deficiency were used, irrespective of the clinical presentation, and the lack of correlation between phenotype and biochemical findings was accepted and subsequently proved to be correct—biochemical defects in complex IV, for example, could have widely different clinical features. Once genetic testing was available, some of the eponyms became linked to specific mtDNA and, later, nDNA mutations, as seen with the mtDNA A3243G mutation association with mitochondrial encephalopathy, lactic acidosis, and stroke-like syndrome (MELAS) [11]. However as more patients were tested it became clear that the genotype–phenotype

correlations did not always hold true with both the situation where a specific mutation could have different clinical presentations but also that some syndromes, such as MELAS, had more than a dozen causative mutations. The discovery of the widely different phenotypes associated with mutations in genes such as *POLG* [12], responsible for mtDNA replication, and *BCS1L*, responsible for complex III assembly, drove home the point that even identification of the gene responsible for the illness did not always correlate with a single set of clinical findings [13]. Therefore, it is often necessary in an individual patient to define the clinical phenotype (or descriptors of clinical findings when such a phenotype has not been defined), a genetic diagnosis (often with an additional mention how certain the mutation causes pathology), microscopic diagnosis and/or a biochemical diagnosis.

Symptoms associated with primary mitochondrial diseases do not, by themselves, lead to a certain diagnosis of a mitochondrial disorder. These disorders progress at different rates, and it is not uncommon for the first symptom to begin more than a decade before the clinical features lead to evaluation. Many symptoms are not specific and even the common physical findings (Table 1) and primary phenotypic findings (Table 2), which have held true over the years, are seen in nonmitochondrial disease. There is somewhat of a “chicken or the egg” argument, as some experts view that any effect on mitochondrial function caused by an external

Table 2 Key physical and select laboratory findings in mitochondrial disease

Ophthalmoplegia
Retinal pigmentary changes
Sudden onset optic atrophy in late adolescence
Progressive neurosensory hearing loss
Large fiber neuropathy confirmed with NCV
Ataxia
Movement disorder
Dementia
Cardiac conduction defect
Lipomatosis involving head and neck muscles
Myopathy confirmed with EMG or muscle examination with light microscopy
MRI or MRS findings consistent with known mitochondrial findings

NCV = nerve conduction velocity; EMG = electromyography; MRI = magnetic resonance imaging; MRS = magnetic resonance spectroscopy

disorder can be thought of as having at least a mitochondrial component, while others prefer to view mitochondrial disease as only those illnesses with disease-causing mutations involving only the mtDNA and MitoCarta. Diabetes mellitus is such a condition, but, for practical purposes, at the time of this article, diagnosing a patient as having *simple* diabetes mellitus is probably more practical in the clinical sense than thinking of the patient as having a complex set of polymorphisms and environmental factors resulting in mitochondrial failure leading to diabetes mellitus. The challenge for the clinician, whether it be the primary care provider or subspecialist, is not only to properly identify patients that have illnesses that mimic mitochondrial disease, but to then decide those patients requiring a diagnostic exploration for a mitochondrial disease, and then to implement the evaluation in an efficient and cost-effective manner. Once the question of a mitochondrial disease has been raised it can be difficult for the physician or patient to limit the testing. There can be false-positive or nonspecific leads that prompt further evaluation, or negative results for nonspecific tests that are dismissed because they are not sensitive. Testing may be invasive (lumbar puncture, muscle biopsy), and is limited by intrinsic and technical issues causing lack of sensitivity and specificity (blood and urine analyte, ETC enzymology), and utilization may be inhibited by expense [6].

Clinical Presentation of Mitochondrial Diseases in Adults

Clinical features of mitochondrial disease vary among patients, including patients with the same identified genotype, as well as family members affected by the same mutation. This is certainly true of diseases caused by point mutations

Table 1 Organ system involvement in mitochondrial diseases

Organ	Clinical features
Muscle	Myopathy, ptosis, ophthalmoplegia, pain, cramping, muscle hypotonia
Brain	Seizures, dementia, stroke and stroke-like episodes, ataxia, dystonia and other basal ganglia movement disorders, developmental regression, intellectual disability, neuropsychiatric symptoms and mood disorders, atypical migraine, autistic spectrum disorders
Nerve	Demyelinating and axonal neuropathies, neuropathic pain, dysautonomia that includes gastrointestinal problems (gastroesophageal reflux, constipation, bowel pseudo-obstruction), fainting, absent or excessive sweating, aberrant temperature regulation
Kidney	Proximal renal tubular dysfunction (Fanconi syndrome), may result in loss of protein (amino acids), magnesium, phosphorous, calcium, and other electrolytes
Heart	Cardiac conduction defects and cardiomyopathy
Liver	Nonalcoholic steatohepatitis, hepatocellular dysfunction
Eyes	Optic atrophy—neuropathy, retinitis pigmentosa
Ears	Sensory-neural hearing loss, aminoglycoside sensitivity
Pancreas	Diabetes and exocrine pancreatic failure
Systemic	Failure to gain weight, linear growth failure, exercise-induced fatigue

or deletions/duplications in the mtDNA, and probably less true in those disorders caused by specific mutation pairs in nDNA, although variability does exist. In those with mutations affecting mtDNA the disease variability among genotypes has been attributed to the percent of mutant heteroplasmy and the tissue-to-tissue variation caused by random segregation of mutation burden between tissues that occurs early in embryonic development [14]. These differences in mutant heteroplasmy can exist within families. However, some animal studies suggest this effect of successive segregation of mutant mtDNA may not be as robust a process as once thought, and therefore other causes that could explain variable patterns of disease function likely exist [15]. Additional variability that could occur with families, and certainly between families, are confounding mutations that serve to modify the severity of the primary mutation. In patients with nDNA disorders the variability can exist, as demonstrated by the common mutation seen in *POLG* disorders with the c.3248G > A (E1143G) mutation within the same gene lessening the severity of the most severe mutations [16].

Organs that have completed active mitosis at birth, including the brain, muscle, nerve, retina, pancreas, liver, and kidney, may be vulnerable for several reasons. These tissues all tend to have a high basal energy demand and would be expected not to function when ATP delivery is compromised. Possibly more importantly, diseased cells within these tissues (muscle satellite cells are an exception) cannot be replaced by healthier neighbor cells, such that would occur in tissues with cellular turnover, such as the skin or mucosa. In dividing tissues such as mucosal membranes, cell populations with *healthier* mitochondria would have a selective advantage over those with *less healthy* mitochondria. Over time, this turnover of cells results in cells with more diseased populations of mitochondria that would selectively be lost over time, so the tissue tends to rid itself of mitochondrial abnormalities. However, in tissues that are not able to replace injured cells with mitosis or by recruiting stem cells, no selection process favors repopulation of the tissue with less affected cells. The mitochondria are undergoing constant replication in all tissues, and this process requires functional mtDNA and the mitochondrial replisome system (the polymerase and associated proteins) to ensure ongoing production of these new mitochondria. Therefore, in nonreplicative tissues, mtDNA mutations or any process that involves mtDNA replication or maintenance will result in a continued translation of abnormal proteins or progressive loss of wild mtDNA number, with the resultant progressive dysfunction of individual cells and, eventually, of the organ itself. These phenomena are clinically relevant because a hallmark of most mitochondrial diseases is earlier onset of symptoms in persons with a heavier burden of genetic defects and worsening disease with age [17, 18].

It remains a mystery why mutations in different genes and even within genes in the mtDNA may lead to one spectrum of

organ failure, while other mutations cause a different pattern in different organs. For example, mutations in the complex I genes may cause Leber's hereditary optic neuropathy (LHON), while other mutations in the same gene may lead to Leigh syndrome. Similarly, mutations in leucine tRNA tend to cause MELAS, while in the lysine tRNA cause myoclonic epilepsy and ragged red fibers, but this is not always the case. There are several well-described phenotypes attributed to mitochondrial DNA mutations (Table 3) [3, 14].

Mitochondrial diseases may also arise from processes other than germline mutations in mtDNA. Somatic (acquired) mutations causing mitochondrial disease have been reported [19]. Some mtDNA mutations result from exposure to an environmental toxin: aminoglycoside-induced ototoxicity is an example where those having this mutation are at high risk of developing hearing loss after exposure to aminoglycosides, despite having normal blood levels of the medication [20]. Mutations may accumulate with aging or with chronic hypoxia, as occurs, for example, following cardiac ischemia [4, 21].

Mitochondrial Signs and System: Clinical Features and Pearls

Muscle

Weakness due to myopathy is a common symptom in adult-onset mitochondrial disease, but is nonspecific and common in all other myopathic disorders, including the congenital myopathies, the muscular dystrophies, inflammatory processes, and disuse atrophy. In general, most myopathic processes are symmetric. Involvement of the eyelid and extraocular muscles is a key sign when present, but may be a feature in myasthenia gravis, myotonic dystrophy [22], and oculopharyngeal muscular dystrophy [23].

Patients may have minimal objective findings early in the course of their disease, possibly because in the early stages of myopathy, fatigability, and weakness are difficult to quantify using manual muscle testing. As the illness progresses, muscle bulk and strength reduce as the muscle undergoes atrophy, and may be replaced by fatty infiltration. Weakness due to myopathy is often more apparent in the more proximal muscles. Once a patient begins using their arms to help themselves out of a chair or rise up off the floor, the weakness is easier to quantify. Other helpful features include atrophy of facial muscles, the intrinsic muscles of the hands or feet, or symmetric atrophy in other muscle groups. Muscles with abnormal collagen may feel fibrotic, and those with fatty infiltration have a doughy consistency.

When evaluating muscle weakness there are two additional clinical points that merit comment. First, it is not easy to differentiate pathogenic weakness caused by mitochondrial disease from disuse atrophy caused by deconditioning,

Table 3 Common mitochondrial phenotypes due to mitochondrial DNA (mtDNA) mutations and deletions, and *POLG***Leber hereditary optic neuropathy (LHON)**

Key features: painless visual loss beginning in young adulthood with optic atrophy, men involved more than women, some recover to variable extent, discontinuation of ethanol or tobacco smoking may help in recovery. In general, visual improvement after 1 year is rare.

Other features: Wolff–Parkinson–White syndrome, multiple sclerosis-type disease

Key genetics: most mutations occur in the mtDNA-encoded complex I genes, and these mutations are homoplasmic (all mtDNA carries the mutation). G11778A (69 % of LHON with 4 % recovery), G3460A (13 % of LHON with 22 % recovery), T14484C (14 % of LHON with up to 65 % recovery); other rare LHON mutations G3635A, G3700A, G3733A, C4171A, T10663C, G13359A, C13382A, C13382G, A14495G, T14502C, C14568T.

Mitochondrial encephalomyopathy, lactic acidosis, and stroke-like syndrome (MELAS)

Key features: varying degrees of cognitive impairment and dementia, elevated lactate in blood and cerebrospinal fluid, strokes, and stroke-like events in nonvascular territories, mainly parietal–occipital, myopathy

Other features: hearing loss, diabetes mellitus, Wolff–Parkinson–White syndrome, seizures, dysmotility, weight loss, atypical migraine, vomiting spells.

Key genetics: most MELAS is due to a few mutations in the mtDNA t-RNA^{Leu} with the A3243G representing 80 %, T3271C (7 %), A3260G (about 5 %), A3252G (< 5 %), and in other mtDNA genes, such as *ND5 m. G13513A*.

Myoclonic epilepsy and ragged-red fibers (MERRF)

Key features: progressive myoclonic epilepsy in young patients that may be absent in milder forms with older onset of illness, clumps of diseased mitochondria accumulate in the subsarcolemmal region of the muscle fiber, which appear as “ragged-red fibers” when muscle is stained with modified Gomori trichrome stain. Adults may present with ataxia and cervical lipomas.

Other features: short stature, dementia, neuropathy, sensorineural hearing loss (SNHL), myopathy, optic atrophy.

Key genetics: mutations in the mtDNA t-RNA^{Lys} m.8344A>G (80 % of patients), with m.8356 T>C, m.8363G>A, m.8361G>A accounting for another 10 %. Mutations at m.611G>A (tRNA^{Phe}) and m.15967G>A (tRNA^{Pro}) account for another <5 %.

Leigh syndrome subacute sclerosing encephalopathy

Key features: after normal development the disease usually begins late in the first or second years of life, but the onset may occur in adulthood; a stepwise decline in neurologic function occurs with long track signs, ataxia, bulbar dysfunction, dystonia, neuropathy, dementia, and ventilatory failure.

Key genetics: there is a vast array of mutations associated with Leigh syndrome involving both mutations in the mtDNA, as well as nuclear DNA, often involving complex IV assembly genes. These include 8993, 8994, pyruvate carboxylase deficiency, pyruvate dehydrogenase deficiency, cytochrome oxidase deficiency, and *SURF1*, *SCO1*, *SCO2*, *COX10*, *COX15* mutations.

Neuropathy, ataxia, retinitis pigmentosa, and ptosis (NARP)

Key features: progressive symptoms as described in the acronym, along with dementia.

Mutations associated with phenotype: 8993, the same mutation associated with the infantile form of Leigh syndrome. When the mutation is found in nearly all mtDNA (near homoplasmy) the

phenotype is Leigh syndrome, and when the mutation heteroplasmy is between approximately 70 % and 90 % it will result in the NARP phenotype. Affected individuals are often diagnosed after their children have been found to have Leigh syndrome caused by the 8993 mutation.

Mitochondrial DNA depletion syndromes

All phenotypes occur owing to loss of normal mtDNA content over time. In Kearns–Sayre syndrome (KSS) the genetic defect is due to a deletion (or, less commonly, a duplication) of usually a fixed 4977 base pair portion of the mtDNA. Other disorders are due to a mutation in one of the components of the mtDNA replisome, most commonly *POLG*, or a gene that alters the nucleotide pool by which mtDNA is assembled.

Kearns–Sayre syndrome (KSS)

Key features: external ophthalmoplegia, retinitis pigmentosa, and sensory-neural hearing loss.

Other features: myopathy, cardiac conduction defects, dementia, seizures, exocrine and endocrine pancreatic failure, bulbar dysfunction with hypernasal speech, cardiomyopathy, neuropathy.

Key genetics: large-scale deletion of a 4977 base pair segment of mtDNA, also caused by mtDNA duplication or the A3243G mutation.

***POLG* spectrum disorders**

Key features: childhood onset of epileptic encephalopathy, cortical visual loss, long-tract findings in a child with either normal development or cognitive delays, liver failure, and extreme valproate sensitivity resulting in liver failure.

Key genetics: mutations in *POLG*, *TWINKLE* or possibly *POLG2*.

Ataxia neuropathy spectrum previously known as sensory ataxia neuropathy dysarthria and ophthalmoplegia syndrome (SANDO).

Key features: cerebellar ataxia, sensory ataxia, axonal peripheral sensorimotor neuropathy, sensory neuropathy and dorsal root ganglionopathy, liver failure.

Other features: depression, headache.

Key genetics: mutations in *POLG*, *TWINKLE* or possibly *POLG2*.

Myoclonic epilepsy myopathy sensory ataxia known previously as mitochondrial recessive ataxia syndrome and spinocerebellar ataxia with epilepsy.

Other features: depression, headache.

Key features: myopathy, epilepsy, and cerebellar ataxia.

Key genetics: mutations in *POLG*, *PEO1*, or possibly *POLG2*.

Autosomal dominant progressive external ophthalmoplegia

Key features: progressive external ophthalmoplegia, which includes ptosis and paralysis of the extraocular muscles.

Other features: myopathy, parkinsonism, premature ovarian failure, and depression.

Key genetics: mutations in *POLG*.

Autosomal recessive progressive external ophthalmoplegia (arPEO)

Key features: progressive external ophthalmoplegia, which includes ptosis, and paralysis of the extraocular muscles.

Key genetics: mutations in *POLG*, *PEO1*, or possibly *POLG2*.

or to differentiate weakness that may occur with normal aging from the early stages of a pathologic mitochondrial disease. A careful history provided by accurate information from the patient may be helpful, but the clinician is often unable to formulate an opinion with certainty. Mitochondrial dysfunction plays

a central role in both aging and muscle deconditioning from disuse, but this misses the point regarding pathogenicity. The second point is that myopathy may occur in persons with Ehlers–Danlos syndromes (EDS), and the overlap in symptoms in EDS and mitochondrial disorders may make it difficult for the clinician to distinguish these disorders. Infants and young children with EDS may be labeled as hypotonic, whereas older children and adults will report weakness and easy fatigability. In EDS and other hypermobility disorders, the patient must first exert force to stretch the tendon and keep it taught before the belly of the muscle can shorten to move the joint against the resistance. Furthermore, muscles surrounding the joint that are not even involved in the primary intended movement must contract across the joint in all planes of freedom in order to stabilize the joint. This extra work is required with every movement and creates perceived loss of muscle strength, early fatigue, and pain. There is also evidence that the maximal extensional weakness is caused by excessive compliance of the series-elastic component of muscle tissue [24]. There is also evidence that there is loss of proprioception, at least in the ankles, which leads to gait instability [25]. In one series 85 % of patients had mild or moderate muscle weakness, 60 % had reduction of vibration sensation, 13 % had an axonal polyneuropathy, and 26 % had myopathic electromyography findings. Muscle atrophy occurred in half of patients with myopathic findings on muscle biopsy of 28 % [26]. Although the degree of muscle symptoms vary for patient to patient, true muscle weakness is a feature seen even in patients with the least pathogenic forms of EDS. What cannot be explained is that some people with hyperextensible joints have no perceived weakness and are quite athletic. Another common feature in many EDS spectrum disorders is bowel dysmotility and dysfunction, a feature also found in those with mitochondrial disorders. The dual features in EDS of weakness and bowel dysmotility create a symptom set common in mitochondrial patients. Creatinine kinase (CK) is generally not elevated in EDS, but this is of limited usefulness because CK is not elevated in many cases of mitochondrial myopathy. The protean nature of EDS and its neuromuscular similarity to mitochondrial disorders suggests that this disorder must be considered in persons undergoing any neuromuscular evaluation, including a mitochondrial evaluation.

Myopathy may be, but not always, associated with mildly elevated levels of the creatine kinase skeletal muscle (MM) fraction, a nonspecific finding in many muscle disorders. Cramping and pain may occur—another nonspecific finding of many muscle diseases. It is not unusual for patients with mitochondrial myopathies to have resting CK values up to a level of about 1000 U/l, although chronically elevated CK also suggests a dystrophic or inflammatory process. Cramping may be a feature of mitochondrial disease, as the relaxation

phase of the muscle contraction–relaxation cycle requires ATP to pump calcium back into the sarcoplasmic reticulum. Cramping is an expected feature in some channelopathies, such as would occur in some ryanodine receptor mutations (*RYR1*) that cause excessive calcium influx from the sarcoplasmic reticulum. The ryanodine receptor type 1 channel deserves special mention because some mutations result in cramping and susceptibility to malignant hyperthermia, as well as susceptibility to heat stroke. Other mutations result in a slow leak of calcium, leaving the sarcoplasmic reticulum depleted of calcium at the time when the depolarizing signal occurs, resulting in lack of a normal muscle contraction; mutations of these types in the same gene result in central core myopathy, centronuclear myopathy, and multiminicore myopathy—disorders that can mimic mitochondrial myopathy in a child [27]. Mutations in *CACNA1S* can also be associated with anesthetic or heat-induced hyperCKemia and malignant hyperthermia [27]. Symptoms of oculopharyngeal muscular dystrophy start in a person's 40s or 50s with difficulty swallowing, a partial ophthalmoplegia (ptosis and restricted up-gaze, but not horizontal gaze), and myopathy along with an elevated CK, from 2–7 times that of normal levels [28]. These signs are nearly identical as what can be seen in several mitochondrial disorders and aside from knowledge this disease exists, which can be confirmed by genetic testing, patients can be easily misdiagnosed as having a mitochondrial disorder. Ptosis with full ophthalmoplegia occurs in myasthenia gravis or CPEO syndromes. CK levels seen in rhabdomyolysis (> 10 times the upper limit of normal or about 2000 U/l) are rare in respiratory chain disorders. The elevated CK in the disorders of glycogen storage and utilization are rarely triggered by fasting, dehydration, illness, or heat exposure. Frank rhabdomyolysis that can occur with viral illness, fasting, heat exposure, or dehydration should shift the inquiry away from the disorders that affect ETC function and towards mutations in *LPIN1*, ryanodine receptor mutations, or other channelopathies, disorders of fatty acid oxidation, such as carnitine palmitotransferase 2 or very long-chain acyl-CoA dehydrogenase. *LPIN1* encodes for lipin-1, a protein expressed in muscle and fat that acts as a transcriptional co-activator through its association with PPAR- α and PGC-1- α , as well as a protein involved in phospholipid and triacylglycerol synthesis. Mutations in this gene may disrupt fatty acid oxidation through a variety of mechanisms. This disorder generally presents in childhood and should be considered in an adult with episodes of rhabdomyolysis since childhood, but there are adult presentations. In addition to *LPIN1*, the genes *LPIN2* and *LPIN3* are now implicated in the same syndrome [29, 30].

Cardiac muscle (see later) and smooth muscles may also be affected in mitochondrial disorders. Poor motility of the esophagus, stomach, and intestines can cause considerable morbidity. Anorexia and weight loss can occur in the MELAS [31, 32] and myoneurogenic gastrointestinal encephalopathy syndrome [33,

[34], and is often mistaken for anorexia nervosa. Although calorie restriction in the otherwise healthy individual is associated with improved health, ostensibly in part from the reduction of free radical production, up-regulation of *SIRT1*, which induces protein synthesis of many mitochondrial enzymes [35], the food avoidance that occurs with some mitochondrial illnesses is due to the extremely poor gastrointestinal motility or intermittent pseudo-obstruction. Conversely, the starvation that occurs in anorexia nervosa will eventually cause mitochondrial failure, including heart failure [36]. Interpreting blood and urine markers of mitochondrial dysfunction is very difficult in patients with starvation of any cause, with abnormal findings leading the clinician down the pathway of concluding the illness is based primarily on a primary defect in energy metabolism, where, in fact, starvation is the primary illness. Ipecac, which, in the past, was often abused by persons with anorexia nervosa, is a mitochondrial poison [37].

There are no simple rules to guide the clinician in determining the etiology of muscle weakness, although a careful history and physical examination, along with information in Table 4, may be helpful in guidance. Being mindful that other muscle disorders may mimic mitochondrial myopathy, along with the different possibilities causing an elevated CK can assist in developing a plan for the laboratory evaluation.

Brain

Central nervous system (CNS) manifestations of mitochondrial disease may include atypical migraine, dementia, seizures, ataxia and movement disorders, and stroke-like episodes [38]. Most adults undergoing initial evaluation for mitochondrial disorders are cognitively normal. Complex migraine and abdominal migraine (cyclic vomiting variant) are part of many mitochondrial disorders. Dementia with or without psychiatric manifestations, including mood disorders, are associated with many mutations in the mtDNA, such as myoclonic epilepsy and ragged red fibers and MELAS, as well as those illnesses caused by mutations in the genes encoding for the mtDNA replisome. Strokes and stroke-like episodes are common in MELAS, but can be seen in other phenotypic disorders. These strokes may present acutely like a thrombotic stroke. The patient may not present with an acute neurologic event that would be classified as a stroke, but the MRI demonstrates a region of brain infarction. The strokes that occur in mitochondrial disorders tend not to have a vascular distribution, and tend to occur in the occipital lobe and in areas of the brain that are metabolically active, such as the basal ganglia, thalamus, and cerebellum. Like thrombotic strokes, the neurologic deficit may last minutes to months and, in some cases, be irreversible. At the time of the initial presentation, it is impossible to clinically differentiate a complicated migraine from a stroke or stroke-like event. Furthermore, if a mitochondrial etiology is not known, consideration of CNS vasculitis or infection should

Table 4 Nonmitochondrial disorders associated with elevated creatinine kinase (CK)-MM

No or minimal elevation
Congenital myopathies
Bethlem myopathy
Chronic mild or moderate elevation
Muscular dystrophies
Primary merosin (laminin alpha-2 deficiency) or secondary merosin deficiency (dystroglycanopathies)
Myotonic dystrophy
Myositis (inclusion body myositis, inflammatory or auto-immune vasculitis)
HMGCoA reductase inhibitors (statins)
Hypothyroidism
Polymyositis (paraneoplastic)
Amyotrophic lateral sclerosis
Acute unaccustomed exercise
Intermittent massive elevation
Channelopathies: ryanodine receptor (<i>RYR1</i>) disorders
<i>LPIN1</i> mutations
Fatty acid oxidation disorders (normal baseline CK, list not all inclusive):
carnitine palmitotransferase 2 deficiency
trifunctional protein deficiency
very-long chain acylCoA dehydrogenase deficiency
Glycogenolysis defects:
myophosphorylase deficiency (high CK at baseline)
phosphorylase b kinase deficiency
Monophasic elevation
Infectious
Viral: CMV, EBV, HIV, herpes simplex, adenoviruses, and others
Bacterial: staphylococcal, streptococcal, and salmonella
Syphilis
Fungal
Parasitic: trichinosis, toxoplasmosis

HMG-CoA = 3-hydroxy-3-methyl-glutanyl coenzyme A; acylCoA = acyl coenzyme A; CMV = cytomegalovirus; EBV = Epstein-Barr virus; HIV = human immunodeficiency virus

be considered [10, 38, 39]. Neuroimaging with diffusion-weighted studies and diffusion tensor imaging (DTI) is an important component of evaluation of any patient with CNS signs or symptoms. Magnetic resonance spectroscopy can show regions of elevated lactic acid concentration [31]. One of the most important reasons for identifying a mitochondrial illness, such as MELAS, in a patient with stroke is because immediate and long-term therapy with L-arginine and L-citrulline may be beneficial in recovery and reduction of future stroke risk [39, 40].

The differential diagnosis of all diseases that can mimic the CNS manifestations of a mitochondrial disorder is beyond the scope of this review. Seizures are a common manifestation

of mitochondrial disorders, but features of the seizure are rarely specific for mitochondrial disorders. However, presentations with difficult-to-treat status epilepticus or epilepsy partialis continua should warrant testing of *POLG* and possibly other genes responsible for mtDNA depletion [41–46]. MRI features suggestive of a mitochondrial process include symmetric lesions in the deep gray matter, including basal ganglia, periaqueductal gray, and brainstem; cortical strokes in nonvascular distribution; progressive atrophy; and hyperintense lesions in the white matter suggestive of leukodystrophy [31, 34, 41, 42]. The CSF may be normal, but findings of elevated protein, lactate, and alanine, as well as low 5'-tetrahydrofolate levels, are likewise suggestive of a mitochondrial disorder [7].

Peripheral Nerve

Peripheral nerve and Schwann cells are highly metabolically active: nerve cells require a tremendous amount of energy to maintain the electrochemical gradient, and the Schwann cell is continuously myelinating the nerve. The clinical effects may be seen as focal lesions on MRI or loss of function in the peripheral nerve. Autonomic neuropathy may occur with temperature instability, inappropriate sweat response, orthostatic hypotension, or gastrointestinal or bladder dysfunction. Neuropathic pain may occur. In some patients the lack of appropriate sweating can be disabling, rendering them susceptible to heat stroke at temperatures that should be only mildly uncomfortable [46, 47]. When dysautonomia (without a large fiber neuropathy) is the primary complaint, identifying a mitochondrial etiology has been illusive, without any unifying biochemical or genetic findings. Despite efforts to biochemically or genetically characterize primary dysautonomia as a mitochondrial disorder, this work has not yielded firm evidence. The use of tilt table examination and quantitative sudomotor axon reflex testing may assist in the interpretation. More common disorders, such as postural orthostatic tachycardia syndrome or atypical demyelinating polyneuropathy syndromes, may mimic mitochondrial disease. There are a number of severe neurologic and systemic disorders where dysautonomia is a prominent feature, but are masked by other more severe symptoms that separate these from primary dysautonomia. Dysautonomia can be seen in diabetes mellitus, alcoholism, and in the many disorders of hereditary neuropathies. Combined systems degeneration (Shy–Dragger syndrome), which remains a clinical diagnosis, shares many features of a mitochondrial disorder, and presents with profound dysautonomia and parkinsonism, with hypophonia, dementia, visual changes, and impotence [48]. Familial transthyretin amyloidosis is a severe disorder caused by mutations in the transthyretin gene (*TTR*) with autosomal dominant inheritance, although most patients present with *de novo* mutations. Symptoms associated with familial transthyretin amyloidosis overlap the mitochondrial

phenotype and include severe dysautonomia, including bowel dysfunction, peripheral neuropathy, blindness, cardiomyopathy, and nephropathy [49].

Heart

The sinoatrial and atrioventricular nodes are the most metabolically active tissues in the body, and the muscular activity of the heart never ceases. Given this constant demand for energy, it remains a mystery why cardiac conduction defects and cardiomyopathy are not more frequent complications of mitochondrial dysfunction at any age. Aside from some well-described conditions, it is rare for a cardiac issue to be the presenting feature of a mitochondrial disorder in adults. Progressive cardiac conduction defects culminating in a complete heart block may develop quickly in Kearns–Sayre syndrome [50]. Wolff–Parkinson–White syndrome can develop in patients with LHON [51] and in MELAS owing to the A3243G mutation [52]. There is no feature of the cardiomyopathy that differentiates a mitochondrial disorder, although hypertrophic cardiomyopathy is most common. There are many mtDNA mutations, mtDNA depletion disorders, and nuclear genes associated with the risk for cardiomyopathy. Cardiac conduction defects remain a manifestation of both Kearns–Sayre syndrome and some patients with MELAS.

Liver

The mtDNA depletion disorders and mutations in a complex IV assembly gene, *SCO1*, probably cause the most fulminant hepatic disease, but these generally cause infantile or childhood disease [53, 54]. However, adult onset hepatic disease can be a feature of pathogenic mutations in *POLG* not presenting until adult life with the syndrome of fatty liver, hepatic fibrosis culminating in nonalcoholic steatohepatitis [55–57; Bruce H. Cohen, personal communication]. Nonalcoholic steatohepatitis can also be a feature of fatty acid oxidation disorders [54–56]. A number of enzymatic disorders result in failure of normal gluconeogenesis due to either cytoplasmic enzyme dysfunction (e.g., glucose-6-phosphatase deficiency) or mitochondrial enzyme dysfunction (e.g., pyruvate carboxylase deficiency), and may cause hepatic dysfunction, but these usually present in childhood. In older patients secondary gluconeogenic defects are seen in some patients with ETC disorders. The fatty acid oxidation disorders, such as very long-chain acyl-CoA dehydrogenase deficiency and carnitine palmitoyltransferase II deficiency have their onset in adolescence or adult life, and may have a hepatic component [54–56]. It is not clear if the metabolic syndrome of obesity, glucose intolerance, and fatty liver has a primary mitochondrial origin linked with yet-to-be identified polymorphisms in genes encoding energy pathway enzymes, or simply the final consequence of years of oxidative

stress caused by excessive food intake in relation to moment-to-moment energy needs, without the ability to properly oxidize the food. Until this question is solved it will remain one of the most important conundrums in the chicken or the egg problem as to the primary role in many common diseases of aging and excessive calorie intake without adequate exercise.

Eyes

Ocular dysfunction is a common manifestation of mitochondrial disorders [58, 59]. Both retinitis pigmentosa (RP) [58–61] and optic atrophy (OA) [58–60] are features of several well-documented mitochondrial genotypes. RP is not uncommon in mtDNA point mutations and mtDNA depletion disorders. Optic atrophy is most frequent in LHON [63, 64], but also can be seen in autosomal dominant OA caused by mutations in *OPA1*, a gene encoding for a protein responsible for proper mitochondrial fusion [62, 65]. OA with severe subacute visual loss is a hallmark of LHON—sometimes the only feature. The OA that occurs in autosomal dominant OA is not as severe as in LHON and presents in the first decade of life [62–65]. The differential diagnosis RP and OA is broad, and there are many genetic and acquired disorders associated with these conditions, but ocular findings should be considered in the context of a suggestive history, family history, or physical examination as a sign of mitochondrial disease.

Ears

Sensorineural hearing loss occurs in some patients with mitochondrial diseases [66, 67]. This often begins as a high-frequency hearing loss and can progress to total deafness. The mtDNA mutation, A1555G, occurs in about 1:1200 people [68] and predisposes to an extreme ototoxic sensitivity to aminoglycoside exposure. Some persons with this mutation may lose hearing without exposure to aminoglycosides. A number of disorders caused by mtDNA point mutations are associated with hearing loss, and caution should be used when choosing an antibiotic and with the use of aminoglycosides. Hearing loss has been observed in those without identified mutations, although these data were published before the most current genetic testing was available [69, 70]. The role of nuclear gene mutations and environmental exposure in worsening the effects of the A1555G mutation has been elucidated [71, 72], and can serve as another example for thinking about the effects of modifying mutations, even outside of the primarily affected gene.

Kidney

The proximal renal tubular cells require an abundant and steady energy supply. Mitochondrial dysfunction will result

in the inability to reabsorb amino acids and electrolytes after filtration, especially in affected infants. Aminoaciduria, renal tubular acidosis, Fanconi syndrome, and nephrotic syndrome are often seen in childhood-onset disorders [73, 74]. Both tubulopathy and focal segmental glomerulosclerosis have been reported in adults with MELAS caused by the A3243G mutation [75, 76].

Endocrine System

Endocrine dysfunction is a common feature of many mitochondrial disorders. The first patient with a reported illness identified to be caused by mitochondrial dysfunction was first evaluated in an endocrine clinic—a woman with hypermetabolism caused by uncoupling of oxidative phosphorylation who did not have hyperthyroidism [77]. Diabetes mellitus is a common feature of mitochondrial diseases [78, 79]. The common MELAS mutation (mtDNA A3243G) is often associated with diabetes mellitus, and in some families with a seemingly less severe phenotype due to the same mutation, the expression of the disorder is solely diabetes mellitus with or without high-frequency hearing loss, without other complicating features. Because of the association, families with diabetes mellitus that seems to manifest along mtDNA inheritance patterns should be considered for the MELAS A3243G mutation [80]. Diabetes mellitus may be seen in other mitochondrial disorders and is a common condition in otherwise healthy adults. Several other endocrine disorders, including adrenal insufficiency, hypoparathyroidism, and hypo- and hyperthyroidism have been described in respiratory chain disorders [81, 82], including 1 study with endocrine dysfunction in as high as 17 % in adults with respiratory chain disorders [83].

Primary ovarian failure, primary and secondary amenorrhea, primary testicular failure, low libido, erectile dysfunction, and immobile spermatozoa have been associated with some mitochondrial disorders, especially those caused by mtDNA mutations, mtDNA deletions, and *POLG* mutations [12, 84, 85]. It would appear reasonable to assume that certain mtDNA mutations would lead to reproductive failure, but, given the numbers of patient's whose mothers have the same mtDNA mutation (point mutation and even deletion), this remains an area in need of further investigation.

Short stature may be associated with growth hormone deficiency or renal tubular acidosis, but not exclusively so, and is likely due to a combination of a variety of factors that are the end result of inadequate production of ATP. Short stature is a near-universal feature seen in the A3243G form of MELAS. Up to 33 % of those with mtDNA disorders have short stature [83, 84, 86]. Growth hormone replacement therapy when growth hormone deficiency is documented may be of benefit, but the data on this therapy are limited [87].

Failure to gain weight is a systemic manifestations that is not easy to characterize as a single organ system issue and often is due to the combination of autonomic gut neuropathy, smooth muscle dysfunction, endocrine issues, hypothalamic function, and global reduction of normal energy production critical for the necessary aspects of normal maintenance of growth and maintaining weight, in addition to the lipodystrophy and muscle atrophy that can additionally occur in those with disorders of mtDNA maintenance.

Chronic Fatigue

A few case reports have described patients labeled with chronic fatigue syndrome (CFS) ultimately diagnosed with an energy metabolism disorder, based on measured deficiencies in respiratory chain or fatty acid oxidation enzymes, or on the basis of carnitine deficiency [88–90]. Although beyond the scope of this article, these earlier findings resulted in considerable interest among clinicians and patients to perform in-depth evaluations of those suffering with CFS. Many of these patients presented with histories that indicated perfect health until the onset of a viral or other otherwise minor illness, most often a febrile illness. The recovery from the illness was never complete and patients do not return to normal activity, being left with excessive physical and mental fatigue, headache, sleep disturbance, gastrointestinal symptoms that mimic pseudo-obstruction without weight loss, and generalized body pain. Patients with chronic fatigue complain of fatigue at rest, which is different from the exercise-induced fatigue seen in mitochondrial disorders, at least early in the course of the mitochondrial illness. Differentiating these patients from those with a primary mitochondrial disease may come down to objective clinical signs (Tables 1 and 2) and results of screening mitochondrial labs (Table 5).

The symptoms of mitochondrial diseases and CFS overlap considerably, and there is no simple way to screen for a mitochondrial dysfunction or mitochondrial disease. In a study of 5 adults with exercise intolerance as their main symptom, somatic mutations in the *cytochrome b* gene were found on muscle biopsy. Four of the 5 patients had elevated resting lactate levels, which may help differentiate CFS from mitochondrial disorders, at least as an initial screening test. In the 4 patients on whom muscle immunohistochemistry was performed, the percentage

of ragged-red fibers or cyclooxygenase-negative fibers was significantly elevated [19, 90], which is not observed in CFS [91]. Many, but not all, patients with mitochondrial diseases have chronic fatigue but, in all likelihood, only a small fraction of those with CFS have a mitochondrial disorder. Evaluating every person with CFS for mitochondrial dysfunction would not be practical and probably should be reserved for patients with objective physical findings suggesting a myopathy, other clinical manifestations, or abnormalities found on a limited evaluation of organic acids, blood lactate, CK, and carnitine levels. A recent study demonstrated the rate of ATP production and ETC enzymatic activity of complexes I, II, III and IV was normal in CFS, although mitochondrial content as determined by muscle citrate synthase levels, was decreased when compared with patients having genetically confirmed CPEO or MELAS due to the *A3243G* mutation. The 16 CFS patients all had a normal physical and neurologic examination, no family history of a neuromuscular or mitochondrial disorder, normal screening general labs, as well as lactate and CK, and no newly diagnosed medical conditions that could explain the CFS. Eleven healthy medical students served as controls, along with 22 patients with MELAS or CPEO. The CS (citrate synthase) activity in CFS patients was 97 ± 32 , significantly lower than healthy controls (180 ± 49 U/mg protein), $p < 0.001$. This study cannot determine if the fatigue was caused by the low CS value or if the lower mitochondrial content as determined by the CS value was the effect of less-than-normal physical activity. However, ATP production rate was normal in CFS, suggesting the individual mitochondria function normally [91].

An Individualized Approach—Lessons Learned

For the patient that has undergone years of unrewarding medical evaluation, the suggestion that their illness is caused by a mitochondrial disorder is usually a welcomed diagnosis. However, it is important not to assume the patient has a mitochondrial disease because of failure to diagnosis another disorder or because the symptoms appear to fit a mitochondrial disease. Careful evaluation of the facts of the case should occur before unnecessary testing is performed. It is not an overstatement that many common and treatable illnesses may present with mitochondrial features (Table 6). It is incumbent on the clinician to recognize and consider evaluation of illnesses that may mimic mitochondrial diseases. The most common diseases with overlapping symptoms include the endocrinopathies (diabetes, thyroid, parathyroid, and adrenal disorders), B12 deficiency, disorders of collagen formation, and autoimmune inflammatory disorders. The science becomes confusing because an estimated one-third of patients with mitochondrial disorders have an endocrinopathy and because hyperthyroidism, with occurs early in the autoimmune thyroid-destructive phase of Hashimoto's disease, can

Table 5 Proposed screening mitochondrial laboratory tests to consider for evaluation of adults with nonclassical presentations

1. Resting lactate without tourniquet.
2. Amino acids (blood) with ammonia.
3. Organic acids (urine).
4. Free and total carnitine with acylcarnitine profile.

Table 6 Common medical disorders that mimic mitochondrial disease

1. Endocrine
 - A. Hyper- or hypothyroidism
 - B. Adrenal insufficiency
 - C. Diabetes mellitus
 - D. Hypoparathyroidism and related disorders
2. General medical illnesses
 - A. Obstructive sleep apnea
 - B. Metabolic syndrome
 - C. The deconditioned state
 - D. Fibromyalgia
 - E. Chronic fatigue syndrome
3. Inflammatory: SLE and other collagen vascular disorders, inclusion body myositis
4. Paraneoplastic: anti-Hu, anti-Yo, anti-NMDA receptor, opsoclonus-myoclonus, GARS
5. Muscle–hepatic disorders
 - A. Congenital muscular dystrophies: central core disease, multiminicore disease, Ullrich–Bethlem myopathy (*COL6* disorders)
 - B. Muscular dystrophies: OPMD, other dystrophies (note: ragged-red fibers are common in the muscular dystrophies)
 - C. Channelopathies (*RYR1* mutations)
 - D. *LPIN* disorders
 - E. Glycogen synthesis disorders
 - F. Fatty acid oxidation disorders
6. Chronic renal failure with acidosis or loss of amino acids (note: systemic carnitine deficiency occurs in patients on dialysis)
7. Vitamin deficiencies: B12 deficiency, other cobalamin disorders, vitamin E deficiency, micronutrient disorders seen in patients having undergone bariatric surgery, on chronic TPN, self-induced restrictive diets, inflammatory bowel disease, or short bowel syndrome

SLE = systemic lupus erythematosus; NMDA = N-methyl-D-aspartic acid; GARS = glycyl-tRNA synthetase; OPMD = oculopharyngeal muscular dystrophy; TPN = total parental nutrition

cause features similar to a mitochondrial cytopathy, and thyroid hormone can uncouple the process of oxidative phosphorylation. As an example of this phenomenon, a patient was sent to our clinic with a ragged-red fiber myopathy and an enzymatic deficiency in complex IV. A review of his chart indicated he was never evaluated for thyroid disease, and was found to have intermittent bursts of thyroid hormone from a multinodular goiter. Had the thyroid disorder been diagnosed, the patient would have likely never undergone a muscle biopsy. In order to better characterize the illness we did sequencing and performed a long-range polymerase chain reaction on the muscle mtDNA and *POLG*, which were normal. The repeated bouts of sustained thyroid hormone surge are the likely cause of the biopsy features and clinical findings in this patient. As a second example, a 70-year-old man was referred to our clinic with a 3 year progressive history of weight loss, myopathy, and small fiber painful neuropathy; his daughter had been diagnosed with lactic acidosis with a biochemical complex I defect 20 years prior. His primary care

Table 7 Nonmitochondrial laboratory tests to consider for evaluation of adults with nonclassical presentations (perform only those labs that have clinical relevance)

1. CBC
2. CMP
3. Free T₄, TSH
4. Fasting early morning cortisol
5. ESR, C-reactive protein, ultra-sensitive C-reactive protein
6. Vitamin B12 level and methylmalonic acid
7. CK
8. Fasting glucose, 2 hour glucose tolerance test and/or HbA1c
9. Paraneoplastic Panel, SPEP, urine monoclonal proteins/M-protein
10. Polysomnogram (even without complaints of snoring or sleep apnea)
11. Polysomnogram and multiple sleep latency test (for excessive daytime sleepiness)
12. ECG (and possibly an echocardiogram)
13. Audiogram
14. Ophthalmologic examination
15. MRI (if there are focal neurological deficits, dementia, ataxia, movement disorder)
16. Awake and sleep EEG (if there are seizures or dementia)
17. Nerve conduction test and EMG (if there is a neuropathy or myopathy)

CBC = complete blood count; CMP = complete metabolic panel; TSH = thyroid-stimulating hormone; ESR = erythrocyte sedimentation rate; CK = creatine kinase; HbA1c = glycated hemoglobin; SPEP = serum protein electrophoresis; ECG = electrocardiogram; MRI = magnetic resonance imaging; EEG = electroencephalogram; EMG = electromyography

physician assumed his illness was linked to the daughter's mitochondrial disease and did not pursue any medical evaluation. The first laboratory evaluation 3 years after symptom onset demonstrated a fasting glucose of 376 mg/dl, which ended the diagnostic evaluation.

A letter of referral from the healthcare provider most familiar with the patient's course is always helpful, especially if the letter can highlight the salient features of the patient's illness and review of any laboratory testing. The initial evaluation should include a comprehensive history

Table 8 Tissue and biochemical testing of tissue for patients with strongly suspected mitochondrial disorders

1. Routine light microscopy and immunohistochemistry of muscle
2. Electron microscopy of muscle or skin
3. Electron transport chain enzymology on fresh or flash-frozen muscle homogenate or on mitochondrial isolate, skin fibroblasts
4. Polarography on fresh mitochondrial isolate, fresh permeabilized muscle fibers or on fibroblast culture
5. Blue native gel or clear native gel electrophoresis for supercomplex structure
6. Western blot analysis of mitochondrial proteins
7. Mitochondrial DNA content analysis

Table 9 Genetic testing to consider for patients with strongly suspected mitochondrial disorders

1. Mitochondrial DNA select point mutation testing (preferred muscle, cheek swab/saliva, urine sediment but can be done on blood).
2. Mitochondrial DNA whole genome testing (preferred muscle, cheek swab/saliva, urine sediment, but can be done on blood).
3. Long-range polymerase chain reaction or Southern blot (preferred muscle, but can be done on cheek swab/saliva or urine sediment; blood not reliable).
4. Sequencing and deletion/duplication testing of specific nuclear genes, or panel of genes.
5. Massive parallel sequencing (NextGen) of large numbers of nuclear genes, including that all known mitochondrial-targeted genes, look-alike disease genes, or whole exome along with a high-density single nucleotide polymorphism microarray.

and physical examination. If there are objective findings that are suggestive of a syndromic mitochondrial disorder the appropriate evaluation should be considered. In some instances there is strong evidence for a specific genetic disorder, in which case targeted genetic testing for one gene or a panel of genes, such as a whole mitochondrial DNA sequence or panel of nuclear genes, is indicated. It is rare, even in patients where there seems certain to be a genetic answer, that I do not perform general screening labs (the relevant components found in Tables 5 and 7). If there are CNS findings, such as dementia, epilepsy, or movement disorders, CSF sampling for protein, glucose, lactic acid, amino acids, neurotransmitters, and 5'-methyltetrahydrofolate levels may be performed. For any objective finding that can be further evaluated with conventional laboratory testing, testing should be pursued, for

example MRI for a focal neurological deficit, nerve conduction velocities (NCV) and electromyography for a neuropathy or myopathy, and electrocardiogram for a rhythm disturbance. For patients without objective physical findings I would want to make certain the laboratory tests for common disorders (Table 6) have been performed recently or considered, and will usually perform the labs relevant to the presentation listed in Table 7. Any findings should be approached using conventional evaluation and therapy.

Before the advent of genetic testing, patients with positive screening analytes suggesting a mitochondrial disorder underwent a muscle biopsy for microscopy and biochemical analysis (Table 8). This is still a valuable approach, but because genetic testing is less invasive and often less expensive, the genetic testing approach should be considered (Table 9). Muscle biopsy is still important for many disorders, and often patients will require both the muscle biopsy and genetic testing. Reasons for performing a muscle biopsy include the diagnosis of a myopathy or neuropathy, or if there is a concern for an inflammatory disorder or vasculopathy. A muscle biopsy can be helpful if the genetic testing was noninformative and the patient has muscle weakness.

With the exception of known pathogenic mutations in the presence of clinical findings, no single laboratory test is likely to be diagnostic of a mitochondrial disease. There are common errors that occur, which result in misdiagnosis (Table 10). Although referral of the patient to a mitochondrial center can clear up these issues, it is important to be aware of the common sources of error. The efficient and cost-effective evaluation of an adult

Table 10 Common pitfalls and errors in interpretation of mitochondrial lab results

1. Blood lactate can be falsely elevated owing to the tourniquet effect or exercise immediately prior to sampling.
2. Carnitine values below the control range can be seen in those on vegan diets or starvation.
3. Abnormal organic acids can be precipitated by consumption of excessive free carbohydrates prior to sample, and 3-methylglutaconic acid can be found in extreme physiologic stress, glucocorticosteroid, or progesterone use.
4. Ragged-red fibers are found in increasing percentage in aging. Quantification is required especially in patients older than 50 years.
5. Descriptions of muscle findings, such as type II fiber type atrophy or disproportionate ratio of fiber types are not a medical diagnosis.
6. Genetic testing in previous years often only sequenced the coding regions of a gene. Newer methods often include critical additional testing, such as sequencing of the promoter regions, intronic regions, and also will include deletion/duplication testing of that gene. It is important to understand the methodology of the particular test in order to determine exactly what testing was performed.
7. It is important to understand concepts of homozygosity, heterozygosity, heteroplasmy, polymorphism, and variants of uncertain significance when reading genetic reports. If there are questions about the clinical relevance of the findings, the clinician should call the laboratory director.
8. Because several mitochondrial enzyme activities are chemical and heat labile, muscle removed from the body for biochemical testing must be obtained without injection of local anesthetic, without use of electrocautery, and be properly frozen within several minutes of leaving the body. The tissue must remain at -80°C until analyzed. The activity of rotenone-sensitive complex I + III activity may disappear within 20 minutes at room temperature. Any deviation from these standard methods can result in false-positive results.
9. Correlation of muscle biochemical findings with the pathology findings is important. Elevated citrate synthase as a marker of mitochondrial proliferation or depletion should be correlated with the pathology report to determine if evidence of mitochondrial proliferation was present on microscopy or ultrastructural examination. Additional laboratory testing can confirm or refute such interpretations.
10. Not understanding laboratory control values and how the control values were obtained can lead to misdiagnosis. The methodology of the control range should be made available upon request and updated with reasonable frequency.
11. The final decision regarding the clinical diagnosis is ultimately the responsibility of the clinician ordering the test and not the laboratory director signing the laboratory report. If there are questions the clinician should discuss the findings with the laboratory director.

with suspected mitochondrial disease is neither intuitive nor easy. A diagnosis may not be reached in patients with abundant objective features having undergone extensive testing. Physicians with experience develop their own diagnostic pathways over time that is most useful to their patients.

Conclusions

Even as genetic discoveries in the last two decades have allowed the field of mitochondrial medicine to mature, and allow for improved understanding of many disorders, there remain many challenges in correctly identifying the patients with possible mitochondrial disorders that require an evaluation and then determining the scope of that evaluation. It is important to understand the basic clinical features of the primary mitochondrial diseases and the well-described mitochondrial phenotypes. If the patient has a well-described phenotype, the evaluation can generally be pursued by performing genetic testing with or without additional biochemical testing. If the patient does not have features of the primary mitochondrial phenotype, careful consideration for medical illnesses that mimic mitochondrial disorders is important. This generally requires a re-evaluation of historical elements of the illness and a review of the laboratory data, along with a physical examination. This rigid process will often lead the clinician towards the correct diagnostic approach. Screening mitochondrial labs may be helpful in all patients that are suspected of having a mitochondrial illness, but who do not meet the rigid primary phenotype criteria, and, if positive, drive additional evaluation. It is important to recognize that patients not meeting a level of diagnostic suspicion do not require extensive testing. Because noninformative results cannot “rule-out” mitochondrial disease, negative results resulting in additional testing can be wasteful and detract from the proper medical evaluation and treatment. Regardless, the challenge of determining which patient warrants an invasive evaluation remains a challenge.

Required Author Forms Disclosure forms provided by the authors are available with the online version of this article.

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