

# Diagnosis and management of mitochondrial disease: a consensus statement from the Mitochondrial Medicine Society

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**Purpose:** The purpose of this statement is to review the literature regarding mitochondrial disease and to provide recommendations for optimal diagnosis and treatment. This statement is intended for physicians who are engaged in diagnosing and treating these patients.

**Methods:** The Writing Group members were appointed by the Mitochondrial Medicine Society. The panel included members with expertise in several different areas. The panel members utilized a comprehensive review of the literature, surveys, and the Delphi method to reach consensus. We anticipate that this statement will need to be updated as the field continues to evolve.

**Results:** Consensus-based recommendations are provided for the diagnosis and treatment of mitochondrial disease.

**Conclusion:** The Delphi process enabled the formation of consensus-based recommendations. We hope that these recommendations will help standardize the evaluation, diagnosis, and care of patients with suspected or demonstrated mitochondrial disease.

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Mitochondrial diseases are one of the most common inborn errors of metabolism, with a conservative estimated prevalence of approximately 1:5,000. Primary mitochondrial diseases are defined as disorders impacting the structure or function of the mitochondria as a result of either nuclear DNA (nDNA) or mitochondrial DNA (mtDNA) mutations.<sup>1</sup>

The field of mitochondrial medicine has only developed over the past 25 years, and clinicians have limited but growing evidence to formulate clinical decisions regarding diagnosis, treatment, and day-to-day patient management. These disorders still lack sufficiently sensitive and specific biomarkers. Most current diagnostic criteria were developed prior to the recent expansion in genetic knowledge that allows precise delineation of specific disease etiologies. Establishing a diagnosis often remains challenging, costly, and, at times, invasive.

There are no published consensus-based practice parameters that clinicians can utilize for initiating diagnosis or patient management. Most mitochondrial medicine specialists use a set of internally established guidelines based on theoretical concepts, limited published recommendations, and personal and anecdotal experience. As the Mitochondrial Medicine Society recently assessed, notable variability exists in the diagnostic approaches used, extent of testing sent, interpretation of test results, and evidence from which a diagnosis of mitochondrial disease is derived.<sup>2</sup> There are also inconsistencies in treatment and preventive care regimens.

Our purpose here was to review the literature on mitochondrial disease and, whenever possible, make consensus-based recommendations for the diagnosis and management of these patients. In the interest of brevity, limited background

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information about mitochondrial diseases, including testing, diagnostics, and treatment approaches is provided. We direct the reader who may be unfamiliar with these topics to several excellent reviews<sup>3-6</sup> and to the supplementary material accompanying this review for comprehensive topic-specific summaries prepared as part of this consensus development endeavor.

## METHODS

Writing Group members were appointed by the Mitochondrial Medicine Society's Consensus Criteria Committee. The panel included 19 mitochondrial medicine specialists throughout North America with different areas of expertise, including neurologists, geneticists, clinical biochemical geneticists, anesthesiologists, and academic diagnostic laboratory directors. Clinician-led subgroups were formed to review relevant literature via a detailed PubMed search and summarize the evidence on selected topics in mitochondrial disease. Because some aspects of mitochondrial disease have been studied more thoroughly, these topics received more attention than others.

We expected that there would be variable but generally limited data available to establish evidence-based clinical practice protocols. Case reports and a limited number of case series are the primary evidence base available for the diagnosis and treatment of mitochondrial disease. Few studies were prospective. An initial approach to categorize the literature based on the Oxford Centre for Evidence-Based Medicine system showed that the majority of the literature met grade 3 or less (case-control, low-quality cohort studies, or expert opinion without explicit critical appraisal). Thus, the panel was asked to develop consensus recommendations using the Delphi method. Delphi is a consensus method developed to utilize expert opinion to make a knowledge-based decision when insufficient information is available.<sup>7</sup> It is increasingly used to develop consensus-based guidelines in medicine and rare diseases.<sup>8,9</sup> Expert panelists review the available knowledge and answer surveys concerning the issues in question. The survey is scored to determine the variation in opinion; if consensus is not reached, then these items are returned to the panelists for a second round, this time with the mean of responses from the first round available. A face-to-face meeting is then held to review areas of persistent disagreement.

The survey instrument, developed using QuestionPro software, generally comprised a five-point bipolar Likert scale; the potential responses were strongly disagree, disagree, neutral, agree, or strongly agree. Each subgroup was tasked with creating key clinical questions to address within its focus area. Each response was assigned a numerical score, such that "strongly disagree" was scored as 1, "disagree" as 2, "neutral" as 3, "agree" as 4, and "strongly agree" as 5. The mean consensus score for each item was then tallied. Items with a mean consensus score of  $>4$  (agree/strongly agree) or  $<2$  (disagree/strongly disagree) were considered to reach consensus (as agree or disagree, respectively).

For items that did not meet consensus, a streamlined survey was returned to the panelists for a second round, with items marked with the group's score so that each panelist was aware of the group mean when they re-scored the survey. After

completion of a second set of surveys, a meeting of the panel was convened in conjunction with the 2014 annual meeting of the United Mitochondrial Disease Foundation Symposium in Pittsburgh, Pennsylvania, where items that did not reach consensus were discussed. Consensus was not reached for all items during the face-to-face meeting, especially on items that were considered to need further research.

To maintain brevity, the full data summary for each topic reviewed is not outlined below. All data summaries used by the working groups along with the initial composite consensus scores are available for review as an online supplement at [bit.ly/mmsdatasummaries](http://bit.ly/mmsdatasummaries) and provide a comprehensive and in-depth review of these topics.

## DIAGNOSIS

### Biochemical testing in blood, urine, and spinal fluid

Most diagnostic algorithms recommend evaluation of selected mitochondrial biomarkers in blood, urine, and spinal fluid. These typically include measurements of lactate and pyruvate in plasma and cerebrospinal fluid (CSF), plasma, urine, and CSF amino acids, plasma acylcarnitines, and urine organic acids.

Lactate elevation occurs because the flux through glycolysis overwhelms the utilization of pyruvate in the mitochondria. Its usefulness is often limited by errors in sample collection and handling.<sup>3</sup> Venous plasma lactate levels can be spuriously elevated if a tourniquet is applied during the collection and/or if a child is struggling during the sampling. Markedly elevated plasma lactate ( $>3$  mmol/l), in a properly collected sample, suggests the presence of mitochondrial dysfunction, which can be due either to primary mitochondrial disease or, secondarily, to organic acidemias, other inborn errors of metabolism, toxins, tissue ischemia, and certain other diseases.

Several studies have shown that in patients with primary mitochondrial disease, truly elevated lactate levels have sensitivity between 34 and 62% and specificity between 83 and 100%.<sup>10-12</sup> The blood lactate/pyruvate ratio is most reliable in differentiating electron transport chain (ETC) disease from disorders of pyruvate metabolism, but only when lactate levels are high.<sup>13</sup> The sensitivity of this ratio is 31%, with a specificity of 100%.<sup>11</sup>

Elevated CSF lactate can be a helpful marker of mitochondrial disease in patients with associated neurologic symptoms.<sup>14</sup> Collection artifacts are less of a problem, although a variety of brain disorders, status epilepticus in particular, can transiently increase CSF lactate.<sup>15</sup> Surprisingly, urine lactate correlates less well with the presence of mitochondrial disease.<sup>16</sup>

Pyruvate elevation is a useful biomarker for defects in the enzymes closely related to pyruvate metabolism, specifically pyruvate dehydrogenase and pyruvate carboxylase.<sup>17</sup> Blood pyruvate levels are also plagued by errors in collection and handling; furthermore, pyruvate is a very unstable compound. A single study has shown a sensitivity of 75% and specificity of 87.2% in patients with primary mitochondrial disease.<sup>11</sup>

Quantitative amino acid analysis in blood or spinal fluid is commonly obtained when evaluating a patient with possible mitochondrial disease. Elevations in several amino acids occur

due to the altered redox state created by respiratory chain dysfunction including alanine, glycine, proline, and threonine.<sup>3</sup> The exact sensitivity and specificity of alanine or the other amino acid elevations in patients with primary mitochondrial disease are not yet known. Elevations may be present in either blood or spinal fluid, and notable findings may only occur during times of clinical worsening. Urine amino acids are most commonly used to assess for mitochondrial disease–associated renal tubulopathy.

Carnitine serves as a mitochondrial shuttle for free fatty acids and a key acceptor of potentially toxic coenzyme A esters. It permits restoration of intramitochondrial coenzyme A and removal of esterified intermediates. Quantification of blood total and free carnitine levels, along with acylcarnitine profiling, permits identification of primary or secondary fatty-acid oxidation defects, as well as some primary amino and organic acidemias. Although acylcarnitine testing is suggested in a variety of mitochondrial reviews,<sup>3</sup> there is limited background literature to clearly support this recommendation. This testing is typically recommended because of the association of a potential secondary disturbance of fatty-acid oxidation in patients with mitochondrial disease and certain mitochondrial phenotypes overlapping other inborn errors of metabolism for which acylcarnitine analysis is diagnostic.

Urinary organic acids often show changes in mitochondrial disease patients. Elevations of malate and fumarate were noted to best correlate with mitochondrial disease in a retrospective analysis of samples from 67 mitochondrial disease patients compared with 21 patients with organic acidemias; other citric acid cycle intermediates and lactate correlated poorly.<sup>16</sup> Mild-to-moderate 3-methylglutaconic acid (3MG) elevation, dicarboxylic aciduria, 2-oxoadipic aciduria, 2-aminoadipic aciduria, and methylmalonic aciduria can all be seen in certain primary mitochondrial diseases.<sup>3,18–20</sup> Although urine organic acid may detect 3MG elevations, specific quantification of 3MG in blood and urine is more reliable, especially when 3MG levels are not markedly elevated.<sup>21</sup>

Elevated creatine phosphokinase and uric acid are common in acute rhabdomyolysis in patients with fatty-acid oxidation disorders, and the elevations are caused by nucleic acid and nucleotide catabolism.<sup>22</sup> Although not extensively studied in primary mitochondrial disorders, patients with primary mitochondrial diseases may have muscle disease (especially with cytochrome b disease and thymidine kinase 2 deficiency),<sup>23</sup> and elevations can also occur with primary or secondary fatty-acid oxidation disorders. Hematologic abnormalities can be detected with a complete blood count. Aplastic, megaloblastic, and sideroblastic anemias, leukopenia, thrombocytopenia, and pancytopenia have been reported in some primary mitochondrial diseases.<sup>24</sup> Multiple primary mitochondrial diseases are associated with liver pathology based on mtDNA depletion and/or general liver dysfunction, and transaminases and albumin levels may help in diagnosis. New biomarkers of mitochondrial disease such as FGF21 and reduced glutathione await validation.<sup>11,12,25,26</sup>

Cerebral folate deficiency is seen in a wide variety of neurologic and metabolic disorders including mitochondrial disease and is diagnosed via measurements of 5-methyltetrahydrofolate in CSF.<sup>27</sup>

Cerebral folate deficiency was initially identified in mitochondrial disease in patients with Kearns–Sayre syndrome (KSS).<sup>28,29</sup> More recent case series in patients with KSS have further confirmed this finding.<sup>30,31</sup> Cerebral folate deficiency has been identified in patients with mtDNA deletions,<sup>32</sup> *POLG* disease,<sup>33</sup> and biochemically diagnosed complex I deficiency.<sup>34</sup> A primary cerebral folate disorder also exists, often due to mutations in the folate receptor 1 (*FOLR1*) gene encoding folate receptor alpha.<sup>27</sup>

#### *Consensus recommendations for testing blood, urine, and spinal fluid*

1. The initial evaluation in blood for mitochondrial disease should include complete blood count, creatine phosphokinase, transaminases, albumin, lactate and pyruvate, amino acids, and acylcarnitines, along with quantitative or qualitative urinary organic acids. *Caution must be taken to ensure that specimens are collected appropriately, especially for lactate and pyruvate measurements.*
2. Postprandial lactate levels are more sensitive than fasting specimens and are preferred when possible. *Caution must be taken to not overinterpret small elevations in postprandial lactate.*
3. The lactate/pyruvate ratio in blood or CSF is of value only when the lactate level is elevated.
4. Quantitative 3MG measurements in plasma and urine should be obtained when possible in addition to urine organic acids in patients being evaluated for mitochondrial disease.
5. Creatine phosphokinase and uric acid should be assessed in patients with muscle symptoms who are suspected of having mitochondrial diseases.
6. Urine amino acid analysis should be obtained in the evaluation of mitochondrial tubulopathy.
7. When CSF is obtained, it should be sent for lactate, pyruvate, amino acid, and 5-methyltetrahydrofolate measurements.
8. Further research is needed regarding other biomarkers such as FGF21, glutathione, and CSF neopterin.

#### **DNA testing**

Primary mitochondrial disorders are caused by mutations in the maternally inherited mtDNA or one of many nDNA genes. mtDNA genome sequencing and heteroplasmy analysis can now effectively be performed in blood, although it may be necessary to test other tissues in affected organs. Newer testing methodology allows for more accurate detection of low heteroplasmy in blood down to 5–10%<sup>35</sup> and 1–2%.<sup>36,37</sup> Overall, the advent of newer technologies that rely on massive parallel or next-generation sequencing (NGS) methodologies have emerged as the new gold standard methodology for mtDNA genome sequencing because they allow significantly improved reliability and sensitivity of mtDNA genome analyses for point mutations, low-level heteroplasmy, and deletions, thereby

providing a single test to accurately diagnose mtDNA disorders.<sup>38</sup> This new approach may be considered as first-line testing for comprehensive analysis of the mitochondrial genome<sup>39</sup> in blood, urine, or tissue, depending on symptom presentation and sample availability. Identification of a causative mitochondrial disease mutation allows for families to end their diagnostic odyssey and receive appropriate genetic counseling, carrier testing, and selective prenatal diagnosis.

It may be necessary to preferentially test other tissues as part of the diagnostic evaluation of a patient for a suspected mitochondrial disorder. Urine is increasingly recognized as a useful specimen for mtDNA genome analysis, given the high content of mtDNA in renal epithelial cells.<sup>40</sup> This finding particularly applies to MELAS (mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes) syndrome and its most common mutation m.3243 A>G in *MTTL1*.<sup>41,42</sup> Skeletal muscle or liver are preferred tissue sources for mtDNA genome sequencing when available, given their high mtDNA content, reliance on mitochondrial respiration, and the possibility that they may harbor a tissue-specific mtDNA mutation that is simply not present in blood.

The mtDNA deletion and duplication syndromes manifest along a spectrum of three phenotypic presentations: KSS, chronic progressive external ophthalmoplegia, and Pearson syndrome. The most commonly used methods for detection of mtDNA deletions previously included Southern blot and long-range (deletion-specific) polymerase chain reaction analysis. However, Southern blot analysis lacks sufficient sensitivity to detect low levels of heteroplasmic deletions. In contrast, array comparative genome hybridization detects deletions and also estimates the deletion breakpoints and deletion heteroplasmy.<sup>43,44</sup> All of these methodologies are being replaced by NGS of the entire mitochondrial genome,<sup>39,45</sup> which provides sufficiently deep coverage uniformly across the mtDNA genome to sensitively detect and characterize either single or multiple deletions.<sup>46</sup> Deletions and duplications may only be detected in muscle or liver in many patients.

The mtDNA depletion syndromes are a genetically and clinically heterogeneous group of disorders characterized by a significant reduction in mtDNA copy number in affected tissues. Abnormalities in mtDNA biogenesis or maintenance underlie the pathophysiology of this class of mitochondrial disorders. They typically result from nDNA mutations in genes that function in mitochondrial deoxynucleotide synthesis or in mtDNA replication. Less frequently, mtDNA depletion can be caused by germline deletions/duplications of mtDNA segments.<sup>47</sup> Diagnosis therefore requires quantification of mtDNA content, typically in affected tissue, with identification of a significant decrease below the mean of normal age, gender, and tissue-specific control when normalized to nDNA tissue content.<sup>48,49</sup> mtDNA content is not assessed by NGS of the mtDNA genome and must be assayed by a separate quantitative real-time polymerase chain reaction.

More than 1,400 nuclear genes are either directly or indirectly involved in mitochondrial function. In addition to single-gene testing, there are many diagnostic laboratories that offer next-generation sequencing-based panels of multiple genes. Some

companies offer panels with a small number of targeted genes, varying from a few to a dozen or so per mitochondrial disease phenotype (e.g., for mitochondrial depletion syndrome). Larger panels of more than 100, 400, or 1,000 nuclear genes are also available. Whole-exome sequencing became clinically available in 2011, and it is an increasingly common diagnostic tool utilized in patients with suspected mitochondrial disease. Numerous research reports describe the detection of novel pathogenic mutations in nuclear mitochondrial genes by whole-exome sequencing, but no clear evidence-based practice recommendation has been established related to the use of single-gene sequencing, nuclear gene panels, or whole-exome sequencing for diagnostic purposes in mitochondrial disease patients in clinical practice.<sup>38,45,50–57</sup>

### Consensus recommendations for DNA testing

1. Massively parallel sequencing/NGS of the mtDNA genome is the preferred methodology when testing mtDNA and should be performed in cases of suspected mitochondrial disease instead of testing for a limited number of pathogenic point mutations.
2. Patients with a strong likelihood of mitochondrial disease because of a mtDNA mutation and negative testing in blood, should have mtDNA assessed in another tissue to avoid the possibility of missing tissue-specific mutations or low levels of heteroplasmy in blood; tissue-based testing also helps assess the risk of other organ involvement and heterogeneity in family members and to guide genetic counseling.
3. Heteroplasmy analysis in urine can selectively be more informative and accurate than testing in blood alone, especially in cases of MELAS due to the common m.3243A>G mutation.
4. mtDNA deletion and duplication testing should be performed in cases of suspected mitochondrial disease via NGS of the mtDNA genome, especially in all patients undergoing a diagnostic tissue biopsy.
  - a. If a single small deletion is identified using polymerase chain reaction–based analysis, then one should be cautious in associating these findings with a primary mitochondrial disorder.
  - b. When multiple mtDNA deletions are noted, sequencing of nuclear genes involved in mtDNA biosynthesis is recommended.
5. When a tissue specimen is obtained for mitochondrial studies, mtDNA content (copy number) testing via real-time quantitative polymerase chain reaction should strongly be considered for mtDNA depletion analysis because mtDNA depletion may not be detected in blood.
  - a. mtDNA proliferation is a nonspecific compensatory finding that can be seen in primary mitochondrial disease, secondary mitochondrial dysfunction, myopathy, hypotonia, and as a by-product of regular, intense exercise.

- When considering nuclear gene testing in patients with likely primary mitochondrial disease, NGS methodologies providing complete coverage of known mitochondrial disease genes is preferred. Single-gene testing should usually be avoided because mutations in different genes can produce the same phenotype. If no known mutation is identified via known NGS gene panels, then whole-exome sequencing should be considered.

### Pathology and biochemical testing of tissue

**Pathology.** A tissue biopsy, typically muscle, has often been thought of as the gold standard for mitochondrial diagnosis, although the test is affected by concerns of limited sensitivity and specificity. Tissue is typically sent for a variety of histological, biochemical, and genetic studies. With newer molecular testing, there is less of a need to rely primarily on biochemical testing of tissue for diagnosis, although selectively testing tissue remains a very informative procedure, especially for a clinically heterogeneous condition such as mitochondrial disease. Tissue testing allows for detection of mtDNA mutations with tissue specificity or low-level heteroplasmy and quantification of mtDNA content (copy number), directs appropriate molecular studies ensuring that genes of highest interest are covered, and helps validate the pathogenicity of variants of unknown significance found in molecular tests. In patients with a myopathy, certain other neuromuscular diseases can be excluded by a muscle biopsy.

There is debate about whether patients need an open biopsy to preserve histology and perform all the necessary testing. A few centers are experienced in performing needle biopsies for mitochondrial testing. Because of potential injury to the mitochondria and the risk of artifactual abnormalities, open mitochondrial tissue biopsies require a different technique than routine biopsies that a surgeon may perform; these considerations are reviewed in [Table 1](#).

Muscle histology routinely includes hematoxylin and eosin (H&E), Gomori trichrome (for ragged red fibers), SDH (for SDH-rich or ragged blue fibers), NADH-TR (NADH-tetrazolium reductase), COX (for COX negative fibers), and combined SDH/COX staining (especially good for COX intermediate fibers). Other standard stains per the institution's pathology department should be routinely utilized to explore

other myopathies in the differential that can be diagnosed (i.e., glycogen, lipid staining).<sup>58</sup> Electron microscopy (EM) examines the mitochondria for inclusions and ultrastructural abnormalities. Pediatric patients are less likely to have histopathological abnormalities, and irregularities may only be noted on muscle EM, although normal results can also be seen.

Hepatic dysfunction due to mitochondrial disease is mostly seen in pediatric patients. A liver biopsy can show selective histologic and ultrastructural features of mitochondrial hepatopathy, such as steatosis, cholestasis, disrupted architecture, and cytoplasmic crowding by atypical mitochondria with swollen cristae. Ultrastructural evaluation should be performed routinely in unexplained cholestasis, especially when accompanied by steatosis and hepatocyte hypereosinophilia.<sup>59,60</sup>

### Consensus recommendations for pathology testing

- Muscle (and/or liver) biopsies should be performed in the routine analysis for mitochondrial disease when the diagnosis cannot be confirmed with DNA testing.
- When performing a muscle biopsy, an open biopsy is preferred in the routine analysis for mitochondrial disease, except when the center performing the biopsy is experienced in obtaining an adequate quality and quantity of tissue via a percutaneous biopsy.
- The vastus lateralis is the preferred site for a muscle biopsy in the evaluation of mitochondrial disease due to this site having been used by most laboratories to establish reference ranges.
- COX, SDH, NADH-TR, and the combined SDH/COX stain along with EM should be obtained in the routine analysis of tissue for mitochondrial disease; EM is strongly recommended in pediatric patients receiving a tissue biopsy because histological findings are often limited.
- Mitochondrial hepatopathy may have characteristic findings on liver biopsy histology.
- When possible, extra tissue should be frozen to allow for additional testing. See [Table 1](#) for special considerations.

**Biochemical testing in tissue.** Functional *in vitro* assays in tissue (typically muscle) have been the mainstay of diagnosis of mitochondrial disorders, especially prior to the recent advances in genomics. Functional assays remain important

**Table 1** Tissue collection and processing instructions for mitochondrial tissue biopsies

- The vastus lateralis muscle should typically be biopsied because most laboratories standardize their assays to results from this muscle tissue
- Avoid muscles that have experienced electromyogram manipulation or severe wasting
- Avoid infiltrating the muscle with lidocaine or use of isometric muscle clamps
- Never use electrocautery in the biopsy procedure until after the specimen has been removed
- Muscle should not be collected in fragments or from subfascial and myotendinous areas
- Similar cautions need to be followed when liver samples are obtained
- Tissue obtained during a biopsy should be quickly snap-frozen in liquid nitrogen, and a piece should be fixed in glutaraldehyde for electron microscopy; another specimen is needed if it is being used for polarographic evaluation
- Proper storage of specimens along with close coordination between pathology and the practitioner collecting the sample are essential to avoid degradation of biochemical enzyme activity

measures of mitochondrial function. All of the mitochondrial disease guidelines and diagnostic criteria developed prior to the recent advances in genetic techniques and understanding include results of such biochemical studies to help establish a mitochondrial disease diagnosis.<sup>61–65</sup>

These tests evaluate the various functions of the mitochondrial ETC or respiratory chain. Functional assays include enzyme activities of the individual components of the ETC, measurements of the activity of components, blue-native gel electrophoresis, measurement of the presence of various protein components within complexes and supercomplexes (achieved via western blots and gel electrophoresis), and consumption of oxygen using various substrates and inhibitors. When possible, it is best to perform ETC assays on the tissue(s) that are most affected (i.e., muscle in exercise intolerance, heart in cardiomyopathy, liver in hepatopathy). There are several points worth noting regarding limitations of biochemical studies in tissue (**Table 2**).

Defects in the synthesis of coenzyme Q<sub>10</sub> (CoQ<sub>10</sub>) lead to a variety of potentially treatable mitochondrial diseases. CoQ<sub>10</sub> levels can be measured directly in muscle, lymphocytes, and fibroblasts, although it is thought that the levels obtained in muscle are most sensitive for diagnosing primary CoQ<sub>10</sub> deficiency. Low CoQ<sub>10</sub> levels can be found as a secondary defect in other disorders.<sup>66–71</sup>

To avoid invasive testing such as an open muscle biopsy, noninvasive mechanisms have been evaluated to diagnosis ETC abnormalities. A small study showed that buccal swab analysis of complex I (20/26) and complex IV (7/7) had an 80% correlation with muscle ETC analysis, but the method needs further validation.<sup>72</sup> Cultured fibroblast ETC activities are also sometimes used in the diagnosis of mitochondrial disease. These results can be normal even when muscle or liver ETC abnormalities are found.<sup>73–78</sup>

### Consensus recommendations for biochemical testing in tissue

1. Biochemical testing in tissue does not always differentiate between primary mitochondrial disease and secondary mitochondrial dysfunction.
2. When obtaining a biopsy in the evaluation of mitochondrial disease, ETC enzymology (spectrophotometry) of complex I–IV activities in snap-frozen tissue or freshly isolated mitochondria should be obtained. The affected tissue should be biopsied when possible. The analysis of isolated complex III should be performed when possible because analysis of complex II/III and I/III alone may not be sufficient.
3. ETC results should be interpreted with the use of internal controls (within an assay) and normalized to marker enzymes (such as citrate synthase and/or complex II) to increase the diagnostic reliability of the results.
4. Fresh tissue analysis can allow functional oxidative phosphorylation/oxytetric measurements of oxygen consumption and adenosine triphosphate production of all five ETC complexes, and it can be sufficient to diagnose mitochondrial dysfunction. These tests are not available in all centers; therefore, they are not considered essential but should be considered in the diagnosis of mitochondrial disease.
5. In some centers, various techniques of evaluating isolated mitochondria, permeabilized myofibers, immunoblot assays, and radiolabeled assays may enhance detection of ETC abnormalities. However, as stand-alone tests they need validation.
6. When interpreting ETC results, one should use published diagnostic criteria. One should cautiously interpret the relevance of ETC enzyme activity above 20% of the control mean. Caution should also be used in providing a primary mitochondrial disease diagnosis based on biochemical abnormalities from tissue testing alone.
7. The findings of significantly reduced ETC components or reduced enzyme activity from isolated components can give supplementary information in evaluating a patient with possible mitochondrial disease.
8. Tissue analysis of ETC complex enzyme activities may be falsely normal depending on a variety of factors, including the timing of the assay and use of less affected tissue. Therefore, ETC findings should not be used as the sole criterion for excluding mitochondrial dysfunction.
9. Muscle CoQ<sub>10</sub> levels are necessary to determine primary CoQ<sub>10</sub> synthesis defects, especially when genetic studies are not diagnostic. Leukocyte CoQ<sub>10</sub> levels are inadequate to determine primary CoQ<sub>10</sub> synthesis disorders. Reduced CoQ<sub>10</sub> levels in muscle can be seen in other conditions.

**Table 2** Points to consider regarding mitochondrial biochemical testing in tissue

- Assays of spectrophotometric quantification of OXPHOS enzyme activities differ across various laboratories and make interlaboratory comparison of test results highly variable<sup>159,160</sup>
- The use of simultaneous control samples may help validate most ETC test results in muscle<sup>160</sup>
- There is little to no margin between patient ranges and control ranges<sup>161,162</sup>
- Enzyme activities around the lowest reference value cannot always be “absolutely” normal or abnormal
- Electron transport enzyme activity measures are secondarily affected by many factors, with physical inactivity being relevant to most mitochondrial patients<sup>163</sup>
- ETC analysis may be completely normal in any tissue tested early in the disease process, especially in mitochondrial DNA depletion and deletion syndromes<sup>164</sup>
- Interpretation of mitochondrial biochemical testing results is aided by utilizing established diagnostic criteria to avoid mitochondrial dysfunction being identified in a subjective fashion and interphysician variability in diagnoses provided<sup>2</sup>

ETC, electron transport chain.

10. Fibroblast ETC assays can help identify mitochondrial dysfunction in some cases, although testing can lead to false-negative results.
11. Buccal swab analysis should not be a first line for mitochondrial testing; additional comparisons of buccal swab ETC results with muscle ETC activity and genetically confirmed patients are needed.

### Neuroimaging

Neuroimaging in the form of computed tomography and magnetic resonance imaging of the brain have been used to assist in the diagnosis of mitochondrial disorders. Some diagnostic criteria protocols include neuroimaging<sup>64,65</sup> but some do not.<sup>62,63</sup>

Depending on the type of mitochondrial disorder and type of central nervous system involvement, neuroimaging may or may not show structural alterations. Stroke-like lesions in a nonvascular distribution, diffuse white matter disease, and bilateral involvement of deep gray matter nuclei in the basal ganglia, mid-brain, or brainstem are all known classic findings in syndromic mitochondrial disease.<sup>79–88</sup> These “classical” changes are selectively also observed in nonsyndromic mitochondrial diseases<sup>89–91</sup> and other metabolic disorders as well. Thus, they are neither sensitive nor specific enough to allow for a primary mitochondrial disease diagnosis without the presence of other abnormalities. Certain mitochondrial disorders such as KSS<sup>92,93</sup> and myoclonic epilepsy with ragged red fibers (MERRF)<sup>94,95</sup> are known to also have other neuroimaging abnormalities such as nonspecific white matter lesions; these findings are not sensitive enough to be considered part of the syndrome’s diagnostic criteria. More florid white matter abnormalities are seen in mitochondrial neurogastrointestinal encephalopathy syndrome (MNGIE), Leigh syndrome, and mitochondrial disorders due to defects in the aminoacyl-tRNA synthetases.<sup>96–98</sup>

In addition to qualitative changes, there are quantitative changes that can be seen on specific acquisition sequences, proton magnetic resonance imaging (MRS), and diffusion tensor imaging. MRS provides a semiquantitative estimate of brain metabolites, including lactate, creatine, and *N*-acetyl aspartate in a single- or multi-voxel distribution. Diffusion tensor imaging detects and quantifies major white matter tracts. MRS and diffusion tensor imaging changes may be found in classic mitochondrial syndromes as well as nonsyndromic patients, but they are not specific to mitochondrial disorders and can be seen in a variety of other metabolic or other brain parenchymal disorders.

### Consensus recommendations for neuroimaging

1. When the central nervous system is involved, brain magnetic resonance imaging should be performed in the evaluation of a patient suspected of having a mitochondrial disease. MRS findings of elevated lactate within brain parenchyma are useful as well. Neuroimaging cannot by itself be the absolute criterion for disease confirmation.

2. Neuroimaging can be useful in following the progression of mitochondrial neurologic disease.
3. Further research is needed regarding the role of MRS and diffusion tensor imaging in helping follow the course of mitochondrial disease.

## TREATMENT AND PREVENTIVE CARE

### Treatment of acute stroke

Stroke-like episodes are a cardinal feature of several mitochondrial syndromes, including MELAS syndrome. There is early evidence to show a therapeutic role of L-arginine and citrulline therapy for MELAS-related stroke. Arginine and citrulline are nitric oxide (NO) precursors. Endothelial dysfunction due to reduced NO production and disrupted smooth muscle relaxation may cause deficient cerebral blood flow and stroke-like episodes in MELAS syndrome.<sup>99–101</sup> Subjects with MELAS syndrome exhibit lower NO and NO metabolites during their stroke-like episodes with decreased citrulline flux, de novo arginine synthesis rate, and plasma arginine and citrulline concentrations.<sup>102,103</sup> It has been observed that the administration of oral and intravenous (IV) arginine in subjects with MELAS syndrome leads to an amelioration in their clinical symptoms associated with stroke-like episodes and decreased severity and frequency of these episodes.<sup>102,104</sup> There is more recent evidence to also show improvement in NO production with arginine and citrulline supplementation.<sup>105</sup> Controlled studies assessing the potential beneficial effects of arginine or citrulline supplementation, their optimum dosing, and safety ranges in stroke-like episodes of MELAS syndrome and other mitochondrial cytopathies associated with these episodes are required.<sup>103,105</sup>

### Consensus recommendations for the treatment of mitochondrial stroke

1. Stroke-like episodes in primary mitochondrial disease typically have correlating visible magnetic resonance imaging abnormalities.
2. IV arginine hydrochloride should be administered urgently in the acute setting of a stroke-like episode associated with the MELAS m.3243 A>G mutation in the *MTTL1* gene and considered in a stroke-like episode associated with other primary mitochondrial cytopathies as other etiologies are being excluded. Patients should be reassessed after 3 days of continuous IV therapy.
3. The use of daily oral arginine supplementation to prevent strokes should be considered in MELAS syndrome.
4. The role of following plasma arginine and citrulline levels and oral citrulline supplementation in the treatment of MELAS requires further research.

### Exercise

Numerous studies of animal models<sup>106,107</sup> and human patients with varying mitochondrial myopathies (both nDNA and mtDNA encoded) demonstrate the benefits of endurance exercise in mitochondrial disease. Studies of endurance training in

mitochondrial patients have shown increased mitochondrial content, antioxidant enzyme activity, muscle mitochondrial enzyme activity, maximal oxygen uptake, and increased peripheral muscle strength. Other findings include improved clinical symptoms and a decrease in resting and post-exercise blood lactate levels. Findings are sustained over time.<sup>108–115</sup>

The majority of studies report no deleterious effects to patients with mitochondrial myopathy from slowly accelerated exercise training, either resistance or endurance. In particular, there are no reports of elevated creatine kinase levels, negative heteroplasmic shifting, or increased musculoskeletal injuries in mitochondrial patients during supervised progressive exercise aimed at physiological adaptation.<sup>109,110,112,113,116</sup>

### *Consensus recommendations for exercise*

1. Exercise-induced mitochondrial biogenesis is an appropriate target to improve function in patients with mitochondrial disease.
2. Endurance exercise can increase mitochondrial enzyme activity in muscle and quality-of-life scores, and can reduce the energy cost of activities of daily living. Resistance exercise can increase muscle strength and recruitment of satellite cells in muscle fibers in mitochondrial patients.
3. A combination of progressive and resistance exercise is optimal for patients with mitochondrial disease and is thought to be safe when instituted in a supervised, progressive fashion with training beginning at a low intensity and duration.
4. Mitochondrial patients should undergo cardiac screening prior to beginning an exercise program.
5. Exercise intolerance is a real phenomenon in patients with mitochondrial disease, but a deconditioned mitochondrial patient should be encouraged to exercise. Physicians should encourage compliance with exercise programs for mitochondrial patients.
6. High-intensity interval training has been shown to induce similar mitochondrial adaptations as compared with endurance exercise in healthy and diabetic adults, but the effectiveness and safety have not been adequately studied in patients with mitochondrial disease.

### **Anesthesia**

Many patients with mitochondrial disease have generally tolerated anesthesia. More recent reports from studies of small populations of mitochondrial patients or limited outcome measures have suggested that anesthetics are generally safe.<sup>117–120</sup> There are also reports of serious and unexpected adverse events in these patients—both during and after an anesthetic exposure—including respiratory depression and white matter degeneration.<sup>121–123</sup> Thus, there remains a physician perception that these patients are vulnerable to a decompensation during these times.

Anesthetics generally work on tissues with high-energy requirements and almost every general anesthetic studied has

been shown to decrease mitochondrial function.<sup>124–130</sup> This occurs more so with the volatile anesthetics<sup>124,126,128</sup> and propofol.<sup>131,132</sup> Propofol and thiopental are typically tolerated when used in a limited fashion, such as during an infusion bolus. Susceptibility for propofol infusion syndrome has been suggested but not yet proven.<sup>133–135</sup>

Narcotics and muscle relaxants are also frequently used in the operating room. These drugs (with the possible exception of morphine) are generally tolerated because they do not seem to alter mitochondrial function.<sup>136,137</sup> However, they can create respiratory depression, and caution must be used in mitochondrial patients who may already have hypotonia, myopathy, or an altered respiratory drive. The risk of malignant hyperthermia does not seem to be increased in mitochondrial patients.

Finally, mitochondrial patients are often vulnerable to metabolic decompensation during any catabolic state. Catabolism is often initiated in these patients because of anesthesia-related fasting, hypoglycemia, vomiting, hypothermia, acidosis, and hypovolemia. Therefore, limiting preoperative fasting, providing a source of continuous energy via IV dextrose, and closely monitoring basic chemistries are important.

### *Consensus recommendations for anesthesia*

1. Patients with mitochondrial diseases are at an increased risk of anesthesia-related complications.
2. Preoperative preparation of patients with mitochondrial disease is crucial to their perioperative outcome. Patients should minimize preoperative fasting and have glucose added to their perioperative IV fluids, unless they are on a ketogenic diet or have been demonstrated to have adverse reaction to higher glucose intake.
3. Caution must be used with volatile anesthetics because mitochondrial patients may potentially be hypersensitive.
4. Caution must be used with muscle relaxants in those mitochondrial patients with a preexisting myopathy or decreased respiratory drive.
5. Mitochondrial patients may be at a higher risk for propofol infusion syndrome and propofol use should be avoided or limited to short procedures.
6. One should consider slow titration and adjustment of volatile and parenteral anesthetics to minimize hemodynamic changes in mitochondrial patients.
7. Local anesthetics are generally well-tolerated in patients with mitochondrial defects.
8. There is no clear established link between malignant hyperthermia and mitochondrial disease.

### **Treatment during illness**

Review of the literature found little evidence supporting the management of patients with mitochondrial disease in the acute setting. It is known that diseased mitochondria create more reactive oxygen species and periodically enter an energy-deficient state. Catabolic stressors require cells to generate higher amounts of energy via increased utilization of protein,



carbohydrate, and fat stores. A catabolic state is induced by physiologic stressors such as fasting, fever, illness, trauma, or surgery. Because of a lower cellular reserve, mitochondrial patients may more quickly enter a catabolic state and create more toxic metabolites and reactive oxygen species during catabolism. These cellular stresses may lead to cell injury and associated worsening of baseline symptoms or the onset of new ones. Recommendations in mitochondrial patients are based on the established management of patients with other inborn errors of metabolism during these vulnerable periods. Treatments include providing IV dextrose as an anabolic substrate, avoiding prolonged fasting, and preventing exposures to substances that impede mitochondrial function when possible.

#### *Consensus recommendations for treatment during an acute illness*

1. Specific decisions about patient management including hospitalization require clinical judgment and should be case-specific. Decisions should reflect the individual patient's presentation as well as an understanding of the etiology for the acute decompensation and the pathophysiology of the underlying mitochondrial disorder.
2. Patients with a mitochondrial disease should carry an emergency care plan that details their underlying disorder and provides management recommendations.
3. Patients with a mitochondrial disease should wear a Medic Alert bracelet.
4. Mitochondrial patients should take precautions to prevent entering catabolism, especially when exposed to medical stressors, including avoiding prolonged fasting and receiving dextrose-containing IV fluids before, during, and after procedures and surgeries. (Dextrose should not be provided or should be provided in limited in quantity as indicated by clinical status in suspected or confirmed disorders of pyruvate metabolism, if the patient is on a ketogenic diet, or the patient has had an adverse response to high glucose delivery.)
5. Evaluation of a mitochondrial patient in the acute setting should include evaluation of routine chemistries, glucose, transaminases, and lactate; all other testing is as clinically indicated, although one must keep in mind the potential for cardiac and neurologic decompensations in these patients.
6. Treatment during acute decompensation should include dextrose-containing IV fluids, stopping exposures to potentially toxic medications, and correction of any metabolic derangements. (Note: dextrose should be provided only in limited in quantity or not at all, as indicated by clinical status in suspected or confirmed disorders of pyruvate metabolism, if the patient is on a ketogenic diet, or the patient has had an adverse response to high glucose delivery.) The IV fluid rate should be based on the clinical situation. Outpatient mitochondrial therapies should be continued when possible.
7. Lipids can be used when needed in mitochondrial patients, even in the presence of secondary fatty-acid oxidation dysfunction.
8. The following medications should be avoided in patients with mitochondrial disease when possible and, if given, they should be used with caution: valproic acid; statins; metformin; high-dose acetaminophen; and selected antibiotics, including aminoglycosides, linezolid, tetracycline, azithromycin, and erythromycin.
9. Repeat neuroimaging should be considered in any mitochondrial patient with an acute change in neurologic status.

#### **Treatment with vitamins and xenobiotics**

Multiple vitamins and cofactors are used in the treatment of mitochondrial disease, although such therapies are not standardized and multiple variations of treatments exist.<sup>138-144</sup> Despite empiric rationale for the use of such vitamins or xenobiotics, few trials have explored the clinical effects of these treatments.<sup>145</sup> There is a general lack of consensus regarding which agents should be used, although most physicians prescribe CoQ<sub>10</sub>, L-carnitine, creatine,  $\alpha$ -lipoic acid (ALA), and certain B-vitamins.<sup>146</sup> A Cochrane review of mitochondrial therapies has found little evidence supporting the use of any vitamin or cofactor intervention.<sup>147</sup>

CoQ<sub>10</sub> in its various forms is the most commonly used nutritional supplement in mitochondrial disease and is an antioxidant that also has many other functions. The evidence base supporting the use of CoQ<sub>10</sub> in mitochondrial diseases other than primary CoQ<sub>10</sub> deficiency is sparse, with few placebo-controlled studies available.<sup>147,148</sup> Most of the supportive data come from either anecdotal open-label treatment or treatment in combination with other cofactors,<sup>149</sup> with minimal benefit reported with CoQ<sub>10</sub> alone.<sup>150</sup>

ALA is relatively commonly used in mitochondrial disease therapy, but no controlled clinical trial has evaluated its use as monotherapy. A single randomized study used ALA in conjunction with creatine and CoQ<sub>10</sub>.<sup>149</sup> Case reports have also described the use of ALA in conjunction with other therapies, including ubiquinone, riboflavin, creatine, and vitamin E, with some clinical benefit.<sup>151</sup>

Although B-vitamins alone or as a multivitamin tablet (e.g., vitamin B50) are commonly recommended for mitochondrial disease patients in clinical practice, no randomized trials have explored the efficacy of this treatment. Riboflavin use has also been seen to improve clinical and biochemical features in other small, open-label studies.<sup>152,153</sup> Riboflavin as part of a combination of vitamins has been used in an open-label study of 16 children who did not demonstrate clear clinical benefit.<sup>154</sup>

There have been no clinical trials exploring the use of L-carnitine for the treatment of mitochondrial disease, although L-carnitine supplementation is commonly used in this patient population. L-Carnitine supplementation has also been used for the treatment of a number of neurometabolic disorders, including organic acidemias and some fatty-acid oxidation disorders;<sup>155</sup> however, even in these conditions, no randomized,

controlled trials have been performed. L-Carnitine was used as a therapy in case studies, typically combined with other vitamins.<sup>154,156,157</sup>

Patients with mitochondrial disease, especially those with mtDNA deletion syndromes such as KSS, have potentially low CSF 5-methyltetrahydrofolate, and folinic acid supplementation is relatively common in patients with mitochondrial disease and neurologic signs or symptoms.<sup>32,158</sup> There have been no clinical trials using folinic acid for mitochondrial disease therapy.

Many other compounds were reviewed and the data can be found in the online summaries. The consensus was that further research regarding these compounds is needed.

### Consensus recommendations for vitamin and xenobiotic use

1. CoQ<sub>10</sub> should be offered to most patients with a diagnosis of mitochondrial disease and not exclusively used for primary CoQ<sub>10</sub> deficiency.
  - a. Reduced CoQ<sub>10</sub> (ubiquinol) is the most bioavailable form and, when used, dosing should be appropriately modified.
  - b. Plasma and/or leukocyte CoQ<sub>10</sub> levels are helpful in monitoring absorption and adherence to treatment. Plasma levels are more variable and less reflective of tissue levels.
2. ALA and riboflavin should be offered to mitochondrial disease patients.
3. Folinic acid should be considered in mitochondrial disease patients with central nervous system manifestations and routinely administered to those with documented CSF deficiency or with disease states known to be associated with deficiency.
4. L-Carnitine should be administered to mitochondrial disease patients when there is a documented deficiency and levels should be monitored during therapy.
5. When beginning supplement therapy, one should begin one at a time when possible, taking into account a patient's clinical status.
6. There is no evidence to suggest that one can adjust a person's diet on the basis of ETC results.
7. Goal levels for most vitamin therapy used are not yet known; it is prudent to replace deficiency states.

### SUMMARY

Evidence-based clinical protocols are the preferred method for diagnostic and medical management recommendations. For mitochondrial disease, there are insufficient data on which to base such recommendations. The Delphi process enabled our group to reach consensus-based recommendations with which to guide clinicians who evaluate and treat patients for mitochondrial disease.

This document is not intended to replace clinical judgment and cannot apply to every individual case or condition that may arise. It does not address the potential for inconsistent

interpretations between diagnostic laboratories regarding which metabolites and at what levels their changes are considered to reach clinical significance. Each clinician should consult the literature and researchers in mitochondrial disease for updated information when needed. These recommendations should be superseded by clinical trials or high-quality evidence that may develop over time. In the interim, we hope that these consensus recommendations serve to help standardize the evaluation, diagnosis, and care of patients with suspected or demonstrated mitochondrial disease.

Although mitochondrial diseases are collectively common, the incidence of each individual mitochondrial disease etiology or subtype is relatively rare. Large-scale clinical trials in patients with mitochondrial diseases remain difficult. With the recent advent of the North American Mitochondrial Disease Consortium, a sufficiently large cohort of mitochondrial disease patients is being studied to foster an improved understanding of their natural history and individual differences. Continued research is needed to better understand this important group of patients and develop ideal evidence-based clinical care protocols. The Mitochondrial Medicine Society will continue to evaluate the needs of mitochondrial patients and treating physicians and to advance education, research, and collaboration in the field.

### DISCLOSURE

G.M.E. has received unrestricted research gift funds from Edison Pharmaceuticals. M.J.F. is a consultant for Mitokyne, research collaborator for Cardero, and a research grant awardee of RAPTOR Pharmaceuticals, and is on the Scientific and Medical Advisory Board for the United Mitochondrial Disease Foundation. A.G. is a consultant for Stealth Peptides. A.L.G. is a consultant for GeneDx. S.P. conducts research for Edison Pharmaceuticals and the North American Mitochondrial Disease Consortium (NAMDC), and is on the Scientific and Medical Advisory Board for the United Mitochondrial Disease Foundation. R.S. conducts research studies for Edison Pharmaceuticals and is part of the NAMDC. F.S. works for a medical college department that owns a Mitochondrial Diagnostic Laboratory and conducts research studies for Edison and Raptor Pharmaceuticals. M.T. is president and CEO of Exerkine, which develops mitochondrial therapies, and receives speaker honoraria from Prevention Genetics and Genzyme.

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