Evaluation of the Child With Suspected Mitochondrial Liver Disease

*Jean P. Molleston, †Ronald J. Sokol, ‡Wikrom Karnsakul, §Alexander Miethke, ||Simon Horslen, ¶John C. Magee, #René Romero, **Robert H. Squires, and ††Johan L.K. Van Hove, for the Childhood Liver Disease Research Education Network (ChiLDREN)

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Mitochondrial respiratory chain defects can affect any tissue, with the most energy-dependent organs being most vulnerable (1). In general, clinical manifestations include multisystem involvement such as brain, muscle, heart, or kidney, with acute or chronic liver dysfunction, sometimes in the presence of lactic acidosis, a biomarker of limited sensitivity (2,3). Heterogeneous clinical presentations can be explained by the fact that the mitochondrial quantity and function are uniquely influenced by both nuclear and mitochondria DNA (mtDNA) or by the fact that cells in various tissues can contain different mixtures of normal and abnormal mitochondrial genomes (heteroplasmy). Most mitochondrial proteins and enzymes are coded by nuclear genes with Mendelian inheritance, whereas some respiratory chain subunits, ribosomal RNAs, and transfer RNAs are encoded by mitochondrial genes that are maternally inherited (4). Mutations, deletions, or duplications in either of these classes can cause disease, and mutations in nuclear genes that control mitochondrial DNA replication, transcription, and translation may lead to mtDNA depletion syndrome or to a translational disorder (5–7).

The respiratory chain, consisting of 5 multimeric complexes (I–V) in the mitochondrial inner membrane, generates energy as adenosine triphosphate via electron transport and oxidative phosphorylation (Fig. 1). Defects in the respiratory chain enzymes or mitochondrial membrane transport proteins result in injury to energy-dependent organs, especially brain, retina, muscle, heart, and liver (8). In addition, hepatic mitochondria oxidize fatty acids forming ketone bodies, an important source of energy for the brain in the fasting state. Fatty acid oxidation defects, an important group of primary bioenergetic defects, can present similarly with hepatopathy or encephalopathy, often with nonketotic hypoglycemia, acidosis, and hyperammonemia, and are thus included in the differential diagnosis and should be simultaneously evaluated (9).

Establishing the diagnosis of primary mitochondrial bioenergetic defects in patients with liver disease requires a high index of suspicion in specific clinical scenarios. A tiered diagnostic evaluation is useful (Table 1). Although mitochondrial hepatopathies are a heterogeneous group of disorders, there are several general laboratory investigations in blood and urine that can reveal an altered redox status suggestive of respiratory chain defects (lactate:pyruvate molar ratios and ketone body ratios). Specific laboratory tests are considered in patients with unique clinical presentations as well, and either tissue analysis or genotyping is used to identify the etiology. Other typically involved organ systems should be evaluated when mitochondrial hepatopathy is suspected (Table 2). Several important management issues should be addressed during this evaluation process. These guidelines outline the evaluation of the infant or child with suspected mitochondrial hepatopathy. Two summary tables (Tables 3 and 4) describing each genetic etiology follow. Reference clinical laboratories for the genetic tests can be found at *www.genetests.org*.

CLINICAL SCENARIOS SUGGESTING POSSIBLE MITOCHONDRIAL LIVER DISEASE

Mitochondrial liver disease can present acutely in a child with no history of hepatic dysfunction, or with chronic liver and

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From the *Section of Pediatric Gastroenterology, Hepatology, and Nutrition, Indiana University School of Medicine, Indianapolis, IN, the †Section of Pediatric Gastroenterology, Hepatology and Nutrition, University of Colorado School of Medicine, Aurora, CO, the ‡Department of Pediatric Gastroenterology and Nutrition, Johns Hopkins University School of Medicine, Baltimore, MD, the §Division of Gastroenterology, Hepatology and Nutrition, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, the ||Division of Gastroenterology and Hepatology, Seattle Children's Hospital, Seattle, WA, the ¶Section of Transplant Surgery, University of Michigan Medical School, Ann Arbor, MI, #Pediatrics, Hepatology, and Liver Transplantation, Emory University School of Medicine, Atlanta, GA, the **Division of Pediatric Gastroenterology and Hepatology, University of Pittsburgh School of Medicine, Pittsburgh, PA, and the ††Department of Pediatrics, Section of Genetics, University of Colorado, School of Medicine, Aurora, CO.

Address correspondence and reprint requests to Jean P. Molleston, MD, Riley Hospital for Children, Indianapolis, IN 46202 (e-mail: jpmolles@iupui.edu).

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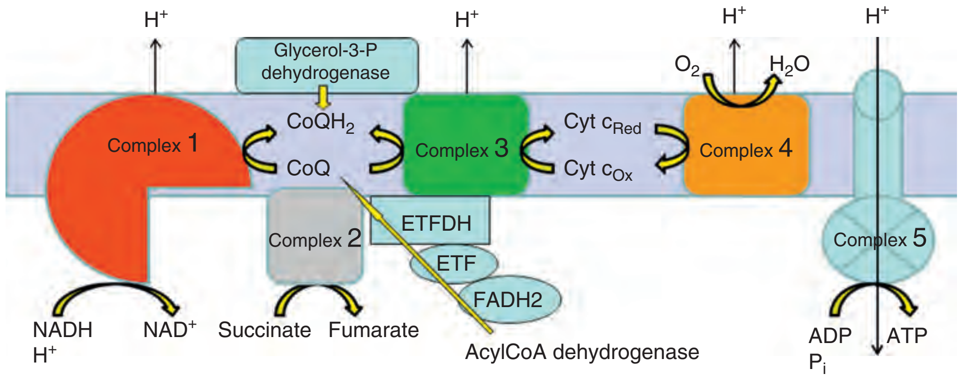

FIGURE 1. The respiratory chain, consisting of 5 multimeric complexes (I–V) in the mitochondrial inner membrane, generates energy as adenosine triphosphate (ATP) via electron transport and oxidative phosphorylation.

TABLE 1. Tiered approach to evaluation of suspected mitochondrial disease

Tier	
1	**Early screening**
	Comprehensive metabolic profile, INR, α-fetoprotein, CPK, phosphorus, complete blood cell count, and ammonia
	Lactate/pyruvate, ideally obtained 1 hour after feeding (normal molar ratio is <20, normal postprandial lactate <2.8 mmol/L)
	Serum ketone bodies: both quantitative 3-hydroxybutyrate and quantitative acetoacetate (3-hydroxybutyrate/acetoacetate ratio <4 is normal) and total free fatty acids to calculate ketone bodies: free fatty acid ratio (10)
	Serum acylcarnitine profile; serum-free and total carnitines
	Urine organic acids (look for elevated lactate, succinate, fumarate, malate, 3-methyl-glutaconic or 2-hydroxyglutaric, 2-ketoglutaric, methylmalonic acid)
	Serum amino acids (look for elevation of alanine [abnormal >500 μmol/L, but more specific if >600 μmol/L)
	Consider: Quantitative 3-methylglutaconic acid (serum or urine) (11)
	Urine acylglycines and 2-ethylmalonic quantification (if multiple acyl-CoA dehydrogenase deficiency is suspected)
	Thymidine (plasma) (especially in cases with coexistent intestinal dysmotility concerning for MNGIE syndrome)
	Coenzyme-Q levels in leukocytes, not serum (for CoQ deficiency; leukocyte levels correlate better with tissue CoQ levels, whereas serum levels reflect nutritional status)
	Quantitative serum methylmalonic acid (elevated in SUCLA and SUCLG1 deficiencies)
	CSF analysis: lactate and pyruvate (if blood lactate is normal but evidence of CNS involvement), amino acids (especially elevated CSF alanine), and protein concentration (note CSF protein can be elevated in POLG1 disease early on, even when lactate is normal)
2	**Genotyping for more common genes**
	Most common with liver involvement: *POLG1* (12,13), *DGUOK* (14–16), *MPV17* (17,18), *SUCLG1* (19), *C10ORF2/TWINKLE* (20), *TRMU* (28,29) (see Tables 3 and 4)
	If neuromuscular features suggest MELAS or pancreatic insufficiency suggests Pearson marrow/pancreas syndrome: mitochondrial DNA point mutations/deletions (21)
	If methylmalonic acid is elevated: *SUCLG1* (19)
	If acylcarnitines and/or urine organic acids suggest specific FAO defects: genotyping for LCHAD (22), CPTI and II deficiency (23,24), or (MADD = glutaric acidemia II = ETF and ETF-DH deficiency) (25), *SLC25A20* for CACT deficiency (26)
	For recurrent acute liver failure: *TRMU, ACAD9, CPTI, SUCLGI* (19,23,27,28). Identification of *TRMU* mutations is urgent in infants with acute liver failure because these patients frequently recover without the need for transplantation (28,29)
	Note: Next-generation sequencing (eg, exome sequencing) will allow for simultaneous evaluation of panels of all nuclear genes encoding mitochondrial proteins and all mitochondrial DNA genes at considerably lower cost in the future, encompassing the above gene tests, and will eventually replace single gene sequencing. It is already available in some countries
3	**Tissue evaluation**
	Liver biopsy:
	Light microscopy, electron microscopy (place specimen in glutaraldehyde); consider oil red O stain for fat on frozen section; consider iron stain if *DGUOK* or *BCS1L* are suspected (Fig. 2)
	Frozen tissue for respiratory chain enzyme activity analysis
	Frozen tissue for DNA quantification (mitochondrial DNA depletion analysis)
	Consider storing frozen tissue for future studies if amount adequate. Consider blue native PAGE gel analysis with in-gel activity staining (Fig. 3)
	Skin biopsy for fibroblast culture. Can be used for FAO enzyme activity, respiratory chain enzyme activities, blue native PAGE testing (30). FAO probe studies (especially if acylcarnitine profile is abnormal), or high-resolution respirometry (31). Note: Because of heteroplasmy of mitochondrial genes or because of differential tissue expression of nuclear genes, abnormalities in patients with mitochondrial hepatopathy can sometimes only be confirmed in liver tissue
	Muscle biopsy, especially if muscle involvement is present: light and electron microscopy. Consider histochemistry for respiratory chain complexes, respiratory chain enzyme assays, blue native PAGE analysis, mtDNA depletion analysis, mtDNA whole genome sequencing, and/or deletion analysis

(Continued)

TABLE 1. (*Continued*)

4	**Further molecular and biochemical evaluation** Additional genes to consider: *TRMU* (28) *BCS1L* (32), *SCO1* (33), *TSFM* (34), *TWINKLE* (5,20), *ACAD9* (27) (the latter especially if episodes of liver failure and fatty acid oxidation defect) (see Tables 3 and 4). Not all of these tests may be clinically available. A microarray is available to evaluate for large deletions or duplications in nuclear or mitochondrial genes (35). Note: Next-generation sequencing (eg, exome sequencing) will allow for simultaneous evaluation of panels of all nuclear genes encoding mitochondrial proteins and many or all mitochondrial DNA genes at considerably lower cost in the future, and will eventually replace single gene sequencing. At present, its efficacy is highest in clinically and biochemically well-characterized patients Targeted molecular analyses based on the results of tissue-based respiratory chain enzyme assays and primary liver disease as presentation: Isolated complex I deficiency: *ACAD9* (27) Isolated complex III deficiency: *BCS1L* (32) Isolated complex IV deficiency: *SCO1* (33) Combined complex I, III, and IV deficiency with incompletely assembled complex V bands on blue native PAGE (this signifies a generic defect in the processing of mtDNA encoded subunits (36)). It can be caused by a deficiency of mtDNA (mtDNA depletion syndromes) or by a defect in transcription or translation If mtDNA content is <5% of normal in liver: mtDNA depletion syndrome: *POLG1, POLG2, TWINKLE, DGUOK*, and *TYMP* (37) With normal or mildly decreased mtDNA but with multiple deletions (assay with long-range PCR or with next-generation mtDNA analysis): same as above, but more often with *POLG* or *TWINKLE* or *TYMP* With normal or (more common) elevated mtDNA: translation defects such as *EF-Tu, EGF-1, TSFM, TRMU, FARS2* (and other tRNA synthase genes, tRNA modification genes, ribosomal genes, translation initiation, elongation and termination factors) (28,34,38). Consider exome or mito-exome sequencing for the many genes associated with the translational machinery Deficiency of combined complex II–III assay but normal isolated assay II and normal III indicates a likely CoQ deficiency. Obtain CoQ levels, and review for causes of CoQ deficiency (39)

CACT = carnitine-acylcarnitine translocase; CNS = central nervous system; CoA = coenzyme A; CoQ = coenzyme Q; CPK = creatine phosphokinase; CPT = carnitine palmitoyl transferase; CSF = cerebrospinal fluid; ETF = electron transport flavoprotein; FAO = fatty acid oxidation; INR = international normalized ratio; LCHAD = long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency; MADD = multiple acyl-CoA dehydrogenase deficiency; MELAS = mitochondrial myopathy encephalopathy, lactic acidosis and stroke-like episodes; MNGIE = mitochondrial neurogastrointestinal encephalopathy; PAGE = polyacrylamide gel electrophoresis; PCR = polymerase chain reaction.

central nervous system (CNS) disease. Fulminant or acute liver failure is 1 important presentation of mitochondrial disease (41). Especially in a young child or in one with preexisting or disproportionate CNS involvement, mitochondrial disease is in the differential diagnosis of acute liver failure. More important, if liver transplant is being considered, careful attention must be paid to potential extrahepatic manifestations of mitochondrial dysfunction. Another clinical presentation is chronic liver disease, manifested by elevated aminotransferases, hepatomegaly, cholestasis, cirrhosis, and especially steatohepatitis; these may be accompanied by other indicators of mitochondrial disease, including hypoglycemia or lactic acidosis. Third, liver disease accompanied by chronic neuromuscular disease or disease in other organ systems may be a sign of mitochondrial disease.

TIERED DIAGNOSTIC EVALUATION

A wide array of tests that are useful in establishing the diagnosis of mitochondrial hepatopathies is available. These tests range from simple, inexpensive, easily available screening tests to extremely expensive, widely ranging genetic studies. In the child who is suspected of having a mitochondrial disease, a tiered approach to diagnostic testing is recommended. Early screening tests (tier 1) may provide clues to abnormalities in energy metabolism, and results of these tests may guide subsequent confirmatory testing to establish a molecular diagnosis. Genotyping is available clinically for the more common mitochondrial diseases (tier 2); the clinical scenario or results of screening tests can inform the choice of genetic tests. For example, a panel screening for specific gene mutations in *DGUOK*, *POLG1*, and *MPV17* responsible for infantile liver failure with lactic acidosis and mitochondrial DNA depletion is readily available and may be useful early in evaluation (see Table 3). The diagnostic role of next-generation sequencing (NGS), which is now allowing sequencing of >100 genes involved in mitochondrial diseases with a single blood test and at relatively low cost (42), or even whole exome or genome sequencing, will become increasingly important and will eventually replace genotyping single genes or small panels of genes in tier 2; however, the identification of multiple gene variants of uncertain significance will require detailed clinical and biochemical confirmation for interpretation. Tissue may also be needed to make a specific biochemical diagnosis, particularly if the liver is the major or sole affected organ (tier 3; Fig. 2). Occasionally, diagnostic findings will only be revealed in liver tissue rather than in blood, muscle, or skin fibroblasts. When further clarification is needed, genotyping for less common disease-causing genes may be required (tier 4); however, the use of NGS earlier on in the evaluation process in the future (in tier 2) may obviate the need for this step in the evaluation paradigm. At present, the diagnostic yield of NGS of all mitochondrial genes is high in patients with well-characterized mitochondrial disease, in particular with biochemical evidence of mitochondrial enzymatic dysfunction (42), but is low in patients with only a clinical suspicion (43). Biochemical studies evaluating the structure and function of mitochondrial subunits in the affected tissue can be performed as needed,

TABLE 2. Evaluation for disease in other organ systems

Organ system	Testing
Brain	Neurologic examination, MR/MR spectroscopy, EEG, CSF
Heart	EKG
Muscle	Muscle biopsy
Eyes	Detailed ophthalmologic examination
Kidney	Electrolytes, serum and urine phosphorus and creatinine, urine amino acids, urinalysis, urine protein
Endocrine	HbA1c, morning cortisol
Pancreas (exocrine)	Fecal pancreatic elastase, endoscopic pancreatic stimulation test
Hearing	Hearing testing

CSF = cerebrospinal fluid; EEG = electroencephalogram; EKG = electrocardiogram; HbA1c = hemoglobin A1c; MR = magnetic resonance.

TABLE 3. Genetic etiologies of mitochondrial hepatopathies presenting in neonates or infants

Mutation/ syndrome	Defect	Onset/age at presentation	Hepatic presentation	Neurological features	Other features	Diagnostic tests
DGUOK (14–16)	mtDNA depletion complex I, III, IV	Acute/early neonatal	Neonatal liver failure, progressive, cholestasis, neonatal hemochromatosis, hepatocellular carcinoma risk, can have isolated liver disease	Hypotonia, developmental regression, nystagmus	Lactic acidosis, hypoglycemia	*DGUOK* sequence analysis
POLG (12,13)	mtDNA depletion complex I, III, IV	Acute/neonatal	Neonatal liver failure, micro- or macrovesicular steatosis, cirrhosis	Encephalopathy, seizures, myopathy, neuropathy, blindness, developmental regression	Vomiting, GERD	*POLG* sequence analysis or panel
MPV17/Navajo neurohepatopathy (17,18)	mtDNA depletion complex I, III, IV	Acute/neonatal	Isolated neonatal/infant liver failure or in multisystem syndrome	Sensorimotor neuropathy, progressive CNS white matter lesions	Acidosis, FTT, corneal anesthesia/abrasions, acral mutilation	*MPV 17* sequence analysis or panel
TWINKLE (PEO1) (C10ORF2) (5,20)	mtDNA depletion DNA helicase	Acute/neonatal	Neonatal liver failure, cirrhosis, elevated liver enzymes	Encephalopathy (athetosis, ataxia, seizures), sensory neuropathy, deafness		*C10ORF2 TWINKLE* sequence analysis
TRMU (28,29)	Decreased mitotranslation (tRNA-modifying enzyme) complex I, III, IV	Acute/neonatal	Neonatal liver failure, some recover, possibly with cirrhosis			*TRMU* sequence analysis
TSFM, EFG1, EF-Tu, MRPS16 (34)	Decreased mitotranslation (elongation)	Acute/neonatal	Liver dysfunction in infancy, hepatomegaly	Hypotonia, dystonia	Hypertrophic cardiomyopathy, tubulopathy	Sequencing individual genes, eg, *TSFM* or exome sequencing
SUCLG1 (19)	mtDNA depletion abnormal succinate synthesis complex I, III, IV	Acute/neonatal	Neonatal liver failure, episodic liver failure	Hypotonia/myopathy (progressive), hearing loss	Acidosis, elevated methylmalonic acid	*SUCLG1* sequence analysis
BCS1L (32)	Complex III assembly deficiency	Acute/neonatal	Neonatal liver failure, cholestasis, hepatic iron overload		Growth failure, amino-aciduria, lactic acidosis, early death (GRACILE syndrome)	*BCS1L* sequence analysis
SCO1 (33)	Complex IV deficiency	Acute/neonatal	Neonatal liver failure, hepatomegaly	Hypotonia	Acidosis	*SCO1* sequence analysis
FARS2 (38)	Phenylalanyl tRNA synthetase	Acute/neonatal	Neonatal liver failure (Alper-like)	Intractable seizures, encephalopathy		*FARS2* sequence analysis on Nexgen
SLC25A20 (26)	Carnitine acylcarnitine translocase deficiency (carnitine, FAO)	Acute/neonatal	Neonatal liver failure, steatohepatitis	Myopathy	Hypoglycemia, cardiomyopathy	*SLC25A20* sequence analysis
HADHA/LCHAD or trifunctional protein deficiency (22)	FAO defect	Acute	Hepatomegaly, fatty liver, elevated LFTs, cholestasis, liver failure, ALF	Encephalopathy, peripheral neuropathy	Acidosis, hypoglycemia, pigmentary retinopathy	HADHA sequence analysis
CPT I deficiency (23)	Carnitine cycle FAO defect	Acute/infancy	Hepatomegaly, liver failure episodes	Reye-like episodes of encephalopathy	Hypoketotic hypoglycemia	*CPTIA* sequence analysis
CPT II deficiency (23,24)	Carnitine cycle FAO defect	Acute/severe neonatal	Liver failure in infancy	Seizures	Hypoketotic hypoglycemia, cardiomyopathy, myopathy	*CPT2* sequence analysis

ALF = acute liver failure; CNS = central nervous system; CoA = coenzyme A; CPT = carnitine palmitoyltransferase; FAO = fatty acid oxidation; FTT = failure to thrive; GERD = gastroesophageal reflux disease; HADHA = hydroxyacyl-CoA deHase/3-ketoacyl-CoA thiolase/enoyl-CoA hydratase alpha subunit; LCHAD = long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency; LFTs = liver function tests.

TABLE 4. Genetic etiologies of mitochondrial hepatopathies presenting as chronic liver disease or with later onset

Mutation/ syndrome	Defect	Onset/age at presentation	Hepatic presentation	Neurological features	Other features	Diagnostic tests
DGUOK (15)	mtDNA depletion complex I, III, IV	Late presentation	Progressive cholestasis, may have iron overload, HCC risk	Hypotonia, developmental regression, nystagmus	Lactic acidosis	*DGUOK* sequence analysis
MPV17/Navajo neurohepatopathy (18)	mtDNA depletion complex I, III, IV	Chronic/neonatal to childhood	Progressive liver disease or in multisystem syndrome	Sensorimotor neuropathy, progressive CNS white matter lesions	Acidosis, FTT, corneal anesthesia, abrasions, acral mutilation	*MPV 17* sequence analysis
POLG/Alper disease (12,13)	mtDNA depletion complex I, III, IV deficiency	Subacute: toddlers, young adults	Later-onset liver failure, macro- or microvesicular steatosis, cirrhosis	Intractable seizures and developmental regression, blindness, neuropathy, ataxia	Vomiting, GERD	*POLG* sequence analysis
TWINKLE (*C10ORF2*) (20)	mtDNA depletion DNA helicase	Chronic	Neonatal liver failure, cirrhosis, elevated liver enzymes	Encephalopathy (athetosis, ataxia, seizures), sensory neuropathy, deafness		*C10ORF2 TWINKLE* sequence analysis
TRMU (28)	Decreased mitotranslation (tRNA-modifying enzyme)	Chronic	Chronic liver disease/cirrhosis, recurrent acute liver failure			*TRMU* sequence analysis
SUCLG (19)	mtDNA depletion, abnormal succinate synthesis (ATP generation)	Chronic or episodic	Episodes of liver failure	Hypotonia/myopathy (progressive), hearing loss	Acidosis	*SUCLG1* sequence analysis
ACAD9 (27)	FAO defect, complex I assembly	Episodic	Episode of liver failure			*ACAD9* sequencing and deletion analysis
TYMP/MNGIES (37)	Thymidine phosphorylase deficiency, complex IV	Chronic	Liver dysfunction, macrovesicular steatosis, cirrhosis	Leukoencephalopathy, ophthalmoplegia, ptosis, peripheral neuropathy, hearing loss	Pseudo-obstruction, GERD	Plasma thymidine and deoxyuridine levels, TP enzyme activity, *TYMP* sequencing
Villous atrophy syndrome (40)	Complex III defect	Chronic/early childhood	Hepatomegaly, raised liver enzymes, steatosis	Cerebellar ataxia, sensorineural deafness, seizures	Vomiting, anorexia, chronic diarrhea (resolved later in life), villous atrophy, lactic acidosis, DM	
Pearson syndrome (21)	mtDNA deletion complex I, III, VI	Chronic/infancy	Cirrhosis with hepatomegaly, cholestasis, raised liver enzymes, progressive liver failure, death in early childhood		Refractory sideroblastic anemia, vacuolization of marrow precursors, variable neutropenia and thrombocytopenia, lactic acidosis, pancreatic insufficiency	mtDNA common mutations and deletions screening
HADHA/LCHAD or trifunctional protein deficiency (22)	FAO defect	Chronic, infancy or later	Hepatomegaly, fatty liver, elevated LFTs, cholestasis, liver failure, acute fatty liver of pregnancy	Encephalopathy, peripheral neuropathy	Acidosis, hypoglycemia, pigmentary retinopathy	HADHA sequence analysis
CPT2/CPT2 deficiency (24)	Carnitine cycle FAO defect	Infantile cardio-hepatopathy, adult myopathy	Liver failure episodes	Seizures	Hypoketotic hypoglycemia, cardiomyopathy, myopathy, rhabdomyolysis	*CPT2* sequence analysis
MADD (glutaric acidemia II) (25)	FAO defect complex II, III	Chronic, infancy up to adulthood	Hepatomegaly, steatosis	Neurologic symptoms, myopathy	Hypoglycemia, acidosis	Sequence of *ETF A*, *B*, or *ETF-DH*

ATP = adenosine triphosphate; CNS = central nervous system; CoA = coenzyme A; CPT = carnitine palmitoyl transferase; DM = diabetes mellitus; FAO = fatty acid oxidation; FTT = failure to thrive; GERD = gastroesophageal reflux disease; HADHA = hydroxyacyl-CoA deHase/3-ketoacyl-CoA thiolase/enoyl-CoA hydratase alpha subunit; HCC = hepatocellular carcinoma; LCHAD = long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency; LFTs = liver function tests; MADD = multiple acyl-CoA dehydrogenase deficiency; MNGIES = mitochondrial neurogastrointestinal encephalopathy syndrome.

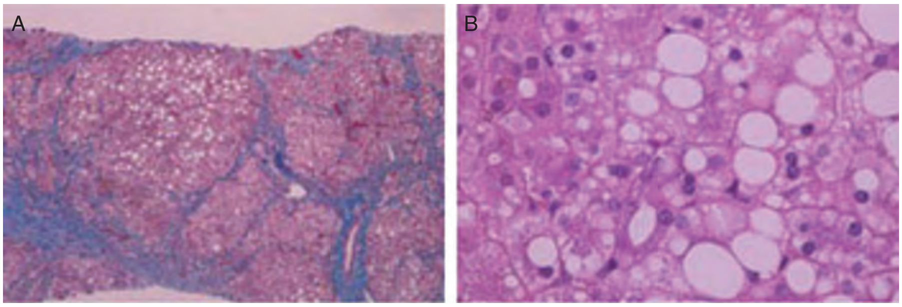

FIGURE 2. A, Micronodular cirrhosis, trichrome stain, in a child with mutations in POLG1. B, Micro- and macrovesicular steatosis, hematoxylin and eosin stain.

specifically to determine whether new genetic variants have a functional effect (Fig. 3). Thus, a combination of biochemical and molecular studies may be needed to confirm the pathologic nature of new gene variants to be described in the future. Table 1 outlines a tiered approach to diagnostic evaluation.

Tables 3 and 4 catalog known mitochondrial hepatopathies and briefly describe the mutation, defect, clinical description, and diagnostic testing. The typical hepatic presentation, ranging from hepatic failure to cholestasis to steatohepatitis to cirrhosis, is briefly outlined; neurologic symptoms and other systems involved are briefly reviewed and references are provided. The disorders are separated into those with a neonatal or an infantile presentation (Table 3) and those with later or more chronic onset (Table 4). There is, however, overlap between these 2, and new diseases and presentations are recognized frequently.

EVALUATION FOR DISEASE IN OTHER ORGAN SYSTEMS

As part of the evaluation for mitochondrial hepatopathies, a systematic approach also needs to be instituted to search for involvement of other affected organ systems (Table 2). This takes on particular significance when liver failure occurs in a child with suspected mitochondrial disease because the decision to consider liver transplantation is especially challenging. Because mitochondrial disease usually involves multiple organ systems and is generally progressive in other organs even following liver transplantation, there are many uncertainties regarding liver transplantation. Possible posttransplant appearance of new progressive symptoms in organs uninvolved before liver transplantation also needs to be considered (44–47). Establishing criteria for liver transplantation in mitochondrial hepatopathies is beyond the scope of this article; however, in the evaluation for transplantation,

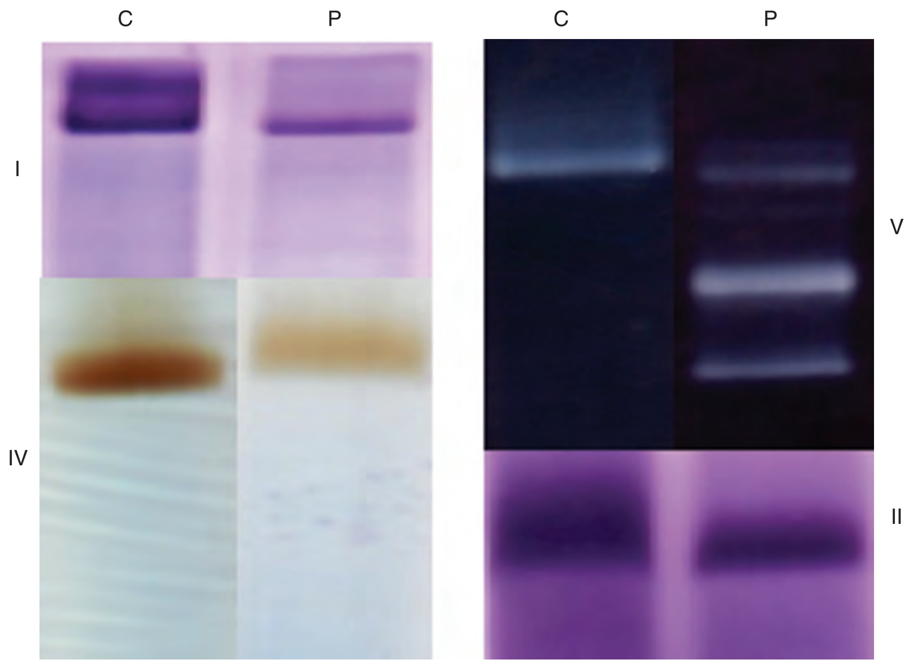

FIGURE 3. A blue native polyacrylamide gel electrophoresis gel analysis with in-gel activity staining of the respiratory chain components in the liver of a patient with DGUOK mitochondrial DNA (mtDNA) depletion disease. The added bands of lower molecular weight for complex V in the patient reflect incomplete assembly of complex V because missing mtDNA encoded subunits. The intensities of the bands for complex I and complex IV are also reduced in the patient. This profile is seen in generalized defects of the synthesis of mtDNA-encoded subunits such as in mtDNA depletion, or in defects of transcription or translation of mtDNA encoded subunits. C = control. P = patient. I, II, IV, V are the respective mitochondrial complexes.

meticulous evaluation for disease in other organ systems is paramount, especially because results of testing for specific disorders can be delayed by weeks. Evaluation of the CNS is critical. Besides a careful neurologic examination, magnetic resonance imaging of the brain is done to evaluate for Leigh disease, cerebellar atrophy, leukodystrophy, and cerebral atrophy. Magnetic resonance spectroscopy can be especially helpful, but blood lactate >3 mmol/L may affect interpretation. Evaluation can also include electroencephalography and cerebrospinal fluid examination (see above). To evaluate for cardiomyopathy, electocardiogram and echocardiogram should be done. A detailed ophthalmologic examination may reveal ophthalmoplegia in *DGUOK* deficiency, retinopathy in long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency or respiratory chain defects, corneal abrasions in *MPV17*, or optic atrophy in POLG disease. Serum electrolytes, serum and urine phosphorus and creatinine, urine amino acids, urinalysis, and urine protein are measured to evaluate renal function because abnormal tubular function may suggest a defect in *BCS1L*. Because diabetes mellitus and even adrenal insufficiency can be seen in mitochondrial disorders, HbA1c and morning cortisol level should be considered. Pancreatic insufficiency is seen in some mitochondrial diseases and may be detected by measuring fecal pancreatic elastase. Hearing screening should be performed.

MANAGEMENT DURING EVALUATION FOR POSSIBLE MITOCHONDRIAL DISEASE

The child with mitochondrial disease can be vulnerable to metabolic perturbations such as hypoglycemia or acidosis; close monitoring is important. It is important to discontinue or avoid medications that may exacerbate hepatopathy or impair mitochondrial function or mtDNA translation or transcription, including sodium valproate, tetracycline, and macrolide antibiotics, reverse transcriptase inhibitors (particularly azathioprine), chloramphenicol, quinolones, and linezolid (48). Use of Ringer lactate intravenous solution should be avoided because the liver may not be able to metabolize lactate; propofol should be avoided during anesthesia or sedated procedures because the drug can interfere with mitochondrial function (49). The goal is to maintain anabolism using a balanced intake of fat and carbohydrates with at least 75% of normal energy intake while avoiding unbalanced intakes (eg, glucose only at high intravenous rate) or fasting for >12 hours (50). In patients with preexisting lactic acidosis, lactate levels should be monitored around procedures to avoid excessive lactic acidosis.

CONCLUSIONS

Mitochondrial disease can present from infancy to adulthood with varying degrees of hepatic and extrahepatic involvement. In the last decade, there has been a rapid expansion of newly recognized mitochondrial diseases and their molecular bases. Available technology to aid in diagnosis has improved substantially. Nonetheless, diagnosis of suspected mitochondrial disease in children is complicated; a systematic clinical, biochemical, and molecular approach can aid in making a timely, accurate, and cost-effective diagnosis.

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