Mitochondrial Hepatopathies

Etiology and Genetics

The hepatocyte mitochondrion can function both as a cause and as a target of liver injury. Most mitochondrial hepatopathies involve defects in the mitochondrial respiratory chain enzyme complexes (Figure 1). Resultant dysfunction of mitochondria yields deficient oxidative phosphorylation (OXPHOS), increased generation of reactive oxygen species (ROS), accumulation of hepatocyte lipid, impairment of other metabolic pathways and activation of both apoptotic and necrotic pathways of cellular death.

Figure 1:

Since the mitochondria are under dual control of nuclear DNA and mitochondrial DNA (mtDNA), mutations in genes of both classes have been associated with inherited mitochondrial myopathies, encephalopathies, and hepatopathies. Autosomal nuclear gene defects affect a variety of mitochondrial processes such as protein assembly, mtDNA synthesis and replication (e.g., deoxyguanosine kinase [dGUOK]) and DNA polymerase gamma [POLG]), and transport of nucleotides or metals. MPV17 (function unknown) and RRM2B (encoding the cytosolic p53-inducible ribonucleotide reductase small subunit) are two genes recently identified as also
causing mtDNA depletion syndrome and liver failure, as has TWINKLE, TRMU, and SUCLG1. Most children with mitochondrial hepatopathies have identified or presumed mutations in these nuclear genes, rather than mtDNA genes.

A classification of primary mitochondrial hepatopathies involving energy metabolism is presented in Table 1. Drug interference with mtDNA replication is now recognized as a cause of acquired mtDNA depletion that can result in liver failure, lactic acidosis, and myopathy in human immunodeficiency virus infected patients and, previously, in hepatitis B virus patients treated with nucleoside reverse transcriptase inhibitors. Current estimates suggest a minimum prevalence of all mitochondrial diseases of 11.5 cases per 100,000 individuals, or 1 in 8500 of the general population.

Additional hepatopathies are related to deficiencies of enzymes involved in mitochondrial fatty acid oxidation (beta-oxidation), a pathway that activates free fatty acids to acyl-CoA esters that are then metabolized to acetyl-CoA. Fatty acid oxidation defects are caused by autosomal recessive mutations in nuclear genes encoding nearly two dozen enzymes and transporter proteins (Figure 2 and Figure 3). Many of these disorders can be identified by screening of blood spots from newborns using tandem mass spectrometry. Recent data from newborn screening programs indicate an overall incidence for all fatty acid oxidation defects of 3-5 per 1,000 births in the general population. While most of these disorders can lead to fatty liver when patients are ill, true hepatocellular disease is common in only a few of them, which will be highlighted in this document.

Table 1: Classification of Primary Mitochondrial Hepatopathies Involving Mitochondrial Energy Metabolism

- Electron transport (respiratory chain complex) defects
  - Neonatal liver failure:
    - Complex I deficiency (NADH: ubiquinone oxidoreductase) (ACAD9)
    - Complex IV deficiency (cytochrome c oxidase) (SCO1)
    - Complex III deficiency (ubiquinol: cytochrome c oxidoreductase) (BCS1L)
    - Multiple Complex deficiencies
  - Mitochondrial DNA depletion syndrome (DGUOK, MPV17, RRM2B, C100RF2/Twinkle, SUCLG1, TRMU and POLG1)
  - Delayed-onset liver failure: Alpers' disease (complex I deficiency, POLG1)
  - Pearson's marrow-pancreas syndrome (mtDNA deletion)
- Mitochondrial neuro-gastrointestinal encephalomyopathy (MNGIE) (TP mutations)
- Chronic diarrhea (villous atrophy) with hepatic involvement (complex III deficiency)
- Navajo neurohepatopathy (MPV17 mutations)

- Fatty acid oxidation and transport defects
  - Carnitine palmitoyltransferase1 and 2 deficiency (CPT1, 2; neonatal or early infancy form)
  - Very long chain acyl-CoA dehydrogenase deficiency (VLCAD; hepatic form)
  - Acyl-CoA dehydrogenase 9 deficiency (ACAD9)
  - Trifunctional protein and isolated long-chain hydroxyacyl CoA dehydrogenase (LCHAD) deficiency

![Figure 2](image-url)
Clinical Features
Defects in OXPHOS can affect any tissue, with the most energy-dependent organs being most vulnerable, resulting in heterogeneous clinical presentations. At presentation, symptoms may involve a single organ, multiple organs, or initially present with single organ involvement and involve increasing number of organs as the disease progresses over time. It is important to consider the diagnosis of a mitochondrial disorder, and potential liver involvement, in patients with progressive, multi-system symptoms that cannot be explained by a known diagnosis. Clinical suspicion for possible mitochondrial hepatopathy should be maintained in 3 clinical scenarios: acute liver failure particularly in young infants or in those with preexisting neurologic or other chronic organ dysfunction; chronic liver disease of multiple forms especially in the setting of steatohepatitis, lactic acidosis or hypoglycemia; and lastly liver disease accompanying neuromuscular disease or dysfunction in other high energy requiring organs such as the heart or kidney. mtDNA depletion syndrome may present as infantile liver failure with lactic acidosis, jaundice, synthetic liver dysfunction, with or without neuromuscular involvement (hypotonia, developmental delay, seizures), and typically results in death in infancy from liver failure. In Alpers' disease (cerebro-hepatic degeneration), a previously normal child develops loss of developmental milestones, hypotonia, vomiting and intractable seizures concurrent with the development of hepatic steatosis and synthetic failure. The onset of liver failure is frequently precipitated by treatment with valproic acid for control of seizures. Non-specific gastrointestinal symptoms, such as
vomiting, diarrhea, constipation, failure to thrive, and abdominal pain, are commonly found in many mitochondrial disorders. Other gastrointestinal manifestations, such as chronic intestinal pseudo-obstruction and exocrine pancreatic insufficiency, are important cardinal manifestations of mitochondrial neurogastrointestinal encephalomyopathy (MNGIE) and Pearson syndrome, respectively.

Disorders of mitochondrial fatty acid oxidation can present with hypoglycemia, myopathy, cardiomyopathy, and hepatic steatosis. Acute liver failure is less common in these disorders compared to respiratory chain disorders and occurs predominantly when the patient is under physiologic stress (especially illness and fasting). Clinical symptoms for each enzyme deficiency tend to overlap and differentiation on clinical criteria is difficult. Moreover, children may be well until stressed when they exhibit acute symptoms. For these reasons laboratory investigations become key in correctly identifying the diagnosis. Mitochondrial trifunctional protein deficiency presents with the most unique clinical picture including skeletal and cardiomyopathy, peripheral neuropathy, and retinopathy with recurrent episodes of hypoglycemia and hyperammonemia. Patients with isolate LCHAD deficiency will have episodes of hypoglycemia and hyperammonemia and can show the characteristic retinopathy, but do not usually have significant myopathy or neuropathy. Hepatocellular dysfunction occurs only during acute metabolic decompensation. Patients with the severe neonatal forms of CPT1, CPT2, and VLCAD deficiency present with profound hypoglycemia +/- hyperammonemia, along with generalized hepatocellular failure. Acute fatty liver of pregnancy and maternal HELLP (hemolysis, elevated liver enzymes and low platelets) syndrome can occur in pregnant women who are carriers of fatty acid oxidation defects, especially when the fetus is affected with the disorder. The mechanism for this phenomenon is unclear. ACAD9 deficiency has been reported to present with isolated, acute, recurrent liver dysfunction or failure with intercurrent illness, or with infantile lactic acidosis.

**Pathogenesis of Liver Injury**

Hepatic steatosis, most often microvesicular, is characteristic of these disorders. Neutral lipid accumulates in the hepatocyte because of continued unbridled uptake by the hepatocyte of free fatty acids released from adipose tissue, impaired beta oxidation of the fatty acids, and increased synthesis of stored triglycerides. The liver is dependent on a rich supply of ATP for its many energy-consuming biological and chemical functions. Thus, a deficiency in production of ATP caused by reduced or abnormal respiratory chain function, deficient replication of mtDNA or loss of functional mitochondria will severely impair many important liver functions, generate oxidative stress and may cause hepatocyte apoptosis or necrosis, reducing the functional mass of hepatocytes even more. Importantly, both the extrinsic and intrinsic pathways of hepatocyte apoptosis
converge on mitochondria as the key mediators of cell death in acute liver injury. Important cell signaling events include an increase in mitochondrial permeability, release of cytochrome c from mitochondria, and subsequent activation of caspase 3 and 7. The exact mechanisms of how mitochondrial dysfunction modulates this final pathway of apoptosis is unknown for many of the inherited mitochondrial hepatopathies. In Alpers' disease, a rapid loss of functional hepatocytes may suddenly occur for unknown reasons.

Cholestasis in mitochondrial liver diseases is caused by poor function of the ATP-dependent bile salt export pump, the canalicular protein providing the main driving force for bile flow, which may exacerbate the hepatocellular injury caused by the accumulation of lipid in the hepatocyte (steatocholestasis). Highlighting the impact of mitochondrial function on BSEP activity is a recent report of a delay in PFIC 2 diagnosis as a result of a secondary mitochondrial injury. Increased reactive oxygen species (ROS) generation is also believed to be an important pathologic process in these disorders. Impaired electron transport in the respiratory chain (see Figure 1), in the face of continued delivery of reducing equivalents, results in generation of superoxide and hydrogen peroxide, oxidation of lipids and thiol proteins in the mitochondrial membrane and secondary damage to mtDNA. This situation may create a vicious cycle, interfering with production of mtDNA-encoded proteins and leading to further impairment of mitochondrial respiration and oxidative phosphorylation, increased ROS generation, and energy deficiency within the hepatocyte. In chronic, more progressive respiratory chain disorders, hepatic fibrosis and micronodular cirrhosis become an important component of the hepatic pathology.

Liver biopsy findings are variable, however certain features are uniform. In respiratory chain defects, microvesicular, and less commonly macrovesicular, hepatic steatosis may be focal or generalized (Figure 4, Figure 5 and Figure 6). Periportal inflammation is variably present. Canalicular cholestasis, portal tract bile duct plugging and bile ductular reaction (proliferation) are common findings. In some cases periportal and centrilobular fibrosis and drop out of broad bands of hepatocytes lead to an appearance of micronodular cirrhosis. Glycogen depletion is common and increased hepatocyte iron deposition may lead to confusion with a primary iron storage disorder. Electron microscopy of liver shows lipid droplets in hepatocytes, and may show either normal mitochondria, usually in increased density in hepatocytes, or swollen, polymorphic mitochondria with decreased matrix density (Figure 7).
Liver biopsy findings in fatty acid oxidation defects typically show panlobular microvesicular or macrovesicular steatosis, generally without significant inflammation, fibrosis or hepatocyte necrosis. However, in some disorders portal fibrosis or frank cirrhosis may develop (such as VLCAD and LCHAD deficiencies). Electron microscopy may show enlarged and abnormally shaped mitochondria, or a condensed matrix with widening of the intracristal spaces. These findings mimic those described in the 1970’s in Reye syndrome, many cases of which were likely undiagnosed fatty acid oxidation defects and other metabolic disorders.

**Diagnosis**

Diagnosing mitochondrial liver disease requires a high index of suspicion. Clinical scenarios that should suggest these disorders include a) association of neuromuscular symptoms with liver dysfunction, b) multi-system involvement in a patient with acute or chronic liver disease, particularly with hepatic steatosis and c) a rapidly progressive course of liver disease, particularly in the presence of lactic acidosis or ketonemia. ChiLDReN investigators recently published recommendations for a coherent approach to the investigation of liver dysfunction in children with suspected mitochondrial liver disease. A tiered approach to these disorders was recommended.

Laboratory findings in the blood and urine that indicate an altered redox status are suggestive of respiratory-chain defects. Persistent elevation of plasma lactate (>2.5 mM) with an elevated molar ratio of plasma lactate to pyruvate (L/P >20) particularly 1 hour post-prandially, and elevated ketone body ratio of beta-hydroxybutyrate to acetoacetate (>4) are highly suggestive of respiratory chain disorders. However, lactic acid and these ratios are not elevated in all patients with these diseases. Elevated ratios are indicative of an increase in reducing equivalents (excess of NADH and lack of NAD) caused by impaired transfer of electrons from NADH to oxygen as a result of disrupted OXHPOS. The L/P ratio is a reflection of the NADH to NAD balance in the cytosol, and the beta-OHB/AA ratio is a reflection of the NADH to NAD ratio within the mitochondrion. Lactate/pyruvate molar ratios in the CSF may be helpful when no elevation in plasma lactate is observed, particularly in patients with CNS involvement.

Urine gas chromatography-mass spectrometry (GC-MS) can detect elevated urinary lactate, Krebs cycle intermediates (succinate, fumarate, and malate), and at times, 3-methyl-glutaconic acid in patients with mitochondrial disorders. These urine organic acids are characteristic of but not specific to respiratory chain disorders. Methylmalonic acid can be elevated in succinyl-CoA ligase deficiency. Serum tyrosine and a-
fetoprotein are elevated in patients with DGUOK deficiency. Urine amino acids may be elevated in the presence of a proximal tubulopathy.

A plasma acylcarnitine profile is the best screening test for a mitochondrial fatty acid oxidation disorder that causes liver disease, along with characteristic urinary organic acid excretion (dicarboxylic and 3-hydroxydicarboxylic acids) detected by GC-MS of urine. This can be augmented with acylcarnitine profiling of extracts or tissue culture medium from cultured skin fibroblasts. Many of these disorders are now identified by state-wide newborn expanded genetic screening programs.

Molecular approaches to diagnosis of these disorders is increasingly common. Sequencing of all fatty acid oxidation defect genes is clinically available. Focused genetic panels for mitochondrial genes most frequently identified with acute liver failure are readily available clinically. Next generation sequencing is allowing an ever expanding number of defective mitochondrial genes to be identified with a single blood test. Unfortunately, this too is not a foolproof technique as there are frequently variants of unknown significance detected which will require additional testing, most often using clinical and biochemical phenotyping to clarify. Exome sequencing has become an effective first line approach but very often requires confirmatory functional testing.

Searching for dysfunction or abnormal histology/biochemistry of the target organ (the liver) remains an important tool for diagnosis. Definitive diagnostic tests for respiratory chain disorders include the following: quantitative measurement of enzymatic activity of respiratory chain complexes in the clinically affected tissues; histology and histochemical staining (e.g., cytochrome c oxidase) of muscle or liver; blue native gel PAGE to identify defective respiratory chain complexes; genotyping for mtDNA and nuclear DNA mutations/deletions; and testing for mtDNA depletion. The appropriate investigations should be carried out on the liver (thus the need for liver biopsy tissue) as well as more standard tissues, such as muscle, since defects can be tissue-specific. Several clinical and research laboratories in North America and Europe provide these analyses.

**Treatment**

Unfortunately, there is no ideal effective therapy for most patients with respiratory chain disorders, including those with liver failure or more slowly progressive liver disease. It is not clear that any currently available medical therapy significantly alters the course of severe disease; however, there are anecdotal reports
suggesting an improvement of neuromuscular symptoms in some patients. Several treatment strategies have been proposed (Table 2). "Mitochondrial cocktails," alleged to promote mitochondrial health, are empiric combinations of various antioxidants, vitamins, cofactors, and electron donors and acceptors. To date, no controlled trials have been performed for liver disease with any of these "mitochondrial cocktails." There is a great need for well-performed double-blind placebo-controlled randomized clinical trials with comparable groups of patients and with sufficient follow-up periods. An experimental drug, EPI-742, an alpha tocopheryl quinone analog, is undergoing testing in a number of mitochondrial disorders.

Management of mitochondrial hepatopathies also includes avoidance of drugs and conditions known to have a detrimental effect on the respiratory chain. Dietary measures, such as avoidance of fasting and high fat intake, have also been advocated. Infections can precipitate rapid metabolic deterioration, and require prompt attention and treatment and appropriate hydration and intravenous dextrose infusion. Ringer’s Lactate solution should be avoided. Many drugs interfere with mitochondrial metabolism, and should be avoided in these patients. Propofol may interfere with mitochondrial function. Seizure control should use phenobarbital with caution, since it can inhibit OXPHOS. Valproic acid should likewise be used with caution because of its effects on respiration and fatty acid metabolism. Several nonsteroidal anti-inflammatory drugs (e.g., aspirin) inhibit or uncouple OXPHOS, and may result in clinical deterioration. Aminoglycoside antibiotics must be avoided in patients with mtDNA mutations, particularly the A1555G rRNA mutation, because of the significant risk of aminoglycoside-induced ototoxicity. Other drugs to avoid include macrolide antibiotics, linezolid, quinolones, chloramphenicol and reverse transcriptase inhibitors.

Most mitochondrial beta-oxidation defects are amenable to treatment with dietary manipulation (low fat intake) and sometimes carnitine supplementation. Patients with defects in long chain fatty acid oxidation need supplementation with large amounts of medium-chain triglycerides (usually 20 to 30% of calories). Patients with severe CPT1 and CPT2 deficiencies have done more poorly, often with fatal initial presentations. Acute liver disease in trifunctional protein and isolated LCHAD, VLCAD, and ACAD9 deficiencies is a sign of acute metabolic decompensation and generally reverses with reestablishment of metabolic equilibrium, sometimes quite rapidly. Deficiency of the essential fatty acid DHA may occur in LCHAD deficiency and at least, in part, cause or exacerbate the retinopathy seen in this disorder. Reduction of 3-OH metabolites (3-hydroxyACP) with MCT oil supplementation is important in preventing retinopathy. Perhaps DHA supplementation is also of benefit.
Although the presence of significant neuromuscular or extra-hepatic involvement in respiratory chain disorders should preclude the use of liver transplantation for patients with end-stage liver disease or acute liver failure, a number of patients with respiratory chain defects isolated to the liver have successfully undergone liver transplantation with excellent long-term outcomes and no extra-hepatic disease expression. Other patients have developed progressive neurologic or other organ involvement following liver transplantation and have a much more guarded prognosis. Extra-hepatic disease, especially neuromuscular, gastrointestinal and cardiac disease, should be ruled out before liver transplantation, however, it may be difficult to differentiate clinical signs of CNS involvement by the primary disorder from secondary signs that accompany liver failure. The precise role for liver transplantation in a patient with mitochondrial disease must be individualized.

Liver transplant has not been commonly pursued in the disorders of fatty acid oxidation. In the milder forms, it appears not to be necessary and in the more severe phenotypes, children have usually not survived to transplant. Given the systemic symptoms in trifunctional protein deficiency, liver transplant is not likely to be effective and palliative care may be indicated. Rapid institution of MCT oil, or triheptanoin, or ketone body therapy rapidly reverses liver dysfunction.

**Table 2: Proposed Pharmacologic Treatments For Mitochondrial Disorders**

<table>
<thead>
<tr>
<th>Electron Acceptors and Cofactors:</th>
<th>Redox bypass of complex I; Free radical scavenger (antioxidant)</th>
<th>Adult: 60-600 mg/day; *Ped: 3 - 15 mg/kg/day</th>
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<tbody>
<tr>
<td>Coenzyme Q10</td>
<td></td>
<td></td>
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<tr>
<td>Idebenone</td>
<td></td>
<td>Adult: 90-270 mg/day; *Ped: 5 mg/kg/day</td>
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<tr>
<td>Thiamine (vitamin B1)</td>
<td>Cofactor of pyruvate dehydrogenase</td>
<td>Adult: 150-300 mg/day</td>
</tr>
<tr>
<td>Riboflavin (vitamin B2)</td>
<td>Acts as flavin precursor for complexes I and II</td>
<td>Adult: 50-200 mg/day</td>
</tr>
<tr>
<td>Vitamin/Drug</td>
<td>Mechanism</td>
<td>Dosage</td>
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<tr>
<td>Menadione (vitamin K3)</td>
<td>Bypass complex III (with vitamin C)</td>
<td>Adult: 40-160 mg/day</td>
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<tr>
<td>Antioxidants:</td>
<td></td>
<td></td>
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<tr>
<td>Vitamin E (TPGS)</td>
<td>Antioxidant</td>
<td>Adult: 400 - 800 IU/day; *Ped: 25 IU/kg/day</td>
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<tr>
<td>Ascorbic acid (vitamin C)</td>
<td>Antioxidant</td>
<td>Adult: 2 - 4 g/day</td>
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<tr>
<td>Carnitine</td>
<td>Antioxidant, conjugates organic acids</td>
<td>Adult maximum of 3 g daily in 3 or 4 divided doses; Child 50-100 mg/kg/day in 3 or 4 divided doses</td>
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<tr>
<td>Other Mechanisms:</td>
<td></td>
<td></td>
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<tr>
<td>Succinate</td>
<td>Donates electrons directly to complex II</td>
<td>Adult: 6 - 16 g/day</td>
</tr>
<tr>
<td>Carnitine</td>
<td>Replace secondary carnitine deficiency</td>
<td>Adult: up to 3 g/day; *Ped: 50 - 100 mg/kg/day</td>
</tr>
<tr>
<td>Creatine monohydrate</td>
<td>Enhances muscle phosphocreatine</td>
<td>Adult: Up to 10 g/day; *Ped: 0.1 - 0.2 g/kg/day</td>
</tr>
<tr>
<td>Dichloroacetate</td>
<td>Reduces lactic acidosis by enhancing pyruvate dehydrogenase activity</td>
<td>Adult: 25 mg/kg/day; *Ped: 25 - 50 mg/kg/day</td>
</tr>
<tr>
<td>Docohexenoic Acid (DHA)</td>
<td>Replenish deficiency</td>
<td>*Ped: 65 mg/day for wt &lt; 20 kg; 130 mg/day for wt &gt; 20 kg</td>
</tr>
</tbody>
</table>
**Outcome and Prognosis**

There is wide variation in clinical outcome and prognosis for individuals with mitochondrial diseases. If there is significant central nervous system or cardiac involvement, the patient generally shows a progressive course of neuromuscular disability with its attendant medical problems. If the liver is severely affected in an inherited disorder involving the respiratory chain, the outlook for recovery or response to current therapies is poor. Infants and young children presenting with lactic acidosis and liver failure caused by mitochondrial hepatopathies have a particularly poor prognosis and may die at a young age. There are also patients who have only minor problems and have mild, manageable symptoms of liver disease or dysfunction of other organs. Liver transplantation can be successful in a limited subgroup of patients without evidence of other major organ involvement (central nervous system, muscle, heart, small intestine or colon, and kidney) or with favorable genotypes. In particular, TRMU portends a favorable prognosis with improvement to resolution of liver dysfunction. However, following liver transplantation, there is always a possibility that extra-hepatic symptoms may develop over time and that the disease was not limited to the liver, but was more slowly progressive in the other involved organ systems. Thus, the long-term prognosis for patients undergoing a successful liver transplantation is always guarded. Fatty acid oxidation defects in general have a better prognosis than respiratory chain deficiency. Long term myopathy and cardiomyopathy may be present but progressive liver disease is uncommon. However, outcomes may be different in patients identified through newborn screening. Clearly, there is a need for a better understanding of the pathophysiology and natural history of these disorders in order to develop improved therapies.

**ChiLDReN Network studies that include patients with mitochondrial liver diseases**

The ChiLDReN Network has one study that includes patients with mitochondrial liver diseases.

The MITOHEP study is a natural history study that includes patients with mitochondrial liver diseases. A natural history study is aimed at acquiring information and data that will provide a better understanding of rare conditions. Participants will be asked to allow study personnel to obtain information from medical records and an interview, and to collect blood, urine, and tissue samples when clinically indicated, in order to understand the causes of these diseases and to improve the diagnosis and treatment of children with these diseases. All of the information obtained in these studies is confidential and no names or identifying
information are used in the study.

**MITOHEP:** A longitudinal study of mitochondrial hepatopathies.

*Eligibility:* Children and adults through age 18 years that have been diagnosed with (or are strongly suspected to have) a mitochondrial liver disease.

[ClinicalTrials.gov Study NCT01148550](https://clinicaltrials.gov/ct2/show/NCT01148550)

**Organizations or foundations that help families dealing with mitochondrial liver diseases**

The ChiLDReN Network works with numerous groups that support patients and families who are dealing with rare liver diseases. Please click here to go to that page on our website (Information for Families). You will see the list of groups and information about them.

**References**
