Genetic Testing for Mitochondrial Disorders
A Guide for Clinicians

KNOWING WHAT TO LOOK FOR   KNOWING WHERE TO LOOK   AND KNOWING WHAT IT MEANS
Mitochondrial Disorders

Introduction
Mitochondrial disorders are a group of related, clinically diverse, genetic diseases with a prevalence of 1/5,000 to 1/8,500 that result from dysfunction of the mitochondrial respiratory chain.\(^1\) They can be caused by defects in either the mitochondrial DNA (mtDNA) or in nuclear genes. The heterogeneous clinical features and genetic causes make diagnosing mitochondrial disorders challenging. An accurate diagnosis is important for patient management and genetic counseling.

Clinical Presentation
Mitochondrial disorders may affect a single organ, but many involve multiple organ systems, particularly those that are highly dependent on aerobic metabolism, such as the brain, skeletal muscle, heart, kidney, and endocrine system (see Figure 1). Patients may present at any age; however, nuclear DNA mutations generally present in childhood and mtDNA mutations are more likely to present in late childhood or in adults. Some affected individuals exhibit clinical features that fall into discrete clinical syndromes, such as Leber’s Hereditary Optic Neuropathy (LHON) and Kearns-Sayre syndrome (KSS) (see Table 1). However, often the clinical features are highly variable and non-specific and many affected persons do not fit into one particular category. Similar clinical features can be caused by different mutations in mtDNA or mutations in many different nuclear genes. Common features of mitochondrial disease may include: \(^1-4\)

Common Symptoms of Mitochondrial Disorders
- Ataxia
- Spasticity
- Chorea
- Muscle weakness
- Hypotonia
- Developmental delay/intellectual disability
- Exercise intolerance
- Diabetes mellitus
- Ptosis
- External ophthalmoplegia
- Optic atrophy
- Failure to Thrive
- Pigmentary retinopathy
- Seizures
- Dementia
- Stroke-like episodes
- Sensorineural deafness
- Migraines
- Cardiomyopathy
- Liver failure
- Recurrent vomiting
- Gastrointestinal reflux
- Delayed gastric emptying
- Chronic diarrhea or constipation
Table 1. **Clinical syndromes of mitochondrial diseases** *(adapted from Chinnery, PF. 2010)*

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Primary symptoms</th>
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<tbody>
<tr>
<td>Alpers-Huttenlocher syndrome</td>
<td>Liver failure, muscle weakness, seizures</td>
</tr>
<tr>
<td>CPEO</td>
<td>Eye muscle paralysis, droopy eyelids (both eyes)</td>
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<tr>
<td>Kearns-Sayre syndrome</td>
<td>High (&gt;1 g/L) protein levels in brain/spinal fluid, cerebellar ataxia, deafness, diabetes mellitus, dementia, hypoparathyroidism</td>
</tr>
<tr>
<td>Pearson’s syndrome</td>
<td>Pancreatic failure, sideroblastic anemia, pancytopenia</td>
</tr>
<tr>
<td>Infantile myopathy and lactic acidosis</td>
<td>Muscle weakness in first year of life, feeding and breathing difficulties</td>
</tr>
<tr>
<td>Leigh syndrome</td>
<td>Subacute relapsing encephalopathy, cerebellar and brain stem signs, infantile onset</td>
</tr>
<tr>
<td>NARP</td>
<td>Late childhood- or adult-onset peripheral neuropathy, lack of muscular coordination, pigmentary retinopathy</td>
</tr>
<tr>
<td>MELAS</td>
<td>Stroke-like episodes at younger than 40, seizures, dementia, lactic acidosis and/or ragged red fibers</td>
</tr>
<tr>
<td>MEMSA</td>
<td>Myopathy, seizures, cerebellar ataxia</td>
</tr>
<tr>
<td>MERRF</td>
<td>Muscle twitching, seizures, cerebellar ataxia, myopathy</td>
</tr>
<tr>
<td>LHON</td>
<td>Subacute bilateral painless visual failure</td>
</tr>
</tbody>
</table>

*CPEO, chronic progressive external ophthalmoplegia; NARP, neurologic weakness with ataxia and retinitis pigmentosa; MELAS, mitochondria encephalopathy with lactic acidosis and stroke-like episodes; MEMSA, myoclonic epilepsy myopathy sensory ataxia; MERRF, myoclonic epilepsy with ragged red fibers; LHON, Leber’s Hereditary Optic Neuropathy*

**Figure 1. Symptoms of Mitochondrial Disorders**
Diagnosing Mitochondrial Disorders

Diagnosing patients with mitochondrial disorders is challenging due to the varied clinical presentation, genetic heterogeneity, and frequent need for invasive testing procedures. The diagnosis is typically considered in patients with progressive disorders involving multiple organ systems and is sometimes obvious if the patient exhibits one of the “classic” syndromes with stereotypical features such as MELAS, MERRF, LHON, NARP, or KSS. If the diagnosis is not obvious, the following studies can be used to help guide the diagnostic process:

- **Family history:** especially if a maternal inheritance pattern is present.
- **Neuroimaging studies:** CT and MRI.
- **Functional studies:** brain stem dysfunction, abnormal BAERS/VERS/EEG, increased signal in the basal ganglia, delayed myelination, white matter abnormalities, cerebellar atrophy and lactate elevation on magnetic resonance spectroscopy (MRS).
- **Laboratory investigations:** lactate, pyruvate, lactate/pyruvate ratio, alanine, acylcarnitine profile and urine organic acids.*
- **Muscle, liver and/or heart biopsy:** assay of electron transport chain activity, light microscopy, and electron microscopy.*
- **Genetic testing**

*Biochemical test results for mitochondrial disorders may not be reliable or reproducible, and rarely can the underlying etiology be determined without molecular studies. Molecular genetic testing is required to make a definitive diagnosis, provide guidance on management and prognosis, and permit accurate risk counseling. For mitochondrial disorders that result from mutations in nuclear genes, molecular genetic testing can also facilitate prenatal diagnosis.

Genetics of Mitochondrial Disorders

- Approximately 1,500 gene products, encoded by both the mitochondrial and nuclear genomes, are involved in maintaining proper mitochondrial respiratory chain function.
- Each mitochondrion has multiple copies of mtDNA and there are hundreds to thousands of mitochondria per cell, dependent on the cell type.
- The mtDNA encodes for ribosomal RNAs (two genes), transfer RNAs (22 genes), and 13 proteins that are part of the respiratory chain. The other genes required for mitochondrial function are encoded in the nuclear genome. (See Figure 2)
- MtDNA mutations can arise de novo (has arisen new in that individual and was not inherited from the mother) or are maternally inherited. In most cases, mtDNA point mutations are inherited, whereas large deletions arise de novo.
- Usually, mtDNA mutations affect only a fraction of the mitochondria; the coexistence of normal and mutant mtDNA is called heteroplasmy.
- When the percentage of mutant mtDNA (mutation load) reaches a certain threshold, which varies by tissue type, age, and specific mutation, the function of that tissue may become impaired.
• The mutation load varies within and between tissues, and the manifestation of mitochondrial disease reflects tissue-specific mutation load. However, access to the relevant tissues for testing is not always possible.

• Due to the **bottle neck effect**, the inheritance of mitochondrial DNA disorders within families is difficult to predict: A mother can pass on a small proportion of mutant mtDNA, or a very high proportion.

• In certain tissues, like blood, there may be selection against some of these mutations, so that cells with normal mtDNA are selectively retained. Therefore, results of genetic testing from the blood may not accurately reflect the heteroplasmy in the relevant tissue(s).

• Mutations in mtDNA may only be identified in specific tissues, particularly those with a lower rate of cell division, such as skeletal muscle, heart, and brain.

• Disorder’s due to nuclear gene mutations that affect mitochondrial function may be inherited in an autosomal dominant, autosomal recessive, or X-linked manner, and genetic testing from blood samples accurately reflects the genetic defect in all tissues.

**Figure 2. Mitochondrial genome and respiratory chain**

The mitochondrial genome and mitochondrial respiratory chain (RC), showing nDNA-encoded subunits (blue) and mtDNA-encoded subunits (colors corresponding to the genes in the mitochondrial genome above).
Genetic Testing for Mitochondrial Disorders at GeneDx

GeneDx has developed a molecular testing approach designed to assist in diagnosing patients with suspected mitochondrial disorders. This approach includes comprehensive testing for many types of mitochondrial disorders. Testing can be performed on blood samples for all nuclear genes and on blood or tissue for mtDNA testing.

Suspected Primary Mitochondrial Disorder

Can Maternal Inheritance be Ruled Out?

No

NGS of Mitochondrial Genome and Deletion Testing

Yes

Does Patient Have Discrete Clinical Syndrome with Positive Lab Results?

No

Yes

Phenotype-specific nuclear gene panels*:

Mito140 Nuclear Gene Panel*

Repeat NGS of Mito Genome and deletion testing on muscle biopsy

Additional comprehensive testing available through GeneDx, including: GenomeDx (aCGH) and XomeDx (WES)

NGS, Next Generation Sequencing; PEO, Progressive External Ophthalmoplegia; MGA, Methylglutaconic Aciduria; mt-Enceph, Mitochondrial Encephalopathy; Pyruv. Met., Pyruvate Metabolism

*Includes sequencing and deletion/duplication.

= Panel is negative.
Testing of Mitochondrial DNA

Full Sequence Analysis and Deletion Testing of the Mitochondrial Genome:
The combination of full sequence analysis plus deletion testing is expected to identify a mitochondrial DNA mutation in approximately 40% of adults and 10-20% of pediatric patients with a primary mitochondrial disorder.\textsuperscript{1,8,9} Next generation sequencing of the mitochondrial genome can detect mtDNA mutations as low as 1.5%- 5% heteroplasmy and large deletions (2kb or larger) as low as 15% heteroplasmy. This test is expected to detect greater than 98% of known pathogenic mutations and deletions of the mitochondrial genome.

Mitochondrial Depletion/Over-Replication Analysis:
Mt-DNA depletion syndrome is a group of mitochondrial disorders characterized by a reduced amount of mitochondrial DNA in tissues. Mitochondrial over-replication can be a cellular response to mitochondrial dysfunction and is characterized by ragged red fibers in the affected muscle specimens of patients with mtDNA mutations in tRNA genes or with large-scale mtDNA deletions.\textsuperscript{11} This test is performed on tissue samples (muscle or liver), and analyzes the copy number of mtDNA per cell, normalized to nuclear DNA (nDNA) copy number by real-time PCR.

Additional tests available for analysis of mitochondrial DNA testing:
- 58 Confirmed Disease-Causing mtDNA Point Mutations and Deletion Testing
- Deletion/Duplication Testing of the Mitochondrial Genome

Testing of Nuclear DNA

Next-generation Sequence Analysis of 140 Nuclear Genes:
This panel includes sequencing and exon-level deletion/duplication testing of 140 nuclear genes important for normal mitochondrial function. The 140 genes encode structural subunits and assembly factors of the oxidative phosphorylation (OXPHOS) complexes, pyruvate dehydrogenase complex, citric acid cycle, and components involved in mitochondrial transport, mitochondrial biogenesis and maintenance, electron transport and ATP synthesis. For the purpose of differential diagnosis, this panel also includes some genes associated with secondary mitochondrial OXPHOS deficiency, such as genes involved in pyruvate metabolism; and some genes associated with diseases with features overlapping that of a primary mitochondrial disease. These genes cover more than 95% of the known nuclear gene mutations associated with primary mitochondrial disorders. Please reference www.genedx.com/mito for more information on the individual genes.
Table 2. Clinical Sensitivity of GeneDx’s Mitochondrial Disease Panels

<table>
<thead>
<tr>
<th>Mitochondrial DNA testing</th>
<th>Detection rate*</th>
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</thead>
<tbody>
<tr>
<td>Full Sequence Analysis and Deletion Testing of the Mitochondrial Genome</td>
<td>~ 30% overall; 40% adults and 10-20% pediatric patients</td>
</tr>
<tr>
<td>Mitochondrial Depletion/Over-Replication Analysis</td>
<td>Unknown</td>
</tr>
<tr>
<td>58 Confirmed Disease-Causing mtDNA Point Mutations and Deletion Testing</td>
<td>~80-85% of patients with primary mitochondrial DNA disorder</td>
</tr>
<tr>
<td>Deletion/Duplication Testing of the Mitochondrial Genome</td>
<td>All large mtDNA deletions associated with mitochondrial disease</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Nuclear DNA Testing</th>
<th>Detection rate*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Comprehensive Mitochondrial Nuclear Gene Panel</td>
<td>30 - 40% (adult) 50 - 60% (children)</td>
</tr>
<tr>
<td>Mitochondrial Encephalopathy/Leigh syndrome Panel</td>
<td>&gt;50%</td>
</tr>
<tr>
<td>Lactic Acidosis/Pyruvate Metabolism Panel</td>
<td>95% for patients with pyruvate dehydrogenase deficiency and majority of patients with lactic acidosis/acidemia</td>
</tr>
<tr>
<td>Progressive External Ophthalmoplegia (PEO)/Optic Atrophy Panel</td>
<td>~ 90% (Familial PEO) ~ 70 - 80% (Familial) and ~ 50% (Sporadic) Optic Atrophy</td>
</tr>
<tr>
<td>Mitochondrial Complex I Deficiency Nuclear Gene Panel</td>
<td>&gt;25%</td>
</tr>
<tr>
<td>Mitochondrial Complex II Deficiency/SDHA Sequence Analysis</td>
<td>Unknown</td>
</tr>
<tr>
<td>Mitochondrial Complex IV Deficiency Nuclear Gene Panel</td>
<td>Unknown</td>
</tr>
<tr>
<td>CoEnzyme Q10 Deficiency Nuclear Gene Panel</td>
<td>&gt;95%</td>
</tr>
<tr>
<td>MtDNA Depletion/Multiple Deletions Panel</td>
<td>70-90%</td>
</tr>
<tr>
<td>POLG Sequence Analysis</td>
<td>5-26%</td>
</tr>
<tr>
<td>PUS1 Sequence Analysis in Mitochondrial Myopathy, Lactic Acidosis, and Sideroblastic Anemia</td>
<td>Unknown</td>
</tr>
</tbody>
</table>

* Due to the high clinical variability and the large nature of these panels these detection rates vary based on previous clinical and genetic test results. Please see panel descriptions and our website (www.genedx.com/mito) for more information.
Each of the targeted tests to follow are composed of a subset of the relevant 140 genes included in the comprehensive nuclear gene panel. These panels include sequencing and exon-level deletion/duplication analysis of the included genes.

Additional tests available for analysis of nuclear DNA testing:

- Mitochondrial Encephalopathy/Leigh Syndrome Nuclear Gene Panel (88 genes)
- Lactic Acidosis/Pyruvate Metabolism Nuclear Gene Panel (85 genes)
- Progressive External Ophthalmoplegia (PEO)/Optic Atrophy Nuclear Gene Panel (21 genes)
- Mitochondrial Complex I Deficiency Nuclear Gene Panel (30 genes)
- Mitochondrial Complex II Deficiency/SDHA Sequence Analysis
- Mitochondrial Complex IV Deficiency Nuclear Gene Panel (18 genes)
- CoEnzyme Q10 Deficiency Nuclear Gene Panel (8 genes)
- Mitochondrial Depletion/Multiple Deletions Nuclear Gene Panel (16 genes)
- Methylglutaconic Aciduria Nuclear Gene Panel (10 genes)
- POLG Sequence Analysis
- PUS1 Sequence Analysis

Genetic Testing Results and Interpretations

Diagnostic genetic testing can be considered to confirm a clinical diagnosis in a patient with a mitochondrial disorder or in patients suspected of having a mitochondrial disorder. Testing should be performed on the symptomatic individual. The three possible outcomes of genetic testing are: positive, negative, and variant of unknown significance. All patients who undergo genetic testing should receive pre-test and post-test genetic counseling so that they can understand the implications of testing. Information about genetic counseling services can be found at www.nsgc.org.

A positive result indicates that a previously understood disease-causing mutation was identified in the individual undergoing the test. Knowledge of a disease-causing mutation can help to assess the patient’s risk of experiencing certain symptoms, and may influence decisions about how to manage the condition. A positive result may also identify certain family members as being at-risk for having the mutation, and carrier testing may be recommended to those individuals.

A negative result in an affected individual does not necessarily rule out a mitochondrial disorder, and the patient should be managed according to his/her clinical symptoms. Possible reasons for a negative result could be: (1) the patient may have a mutation in a gene not covered in the testing panel, (2) the patient may have a type of mutation which would not be detected by the test method performed, or (3) the patient does not have a mitochondrial disorder.
A variant of unknown significance (VUS) indicates that a change in the DNA was identified in the individual undergoing the test; however, the change is not known to be associated with a disorder. To clarify the clinical significance of the variant, testing other family members may be helpful. Sometimes the test results may remain uncertain until additional information is obtained from research studies.

Specimen Requirements

**Blood (preferred for nuclear DNA testing):** Adults: 8-10 mL; Children: 4-6mL; Infants: 1-2mL. Ship overnight at ambient temperature, using a cool pack in hot weather. Specimens may be refrigerated for 7 days prior to shipping. **DO NOT FREEZE BLOOD.**

**Extracted DNA:** A minimum amount of 50 micrograms of high quality DNA, with a concentration of at least 50 ng/μl (50 nanograms per microliter).

**Tissue (preferred for mt DNA testing):** Please submit 50 mg, frozen within minutes after collection, stored at -80°C and shipped on dry ice with overnight delivery.

For more information on other specimens and shipping instructions, please go to www.genedx.com/order-a-test/specimen-types.

Where can I find more information?

More information about mitochondrial disorders can be found at the following websites:

- GeneDx mitochondrial disorders page: [www.genedx.com/mito](http://www.genedx.com/mito)

- United Mitochondrial Disease Foundation, a patient organization that promotes research and education for the diagnosis, treatment, and cure of mitochondrial disorders: [www.umdf.org](http://www.umdf.org)

- Gene Reviews, a database of genetic diseases: [www.geneclinics.org](http://www.geneclinics.org)

- National Society of Genetic Counselors, an organization that can help you find a counselor near you: [www.nsgc.org](http://www.nsgc.org)
About GeneDx

GeneDx is a highly respected genetic testing company founded in 2000 by two scientists from the National Institutes of Health (NIH) to address the needs of patients and clinicians concerned with rare inherited disorders. Currently, GeneDx also offers oligonucleotide microarray-based testing for detecting chromosomal abnormalities, testing for inherited eye disorders and autism spectrum disorders, and gene panels for testing various forms of inherited cardiac disorders, mitochondrial disorders, neurological disorders and inherited cancer disorders. At GeneDx, our technical services are matched by our scientific expertise and customer support. Our growing staff includes more than 40 geneticists and genetic counselors specialized in clinical genetics, molecular genetics, metabolic genetics and cytogenetics who are just a phone call or email away. We invite you to visit our website www.genedx.com to learn more about us and the services we offer.

References