Genetic Testing for Mitochondrial Disorders at GeneDx

Next Generation Sequence Analysis of 24 Nuclear Genes Important for Normal Mitochondrial Function

Clinical features:
Mitochondrial disorders are clinically heterogeneous and result from dysfunction of the mitochondrial respiratory chain, which can be caused by mutations in mitochondrial DNA (mtDNA) or in nuclear genes. Mitochondrial disorders may affect a single organ, but many involve multiple organ systems particularly those that are highly dependent on aerobic metabolism (brain, skeletal muscle, heart, kidney and endocrine system). Patients may present at any age; however, nuclear DNA mutations generally present in childhood and mtDNA mutations generally present in late childhood or in adults. Some affected individuals exhibit clinical features that fall into a discrete clinical syndrome, such as Leber’s Hereditary Optic Neuropathy (LHON), Kearns-Sayre syndrome (KSS), chronic progressive external ophthalmoplegia (CPEO), mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes (MELAS), myoclonic epilepsy with ragged-red fibers (MERRF), neurogenic weakness with ataxia and retinitis pigmentosa (NARP) or Leigh syndrome (LS). 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 mtDNA mutations or nuclear gene mutations. Common features of mitochondrial disease may include ptosis, external ophthalmoplegia, proximal myopathy, exercise intolerance, cardiomyopathy, sensorineural deafness, optic atrophy, pigmentary retinopathy, diabetes mellitus, encephalopathy, seizures, dementia, migraine, stroke-like episodes, ataxia, spasticity, chorea and dementia. Recently, it has been estimated that approximately 7% of patients diagnosed with autism may have an underlying disorder of mitochondrial function.

The prevalence of mitochondrial disorders has been estimated 1/5000 to 1/8500.

Genetics:
Approximately 1500 gene products are involved in maintaining proper mitochondrial respiratory chain function. The mtDNA encodes for ribosomal RNAs (two genes), transfer RNAs (22 genes) and 13 proteins that are part of the respiratory chain. Other genes required for mitochondrial function are nuclear. Mutations in mtDNA arise de novo or are maternally inherited. In most cases, mtDNA point mutations are inherited, whereas gross deletions arise de novo. Each mitochondrion has multiple copies of mtDNA and there are hundreds to thousands of mitochondria per cell, dependent on the cell type. Usually, mtDNA mutations affect only a fraction of the mtDNA; the coexistence of normal and mutant mtDNA is called heteroplasmy. When the percentage of mutant mtDNA (mutation load) reaches a certain threshold that varies by tissue type, age, and specific mutation the function of that tissue may become impaired. As the mutation load varies within and between tissues, the manifestation of mitochondrial disease may reflect tissue-specific mutation load. Disorders due to nuclear gene mutations that affect mitochondrial function may be inherited in an autosomal dominant, autosomal recessive or X-linked manner.

Reasons for referral:
1. Molecular confirmation of a clinical diagnosis
2. Testing of patients suspected of having a mitochondrial disorder
3. Prenatal diagnosis for known familial mutation(s) in nuclear genes in at-risk pregnancies.
4. Genetic counseling

Diagnosing mitochondrial disorders:
The diagnostic work-up of patients suspected of having a mitochondrial disorder is challenging and complex. Mitochondrial dysfunction should be considered in any progressive multi-system disorder; however, sometimes only a single symptom is present. The diagnosis may be straightforward in those who have a recognizable phenotype; however, many patients present with a complex picture of clinical abnormalities. Biochemical testing including plasma and CSF lactic acid concentrations may be normal. In many cases, muscle biopsy is analyzed for histological and histochemical evidence of mitochondrial disease and respiratory chain complex studies are performed; however, even these may be normal or inconclusive in a patient with a mitochondrial disorder. GeneDx has developed a molecular testing approach designed to assist in diagnosing patients with suspected mitochondrial disorders. This approach includes
deletion/duplication testing (see separate info sheets), mtDNA common point mutation analysis (see separate info sheet), mtDNA depletion/over-replication analysis (see separate info sheet), and sequence analysis of the entire mitochondrial genome (see separate info sheet for the mitochondrial genome) and nuclear genes.

**Next-generation sequencing of 24 nuclear genes important for normal mitochondrial function**

Twenty-four nuclear genes important for normal mitochondrial function are analyzed using a new technology developed for high-throughput sequencing (“next-generation sequencing”) to achieve high sensitivity with high efficiency.

**Testing method for next-generation sequencing of 24 nuclear genes**

Using genomic DNA obtained from blood (2-5 mL in EDTA), approximately 222 exons of the 24 genes including their splice junctions are sequenced using a novel solid-state sequencing-by-synthesis process that allows sequencing a large number of amplicons in parallel. For analysis, DNA sequence is assembled and compared to the published genomic reference sequences. The presence of any potentially disease-associated sequence variant(s) is confirmed by conventional dideoxy DNA sequence analysis.

**Genes included in next-generation sequencing panel (organized by gene-function/role)**

The 24 nuclear genes included in this panel 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 electron transport. These genes cover more than 50% of the nuclear gene mutations associated with primary mitochondrial disorders. It is estimated that this panel would detect a disease-causing mutation in 30-40% pediatric and ~20% of adult patients with primary mitochondrial disorders. The technical sensitivity of this testing approach is estimated to be 98% for mutations identifiable by sequence analysis. Gross deletions and duplications would not be identified using this method. Please see separate 24 nuclear gene panel gene descriptions link for information on individual genes.

**Genes associated with the biogenesis and maintenance of the mitochondrial genome:** including genes involved in mtDNA replication, maintenance of mitochondrial deoxyribonucleotides pools, ATP and ADP shuttling, and the salvage pathway of deoxynucleotide synthesis in the mitochondria. Mutations in genes associated with mtDNA biogenesis and maintenance may cause mtDNA depletion syndrome (MDS). The following 12 genes cover more than 95% of known nuclear gene mutations associated with mtDNA depletion syndrome. It is estimated that this panel would detect a disease-causing mutation in >60% of patients with a mtDNA depletion syndrome. The technical sensitivity of this testing approach is estimated to be 98% for mutations identifiable by sequence analysis. Gross deletions and duplications would not be identified using this method.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Function</th>
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<tbody>
<tr>
<td>C100RF2 (Twinkle/PEO1)</td>
<td>RRM2B</td>
</tr>
<tr>
<td>DGUOK</td>
<td>SLC25A4 (ANT1)</td>
</tr>
<tr>
<td>MPV17</td>
<td>SUCLA2</td>
</tr>
<tr>
<td>OPA1</td>
<td>SUCLG1</td>
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<tr>
<td>POLG (POLG1)</td>
<td>TK2</td>
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<tr>
<td>POLG2</td>
<td>TYMP (ECGF1, TP)</td>
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</table>

**Gene involving Coenzyme Q10 (CoQ10):** CoQ10 is located in the inner mitochondrial membrane where it functions to shuttle electrons from complexes I and II to complex III in the respiratory chain. CoQ10 deficiency is a clinically heterogeneous condition that is potentially treatable with exogenous CoQ10.

**COQ2**

**Gene associated with Deafness-Dystonia-Optic Neuronopathy Syndrome (DDON):** Deafness-dystonia-optic neuronopathy (DDON) syndrome (aka Mohr-Tranebjaerg syndrome) is a rare neurodegenerative disease with early-onset deafness, dystonia and other neurological abnormalities including cortical blindness, spasticity, dementia and mental retardation. Females may have mild hearing impairment and focal dystonia. DDON syndrome occurs as either a single-gene disorder resulting from mutation in TIMM8A or a contiguous gene deletion syndrome at Xq22, which also includes X-linked agammaglobulinemia caused by disruption of the BTK gene, located telomeric to TIMM8A.

**TIMM8A (DDP1)**
**Gene involving complex III:** Complex III catalyzes electron transfer from succinate and nicotinamide adenine dinucleotide-linked dehydrogenases to cytochrome c. Complex III is made up of 11 subunits, of which all but one (cytochrome b) are encoded by nuclear DNA. Complex III deficiencies are rare and manifest heterogeneous clinical presentations.

**BCS1L**

**Genes involving complex IV:** Complex IV (cytochrome c oxidase or COX) is the terminal component of the respiratory chain catalyzing the reduction of molecular oxygen to water. COX is composed of 3 catalytic subunits encoded by mtDNA genes and ten accessory subunits encoded by nuclear genes. The accessory subunits are involved in structural stabilization, assembly of the complex and in the modulation of its catalytic activity. Patients with COX deficiency can present with a broad and heterogeneous range of clinical phenotypes. The following 7 genes cover about 90% of known nuclear mutations associated with mitochondrial complex IV deficiency.

- COX6B1
- SCO2
- COX10
- SURF1
- COX15
- TACO1
- SCO1

**Genes associated with Leigh syndrome (LS):** Leigh syndrome is an early-onset progressive neurodegenerative disorder with a characteristic neuropathology consisting of focal, bilateral lesions in one or more areas of the central nervous system, including the brainstem, thalamus, basal ganglia, cerebellum, and spinal cord. The lesions are areas of demyelination, gliosis, necrosis, spongiosis, or capillary proliferation. Clinical symptoms depend on which areas of the central nervous system are involved. The most common underlying cause of LS is a defect in oxidative phosphorylation and LS may be a feature of a deficiency in any of the mitochondrial respiratory chain complexes. The following 9 genes cover more than 50% of known nuclear mutations associated with Leigh syndrome (LS). Approximately 60-70% of Leigh syndrome is caused by nuclear gene mutations. It is estimated that this panel would detect a disease-causing mutation in 30-40% of patients with familial LS. The technical sensitivity of this testing approach is estimated to be 98% for mutations identifiable by sequence analysis. Gross deletions and duplications would not be identified using this method.

- BCS1L
- PDHA1
- COQ2
- SCO2
- COX10
- SURF1
- COX15
- TACO1
- DLD

**Genes associated with progressive external ophthalmoplegia (PEO):** Autosomal-dominant or autosomal recessive progressive external ophthalmoplegia (adPEO or arPEO) is a mitochondrial disorder that is characterized by accumulation of multiple mitochondrial DNA (mtDNA) deletions in post-mitotic tissues. The disorder is heterogeneous, with mutations in six nuclear genes associated with adPEO. These six genes encode both subunits of DNA polymerase gamma (POLG and POLG2), the DNA helicase Twinkle (C10ORF2), the adenine nucleotide translocator ANT1 (SLC25A4), optic atrophy 1 (OPA1) and ribonucleotide reductase (RRM2B). Mutations in POLG gene are more commonly associated with autosomal recessive progressive external ophthalmoplegia with multiple mtDNA deletions (arPEO). Defects in these proteins affect mtDNA maintenance, probably leading to stalled replication forks, consequent mtDNA deletion formation, and progressive respiratory chain deficiency. The following 6 genes cover more than 90% of the known nuclear mutations associated with autosomal dominant progressive external ophthalmoplegia (adPEO). It is estimated that this panel would detect a disease-causing mutation in ~80-90% of patients with familial PEO. The technical sensitivity of this testing approach is estimated to be 98% for mutations identifiable by sequence analysis. Gross deletions and duplications would not be identified using this method.

- C10ORF2 (Twinkle/PEO1)
- POLG2
- POLG (POLG1)
- RRM2B
- SLC25A4 (ANT1)

Information Sheet for mt nuclear nextgen panel  Page 3 of 4  © GeneDx  9/2010
Specimen Requirements and Shipping/Handling:
- Blood: Whole blood in EDTA; Adults: 8-10 ml; Children: 4-6 ml; Infants: 2-3 ml. Ship blood overnight at ambient temperature, using a cool pack in hot weather. Blood specimens may be refrigerated for up to 7 days prior to shipping.
- Extracted DNA is discouraged. Please call first if sending extracted DNA
- Buccal Brushes: NOT accepted for this test.
- Cultured fibroblasts NOT accepted for this test
- Prenatal Diagnosis: 10mL amniotic fluid, 5 mg CV, or 2 T25 flasks. Ship overnight at ambient temperature, using a cool pack in hot weather. Call to discuss requirements for parental blood. Keep backup cultures.

Required Forms:
- Sample Submission (Requisition) Form – complete all relevant pages
- Payment Options Form or Institutional Billing Instructions

Prices and Turn-Around Times - Fees are subject to change without notice:

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<tr>
<th>Test#</th>
<th>Description</th>
<th>CPT Codes - All codes and units apply</th>
<th>Contract Price</th>
<th>Turn Around Time</th>
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<td>24-nuclear gene panel</td>
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*Please see our website for CPT codes/prices for carrier and prenatal testing: http://www.genedx.com.

Possible ICD9 Codes:

- 277.87 Disorder of mitochondrial metabolism
- 276.2 Lactic acidosis
- 250 Diabetes
- 330.8 Leigh syndrome
- 389.10 Hearing loss, sensorineural
- 425.1 Hypertrophic cardiomyopathy