Genetic Testing for Mitochondrial Disorders at GeneDx:
MtDNA Depletion/ Multiple Deletions Nuclear Gene Panel
Sequence Analysis and Exon-Level Deletion/Duplication Testing of 16 Nuclear Genes

<table>
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<tr>
<th>Gene</th>
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<tbody>
<tr>
<td>AGK</td>
<td>MFN2</td>
<td>POLG</td>
<td>SUCLA2</td>
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<tr>
<td>APTX</td>
<td>MPV17</td>
<td>POLG2</td>
<td>SUCLG1</td>
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<td>C100RF2</td>
<td>OPA1</td>
<td>RRM2B</td>
<td>TK2</td>
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<tr>
<td>DGUOK</td>
<td>OPA3</td>
<td>SLC25A4</td>
<td>TYMP</td>
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<td>(ANT1)</td>
<td>(ECGF1, TP)</td>
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Genes associated with the biogenesis and maintenance of the mitochondrial genome include many nuclear 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) or multiple mtDNA deletions. MtDNA depletion syndrome (MDS) is characterized by profoundly decreased mitochondrial DNA copy numbers in affected tissues; three main types of clinical presentations are myopathic, encephalomyopathic and hepatocerebral. This disorder is inherited as an autosomal recessive trait and is genetically heterogeneous. Multiple deletions of mtDNA caused by mutations in nuclear genes are commonly associated with autosomal dominant or autosomal recessive progressive external ophthalmoplegia (adPEO or arPEO).

Clinical features of mitochondrial disorders:
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 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, cardiomypathy, sensorineural deafness, optic atrophy, pigmentary retinopathy, diabetes mellitus, encephalopathy, seizures, dementia, migraine, stroke-like episodes, ataxia, spasticity, chorea and dementia. It has been estimated that approximately 7% of patients diagnosed with autism may have an underlying disorder of mitochondrial function (1). The prevalence of mitochondrial disorders has been estimated 1/5000 to 1/8500. (2-4)

Genetics of mitochondrial disorders:
To date, around 120 nuclear genes have reported disease-causing mutations associated with a primary mitochondrial disorder. 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

Post-PCR Next-Generation Sequencing and Deletion/Duplication Analysis of Nuclear 16 Genes Associated with MtDNA Depletion/ Multiple Deletions

Method:
Using genomic DNA obtained from blood (2-5 mL in EDTA), the coding exons of 16 genes associated with mtDNA depletion/multiple deletions including their splice junctions are PCR amplified and sequenced using a novel solid-state sequencing-by-synthesis process that allows sequencing a large number of amplicons in parallel (5). DNA sequence is then 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. In addition, targeted array CGH analysis with exon-level resolution is performed concurrently to evaluate for a deletion or duplication of one or more exons in the genes included in the panel(s). The technical sensitivity of next-generation sequencing is estimated to be 98%. Next-generation sequencing will not detect large chromosomal aberrations and deletions, insertions, or rearrangements greater than or equal to 5 base pairs. Targeted array CGH is expected to detect most exonic deletions or duplications as small as 150-300 bp.

Advantages of Post PCR Next-Generation Sequencing Versus Capture Next-Generation Sequencing:
Pseudogenes and homologous sequences can lead to both false positive and false negative results. Approximately 25% of human genes and approximately 30% of the genes included in this panel have homologous, but non-functional pseudogenes in the genome (www.pseudogene.org). In contrast to capture next-generation sequencing (NGS), post-PCR NGS uses PCR-based sequence enrichment with unique primers that selectively amplify only the gene of interest, thus avoiding many pseudogene problems.

Test Sensitivity:
The genes in this panel account for more than 95% of known nuclear gene mutations associated with mtDNA depletion syndrome or familial PEO with multiple mtDNA deletions. It is estimated that this panel will detect a disease-causing mutation in about 69% of patients with myopathic mitochondrial DNA depletion syndrome and in more than 84% of patients with hepatocerebral mitochondrial DNA depletion syndrome (6). Approximately 90% of patients with familial PEO are expected to have mutation(s) in one of these genes (7, 8, 9).

MtDNA Depletion/Multiple Deletions Nuclear Gene Panel (16 genes)

<table>
<thead>
<tr>
<th>MtDNA Depletion</th>
<th>15 genes: AGK; APTX; C100RF2 (Twinkle, PEO1); DGUOK; MFN2; MPV17; OPA1; POLG (POLG1); POLG2; RRM2B; SLC25A4 (ANT1); SUCLA2; SUCLG1; TK2; TYMP (ECGF1, TP)</th>
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<tr>
<td>Multiple MtDNA Deletions</td>
<td>11 genes: C100RF2 (Twinkle, PEO1); MFN2; MPV17; OPA1; OPA3; POLG (POLG1); POLG2; RRM2B; SLC25A4 (ANT1); TK2; TYMP (ECGF1, TP)</td>
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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: A minimum amount of 20 micrograms of high quality DNA, with a concentration of at least 50 ng/ul (50 nanograms per microliter).
- Buccal Brushes: NOT accepted for this test.
- Cultured fibroblasts NOT accepted for this test.
- Prenatal Diagnosis (for specific known familial mutation(s) or deletion(s) only): please refer to the specimen requirements table on our website at: http://www.genedx.com/test-catalog/prenatal/. Ship specimen overnight at ambient temperature, using a cool pack in hot weather.
**Required Forms:**
- Sample Submission (Requisition) Form – complete all relevant pages
- Payment Options Form or Institutional Billing Instructions

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<th>Test#</th>
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<tr>
<td>594</td>
<td>MtDNA Depletion/Multiple Deletions Nuclear Gene Panel (sequencing and del/dup analysis for 16 genes)</td>
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**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

**References:**