Genetic Testing for Mitochondrial Disorders at GeneDx:  
Mitochondrial Encephalopathy/ Leigh Syndrome Nuclear Gene Panel  
Sequence Analysis and Exon-Level Deletion/Duplication* Testing of 88 Nuclear Genes

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*Exon-level deletion/duplication testing is not currently available for these genes

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. The clinical features of mitochondrial encephalopathy may overlap that of Leigh syndrome.

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, cardiomyopathy, sensorineural deafness, optic atrophy, pigmented 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. (2-5)

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 88 Nuclear Genes Associated with Mitochondrial Encephalopathy/Leigh Syndrome

Method:
Using genomic DNA obtained from blood (2-5 mL in EDTA), the coding exons of the 88 genes 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. (6). 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 most of 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. The 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:
Mutation(s) in about 88 genes have been reported to be associated with Leigh syndrome or mitochondrial encephalopathy. The genes included in this panel include more than 95% of known nuclear gene mutations associated with Leigh syndrome, Leigh-like syndrome, and mitochondrial encephalopathy. It is estimated that this panel would detect the disease-causing mutation(s) in more than 50% of patients with familial Leigh syndrome.
Mitochondrial Encephalopathy/ Leigh Syndrome Nuclear Gene Panel (88 genes)

Leigh (Leigh-like) Syndrome
50 genes: ADCK3 (CABC1; COQ8); AIFM1; APTX; BCS1L; C8orf38 (NDUFAF6); COQ2; COQ9; COX10; COX15; DLAT; DLD; ETFDH; FOXRED1; GFER; GFM1 (EFG1); LRPPRC; MTFMT; NDUFA1; NDUFA10; NDUFA2; NDUFA9; NDUFA12; NDUFA4 (C6orf66); NDUFA7 (C2orf56); NDUFS1; NDUFS2; NDUFS3; NDUFS4; NDUFS7; NDUFS8; NDUFS11; PC; PDHA1; PDHB; PDHX; PDP1; PDSS1; PDSS2; SCO2; SDHAF1; SERAC1; SLC19A3; SUCLA2; SURF1; TACO1; TIMM44; TPK1; TTC19; TUFM

Mitochondrial Encephalopathy
69 genes: ACAD9; ACO2; AFG3L2; APTX; ATPAF2 (ATP12); BCS1L; BOLA3; C8orf38; C10orf2; COQ2; COX10; COX14 (C12orf62); COX15; COX20 (FAM36A); COX6B1; DARS2; DGUK; DLD; DNML1; EARS2; ETH1; FARS2; FASTKD2; FH; GFM1 (EFG1); GFM2; HLCS; HSPD1; LIAS; LRPPRC; MARS2; MFN2; MPV17; MTPAP; NDUFA1; NDUFA10; NDUFA11; NDUFAF2; NDUFAF4 (C6orf66); NDUFS1; NDUFS2; NDUFS3; NDUFS4; NDUFS5; NDUFS7; NDUFS8; NDUFS11; PC; PDHA1; POLG; RARS2; RMND1; SCO1; SCO2; SDHAF1; SERAC1; SLC19A3; SUCLA2; SUCLG1; SURF1; TK2; TMEM70; TPK1; TSFM; TTC19; TUFM; TYMP (ECGF1, TP)

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

Test# Description
575 Mitochondrial Encephalopathy/ Leigh Syndrome Nuclear Gene Panel (sequencing and del/dup analysis for 88 genes)

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: