



Test Information Sheet

Genetic Testing for Mitochondrial Disorders at GeneDx

Testing for 16 Common Mitochondrial DNA(mtDNA) Point Mutations

Deletion/Duplication Testing of mtDNA

Deletion/Duplication Testing of 116 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 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 function¹. The prevalence of mitochondrial disorders has been estimated 1/5000 to 1/8500.^{2, 3, 4, 5}

Genetics:

Approximately 1500 gene products are involved in maintaining proper mitochondrial respiratory chain function.² 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*.⁶ 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.⁶ As the mutation load varies within and between tissues, the manifestation of mitochondrial disease may reflect tissue-specific mutation load.⁴ In certain tissues, like blood, there may be selection against some of these mutations, so that cells with normal mtDNA are selectively retained. 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.⁶ 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.⁶ 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, mtDNA common point mutation analysis, mtDNA depletion/over-replication analysis (*see separate info sheet*), and sequence analysis of the entire mitochondrial genome and nuclear genes (*see separate info sheets*).

Deletion/Duplication and Mitochondrial DNA Common mutation Test

Test components, methods and sensitivity:

- 1) **Deletion/duplication testing by gene-specific array CGH for 116 nuclear genes.** These 116 nuclear genes are essential for pyruvate metabolism, Krebs cycle, fatty acid oxidation, the metabolism of branched-chain amino acids, respiratory chain function and more. The array is designed to detect full or partial deletions/duplications of the genes in this panel using probes within and closely flanking exons of the common transcript and the alternative transcripts.
- 2) **Detection and quantification of mtDNA deletion/duplication by array CGH.** This test uses a custom-designed microarray with more than 1,600 60-mer probes per mtDNA molecule with probes spaced approximately every 10 bp. Based on this design, any single deletion/duplication larger than 200 bp can be reliably detected; In addition, heteroplasmy can be estimated.²⁹ Large deletions of the mitochondrial genome have been reported in approximately 1.5%-40% of patients with mitochondrial disorders.^{7, 8, 25, 26, 27} The wide range is likely due in part to differences in the populations studied and the types of tissue tested. MtDNA deletions larger than 2 kb account for >95% of the reported disease causing mtDNA deletions and are responsible for >99% cases of mtDNA deletion-associated mitochondrial disease (www.mitomap.org). Due to the large number of probes, deletion/duplication analysis, as performed by GeneDx, is expected to be highly sensitive and able to detect all large mtDNA deletions/duplications associated with mitochondrial disease including those associated with Kearns-Sayre syndrome (KSS), chronic progressive external ophthalmoplegia (CPEO), and Pearson syndrome (Table 1). Heteroplasmy less than 15% may not be detected by this method.

Table 1. Characteristics of Mitochondrial DNA Deletion Syndromes

mtDNA Deletion Syndromes	Disease Characteristics	Characteristics of mtDNA Deletions ⁹
KSS	A triad of (1) onset < 20 y/o, (2) pigmentary retinopathy, and (3) PEO, plus at least one of the following: cardiac conduction block, cerebrospinal fluid protein concentration greater than 100 mg/dL, or cerebellar ataxia	~90% have a large-scale 1.3-10 kb deletion usually present in all tissues, but most abundant in muscle, and often undetectable in blood cells. A deletion of 4977 bp is the most common. Over 150 deletions have been associated with KSS. Large-scale duplications have also been reported.
CPEO	Ptosis, ophthalmoplegia, and variably severe proximal limb weakness may be the early sign of KSS.	Deletion/duplication analysis is estimated to identify a deletion in approximately 50% of patients. Deletions are confined to skeletal muscle.
Pearson Syndrome	Sideroblastic anemia, exocrine pancreas dysfunction, usually fatal in infancy: children who survive the disease usually go on to develop KSS.	Deletions are usually more abundant in blood than other tissue types. Deletion load gradually decreases in blood and increases in muscle as the disease evolves to PEO and KSS over time.

- 3) **Detection and quantification of 16 common mitochondrial mutations by real-time ARMS qPCR .** Real-time ARMS qPCR will be used for the detection of 16 common mtDNA mutations and quantification of heteroplasmy. Mutations in heteroplasmy of ≥ 1% are expected to be reliably detected. The percentage of patients suspected of having a mitochondrial disorder who harbor one of these 16 common mutations (Table 2) is

not known, as this exact panel has not been studied previously in a large population. Currently available information about the frequencies of these common mutations in specific syndromes is provided in Table 2 below.

Table 2: 16 common mtDNA mutations in the GeneDx panel and associated disorders

mtDNA mutations	Examples of Associated Disorders
3243A>G	MELAS (3243A>G present in ~80% of cases) ⁶ Maternally Inherited Diabetes and Deafness (MIDD) (3243A>G present in ~ 2%-7% of patients) ¹¹ Leigh Syndrome ⁶ Hypertrophic Cardiomyopathy (3243A>G present in ~10% of Finnish patients) ¹¹
3271T>C	MELAS (3271T>C present in ~7.5% of cases) ¹⁰
3460G>A	LHON (Together 3460G>A, 11778G>A and 14484T>C account for 95% of patients with LHON) ¹²
4300A>G	Maternally Inherited Hypertrophic Cardiomyopathy (MICM) ¹⁶
8344A>G	MERRF (8344A>G present in over 80% of patients) ¹⁴
8356T>C	MERRF ¹⁴
8363G>A	MERRF ¹⁴ Maternally Inherited Cardiomyopathy (MICM) ¹⁴
8993T>G	Leigh Syndrome (LS) (~10-20% of patients have either 8993T>G or 8993T>C) ¹⁵ NARP (Mutation at nucleotide 8993 is estimated to be present in 20% to greater than 50% of patients. 8993T>G is more common than 8993T>C.) ¹⁵
8993T>C	Leigh Syndrome (LS) (~10-20% of patients have either 8993T>C or 8993T>G) ¹⁵ NARP (Mutation at nucleotide 8993 is estimated to be present in 20% to greater than 50% of patients. 8993T>C is less common than 8993T>G.) ¹⁵
9176T>G	Leigh Syndrome (LS) (present in ~ 1-5% of patients) ¹⁵ NARP (present in ~ 1-5% of patients) ¹⁵
9176T>C	Leigh Syndrome (LS)/NARP (present in ~ 1-5% of patients) ¹⁵
11778G>A	LHON (Together 11778G>A, 3460G>A and 14484T>C account for 95% of patients with LHON. Of the three 11778G>A is the most common, present in ~70% of Caucasian patients and 90% of Asian patients) ¹²
13513G>A	MELAS (rare) ¹⁸ Leigh Syndrome (LS) (present in ~ 1-5% of patients) ¹⁵
14459G>A	LHON (rare) ¹⁹
14484T>C	LHON (Together 14484T>C, 3460G>A and 11778G>A account for 95% of patients with LHON. ¹² 14484T>C is the most common cause of LHON in French Canadians ¹³)
14709T>C	Maternally Inherited Diabetes and Deafness (MIDD) (Present in ~7% of patients) ²⁰
MELAS: Mitochondrial Encephalomyopathy, Lactic Acidosis, and Stroke-like episodes; MERRF: Myoclonic Epilepsy and Ragged Red Muscle Fibers; LHON: Leber Hereditary Optic Neuropathy	

Combined sensitivity of the deletion/duplication and mtDNA common mutation panel

Three separate studies have evaluated large cohorts of patients with a suspected mitochondrial disorder for deletions of the mitochondrial genome and for between 3 and 6 common mtDNA point mutations. In the three studies, not all patients were examined for the same common mtDNA point mutations. Between 0.9% and 19% of patients harbored either a large deletion or a mtDNA common mutation.^{6, 50, 40} In another study, using a combination of multiplex PCR/ASO to detect 11 common mutations and Southern analysis, a common mtDNA mutation or a large mtDNA deletion was detected in approximately 7% of the 2000 samples submitted for mtDNA analysis.⁴¹ Due to the larger number of common mutations included and a sensitive method for deletion/duplication analysis (array CGH) of mtDNA and 116 relevant nuclear genes, the combined clinical sensitivity of this test (possibility of finding a pathogenic mutation) is predicted to be higher.

Specimen Requirements and Shipping/Handling

Special Considerations for Mitochondrial Disorders: While mutations in nuclear genes are easily detectable in whole blood specimens, some mtDNA mutations and deletions/duplications may only be detectable in other tissues. Tissue biopsies are preferable for mtDNA analysis, therefore, sending a blood sample together with a tissue biopsy from the same patient is recommended.

- **PREFERRED:** TISSUE BIOPSIES (muscle or liver) AND BLOOD SPECIMEN: For tissue, please submit ≥ 50 mg, frozen within minutes after collection, stored at -80°C and shipped on dry ice with overnight delivery. Whole blood in EDTA; Adults: 8-10 ml; Children: 4-6 ml; Infants: 2-3 ml. Ship blood separately, overnight at ambient temperature, using a cool pack in hot weather. Blood specimens may be refrigerated for up to 7 days prior to shipping. **DO NOT FREEZE BLOOD**
- **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
- **Prenatal Diagnosis for a known familial nuclear DNA mutations ONLY:** Call to discuss specimen requirements for prenatal sample and for parental blood.
- **Buccal Brushes:** NOT accepted for this test.
- **Cultured fibroblasts** NOT accepted for this test

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:

Test# 390	Mutation detection in a new patient Test for 16 common mtDNA mutations + deletion/duplication analysis of mtDNA and 116 nuclear genes	\$ 1500	Approx. 4-5 weeks
Test# 9014	Testing for a single mtDNA mutation (available for 16 common mtDNA mutations only) with heteroplasmy detection	\$ 500	Approx. 2-3 weeks
Test# 903	Testing for a single mtDNA mutation with heteroplasmy detection (custom mutation)	\$ 800	Approx. 4-6 weeks
Test# 906	Testing for a specific familial large deletion/duplication by aCGH	\$ 500	Approx. 3-4 weeks
Test# 902	Prenatal diagnosis for a specific known nuclear DNA mutation only* (including maternal cell contamination studies)	\$ 2000	Approx. 2 weeks

CPT codes for mutation detection in a new patient - All codes and units apply:

Test # 390 16 common mtDNA point mutations + Deletion/duplication testing for mtDNA and 116 nuclear gene

83891 x 1 unit	= \$ 10
83898 x 32 units	= \$ 340
83894 x 32 units	= \$ 150
83904 x 32 units	= \$ 490
88271 x 81 units	= \$ 450
83912 x 2 units	= \$ 60

TOTAL = \$ 1500

Test# 9014 Testing for single mtDNA mutation (16 common mtDNA mutations only) with heteroplasmy detection

83891 x 4 units	= \$ 40
83898 x 4 units	= \$ 120
83894 x 4 units	= \$ 40
83904 x 8 units	= \$ 200
83892 x 2 units	= \$ 40
83912 x 2 units	= \$ 60

TOTAL = \$ 500

Test # 903 Testing for single mtDNA mutation with heteroplasmy detection (custom mutation)

83891 x 2 units = \$ 30
83898 x 12 units = \$ 670
83892 x 2 units = \$ 40
83912 x 2 units = \$ 60

TOTAL = \$ 800

Test #906 Testing for known exon-level deletion/duplicaion

83891 x 2 units = \$ 12
88386 x 1 units = \$ 488

TOTAL (1 gene) = \$ 500

** Please see our website for CPT codes/prices for additional gene-specific testing, 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

References: *Please see separate link on Genetic Testing for Mitochondrial Disorders page for list of references*