Testing for 65 Confirmed Disease-Associated Mitochondrial DNA (mtDNA) Point Variants and mtDNA DeletionTesting

Variant List: G583A, C1494T, A1555G, G1606A, G1644A, A3243G, C3256T, A3260G, T3271C, T3291C, A3302G, C3303T, G3376A, G3460A, G3635A, G3697A,G3700A, G3733A, G3733C, G3890A, C4171A, G4298A, A4300G, G4308A, G4332A, A5537insT, G5650A, G5703A, A7445G, C7471CC (='7472insC'), G7497A, T7511C, A8344G, T8356C, G8363A, T8528C, T8993C, T8993G, T9176C, T9176G, T9185C, T10010C, T10158C, T10191C, G10197A, T10663C, C11777A, G11778A, G12147A, G12315A, T12706C, G13051A, G13513A, A13514G, G14459A, C14482G, C14482A, T14484C, T14487C, A14495G, C14568T, T14674C, T14709C, T14849C, T14864C and large deletions

Clinical Features:

Mitochondrial disorders are clinically heterogeneous and result from dysfunction of the mitochondrial respiratory chain, which can be caused by pathogenic variants in mitochondrial DNA (mtDNA) or in nuclear genes. Some affected individuals exhibit clinical features that fall into a discrete clinical syndrome, such as Leber's Hereditary Optic Neuropathy (LHON), 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). Although the majority of mtDNA variants are rare, common mtDNA variants have been identified and are often associated with discrete clinical syndromes, whereas, other mtDNA variants have been confirmed as pathogenic because they have been described in multiple independent families. The table below lists the 65 confirmed disease-associated variants according to MITOMAP that are included in this panel.[15]

Genetics:

Variants in mtDNA arise de novo or are maternally inherited. In most cases, mtDNA point variants are inherited, whereas gross deletions arise de novo[12]. Each mitochondrion has multiple copies of mtDNA and there are hundreds to thousands of mitochondria per cell, depending on the cell type. Usually, mtDNA variants affect only a fraction of the mtDNA; the coexistence of normal and mutant mtDNA is called heteroplasmy. When the percentage of mutant mtDNA (variant load) reaches a certain threshold that varies by tissue type, age, and specific variant the function of that tissue may become impaired.[12] As the variant load varies within and between tissues, the manifestation of mitochondrial disease may reflect the tissue-specific variant load.[13] Many factors can affect the percent heteroplasmy these include physiologic processes that are affected by the mtDNA variant, the function of the tissue, and the rate of cell division in that tissue. Variants 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.12 Large deletions of mtDNA associated with Pearson syndrome are detectable in blood, while large deletions associated with KSS and CPEO are detectable in skeletal muscle.

Test Methods:
Using genomic DNA, the entire mitochondrial genome is amplified by long-range PCR and sequenced for the detection of 65 confirmed disease-associated variants and large mtDNA deletions using a novel solid-state sequencing-by-synthesis process that allows sequencing a large number of amplicons in parallel.[14] DNA sequences are assembled and compared to the published mitochondrial genome reference sequences for analysis. The presence of any variants is confirmed by conventional dideoxy sequence analysis or other methods.

Test Sensitivity:
The 65 mtDNA point variants include all mtDNA point variants that have been confirmed to be disease-associated to date. These variants account for >80% of MELAS, >80% of MERRF, >95% of LHON, >50% of MIDD, approximately 50% of NARP and approximately 20% of LS cases.[15] Approximately 90% of individuals with Pearson syndrome or KSS, and 50% of patients with CPEO have a large-scale (2-10 kb) mtDNA deletion.18 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.[15] Overall, this test can detect pathogenic primary mtDNA defects in approximately 85% of patients. For the 65 point variants, heteroplasmy as low as 1.5% is expected to be detected and for large-scale mtDNA deletions (2 kb or larger) heteroplasmy as low as 2.5%-5% is expected to be detected. However, for large-scale mtDNA deletions observed at less than 15% heteroplasmy a quantitative value will not be provided.

Specimen Requirements and Shipping/Handling:
Special Considerations for Mitochondrial Disorders: While variants in nuclear genes are easily detectable in whole blood specimens, some mtDNA variants 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: A minimum amount of 5 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

mtDNA pathogenic variants	Examples of Associated Disorders
G583A	MELAS, Mitochondrial Myopathy and Exercise Intolerance[15]
C1494T	Maternally Inherited Deafness or Aminoglycoside-Induced Deafness[15]
A1555G	Maternally Inherited Deafness or Aminoglycoside-Induced Deafness[15]
G1606A	Ataxia, Myoclonus and Deafness[15]
G1644A	Hypertrophic Cardiomyopathy Plus MELAS[15]
A3243G	MELAS (3243A>G present in ~80% of cases)[1] Maternally Inherited Diabetes and Deafness (MIDD) (3243A>G present in ~ 2%-7% of patients)[2] Leigh Syndrome[1] Hypertrophic Cardiomyopathy (3243A>G present in ~10% of Finnish patients)[2] Sensorineural Hearing Loss, Focal Segmental Glomerulosclerosis, Cardiac Plus Multi-Organ Dysfunction[15] Chronic Progressive External Ophthalmoplegia / Mitochondrial myopathy[19]
C3256T	MELAS[15]
A3260G	Maternal Myopathy and Cardiomyopathy[15]
T3271C	MELAS (3271T>C present in ~7.5% of cases)[3]
T3291C	MELAS, Myopathy, Deafness plus Cognitive Impairment[15]
A3302G	Mitochondrial Myopathy[15]
C3303T	Maternal Myopathy and Cardiomyopathy[15]
G3376A	LHON-MELAS Overlap Syndrome[15]
G3460A	LHON (Together 3460G>A, 11778G>A and 14484T>C account for 95% of patients with LHON)[4]
G3635A	LHON[15]
G3697A	MELAS/ Leigh Syndrome/ LHON and Dystonia[15]
G3700A	LHON[15]
G3733A	LHON[15]
G3733C	LHON[16]
G3890A	Progressive Encephalomyopathy / Leigh Syndrome / Optic Atrophy[15]
C4171A	LHON[15]
G4298A	Chronic Progressive External Ophthalmoplegia/ Multiple Sclerosis[15]
A4300G	Maternally Inherited Hypertrophic Cardiomyopathy (MICM)[5]
G4308A	Chronic Progressive External Ophthalmoplegia[15]
G4332A	Encephalopathy/ MELAS[15]
A5537insT	Leigh Syndrome[15]
G5650A	Myopathy[15]
G5703A	Chronic Progressive External Ophthalmoplegia/ Mitochondrial Myopathy[15]
A7445G	Sensorineural Hearing Loss[15]
C7471CC (='7472insC')	Progressive Encephalopathy/ Ataxia, Myoclonus and Deafness/ Moto Neuron Disease-Like[15]
G7497A	Mitochondrial Myopathy/ Exercise Intolerance[15]

T7511C	Sensorineural Hearing Loss[15]
A8344G	MERRF (8344A>G present in over 80% of patients)[6]
T8356C	MERRF[15]
G8363A	MERRF[6] Maternally Inherited Cardiomyopathy Plus Deafness (MICM)[6, 15] Autism/ Leigh Syndrome/ Ataxia Plus Lipomas[15]
T8528C	Infantile Cardiomyopathy[15]
T8993C	Leigh Syndrome (LS) (~10-20% of patients have either 8993T>C or 8993T>G)[7] NARP (A 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.)[7]
T8993G	Leigh Syndrome (LS) (~10-20% of patients have either 8993T>G or 8993T>C)[7] NARP (A 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.)[7]
T9176C	Leigh Syndrome (LS)/NARP (present in ~ 1-5% of patients)[7]/ Familial Bilaterial Striatal Necrosis[15]
T9176G	Leigh Syndrome (LS) (present in ~ 1-5% of patients)[7] NARP (present in ~ 1-5% of patients)[7] Spastic Paraplegia[15]
T9185C	Leigh Disease/ Ataxia Syndromes/ NARP-Like Disease[15]
T10010C	Progressive Encephalopathy[15]
T10158C	Leigh Disease[15]
T10191C	Leigh Disease/ Leigh-Like Disease/ Epilepsy, Strokes, Optic Atrophy and Cognitive Decline[15]
G10197A	Leigh Disease/ Dystonia/ Stroke/ LHON and Dystonia[15]
T10663C	LHON[15]
C11777A	Leigh Disease[15]
G11778A	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)[4] Progressive Dystonia[15]
G12147A	MERFF-MELAS/ Encephalopathy[15]
G12315A	Chronic Progressive External Ophthalmoplegia/ Kearns Sayre Syndrome[15]
T12706C	Leigh Disease[15]
G13051A	LHON15
G13513A	MELAS (rare)[8] Leigh Disease/ MELAS/ LHON-MELAS Overlap Syndrome[15, 17] Leigh Syndrome (LS) (present in ~ 1-5% of patients)[7]
A13514G	Leigh Disease/ MELAS[15]
G14459A	LHON (rare)[9]/ Leigh Disease[15]
C14482G	LHON[15]
C14482A	LHON[15]
T14484C	LHON (Together 14484T>C, 3460G>A and 11778G>A account for 95% of patients with LHON.[4] 14484T>C is the most common cause of LHON in French Canadians[10])
T14487C	Dystonia/ Leigh Disease/ Ataxia[15]
A14495G	LHON[15, 16]
C14568T	LHON[15]

T14674C	Reversible COX Deficiency Myopathy[15]
T14709C	Mitochondrial Myopathy Plus Diabetes Mellitus & Deafness/ Encephalopathy[15]
T14849C	Exercise Intolerance / Septo-Optic Dysplasia[15]
T14864C	MELAS[15]

Abbreviations:
MELAS – Mitochondrial Encephalomyopathy, Lactic Acidosis, and Stroke-like Episodes
LHON – Leber Hereditary Optic Neuropathy
MERRF – Myoclonic Epilepsy and Ragged Red Muscle Fibers

MtDNA deletion syndromes predominately consist of three overlapping phenotypes that usually occur in a single individual in a family.18 The three phenotypes are Kearns-Sayre syndrome (KSS), CPEO and Pearson Syndrome.

Characteristics of Mitochondrial DNA Deletion Syndromes

mtDNA Deletion Syndromes	Disease Characteristics	Characteristics of mtDNA Deletions[18]
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	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

go on to develop KSS. over time.

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