Targeted Variant Analysis of the Mitochondrial DNA (mtDNA)

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. 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 variants generally present in childhood and mtDNA variants 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 variants or nuclear gene variants. 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.[1] The prevalence of mitochondrial disorders has been estimated 1/5000 to 1/8500.[2-5]

Inheritance Pattern/Genetics:

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. Variants in mtDNA arise *de novo* or are maternally inherited. In most cases, mtDNA point variants 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 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.[6] As the variant load varies within and between tissues, the manifestation of mitochondrial disease may reflect tissue-specific variant load.[4] 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.[6]

Targeted mtDNA Test Options:

Test Code	Name	Method	Detectable Variant Types	Heteroplasmy Sensitivity	Acceptable Sample Types
9017	One mtDNA Mutation with Estimated Heteroplasmy Level	Capillary Sequencing	Sequencing	25%-100%**	Tissue biopsy, blood in EDTA, buccal
9020	Two mtDNA Mutations with Estimated Heteroplasmy Level	Capillary Sequencing	Sequencing	25%-100%**	Tissue biopsy, blood in EDTA, buccal
453	One to Three mtDNA Mutations with Heteroplasmy Level	Next-Generation Sequencing	Sequencing; Large-scale Deletions	1.5%-100%	Tissue biopsy, blood in EDTA, buccal
T822	One to Three mtDNA Mutations with Heteroplasmy Level - Urine	Next-Generation Sequencing	Sequencing	5%-100%	Urine

**Heteroplasmy levels below 25% may appear negative, and heteroplasmy levels above 75% may appear homoplasmic.

Test Methods for Test Codes 9017 and 9020:
Using genomic DNA from the submitted specimen, the mitochondrial genome is PCR-amplified. Bi-directional sequence is obtained and the relevant portion(s) of the DNA sequence is analyzed and compared to the published gene sequence. The methods used by GeneDx are expected to be greater than 99% sensitive in detecting variants identifiable by sequencing. Levels of mutant heteroplasmy 25% or lower may not be detected, and levels of mutant heteroplasmy 75% or higher may appear to be homoplasmic by this method.

Test Methods for Test Codes 453 and T822:
Using genomic DNA from the submitted specimen, the entire mitochondrial genome is amplified and sequenced using a solid state sequencing by-synthesis process and the relevant portion(s) of the DNA sequence is analyzed and compared to the revised Cambridge

Reference Sequence (rCRS). Test code 453 is expected to detect heteroplasmy as low as 1.5% or greater; test code T822 is expected to detect heteroplasmy as low as 5% or greater.

References:

1. Oliveira et al. (2005) Dev Med Child Neurol 47 (3):185-9 (PMID: 15739723)
2. Zhu et al. (2009) Acta Biochim. Biophys. Sin. (Shanghai) 41 (3):179-87 (PMID: 19280056)
3. Chinnery PF. Mitochondrial Disorders Overview. 2000 Jun 8 [Updated 2014 Aug 14]. In: Adam MP, Ardinger HH, Pagon RA, et al., editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2019. Available from: https://www.ncbi.nlm.nih.gov/books/NBK1224/
4. Tarnopolsky et al. (2005) Med Sci Sports Exerc 37 (12):2086-93 (PMID: 16331134)
5. van Adel, B. and Tarnopolsky, M. (2009) J Clin Neuromuscul Dis 10 (3):97-121 (PMID: 19258857)
6. Longo et al. (2003) Neurol Clin 21 (4):817-31 (PMID: 14743651)