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	Lower Limit of Heteroplasmy Detected	Acceptable Sample Types
9017	One Known mtDNA Variant (Estimated Heteroplasmy)	Capillary Sequencing	Sequencing	25%**	Tissue biopsy, blood in EDTA, buccal
9020	Two Known mtDNA Variants (Estimated Heteroplasmy)	Capillary Sequencing	Sequencing	25%**	Tissue biopsy, blood in EDTA, buccal
453	Known mtDNA Variant(s) by NGS-Test 453	Next-Generation Sequencing	Sequencing; Large-scale Deletions	1.5% for point variants and 5% for large deletions	Tissue biopsy, blood in EDTA, buccal
T822	Known mtDNA Variant(s) Testing by NGS-Urine-Test T822	Next-Generation Sequencing	Sequencing	5%	Urine

**Heteroplasmy levels below 25% may appear negative.

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. Alternative sequencing or other detection methods may be used to analyze or confirm mtDNA variants.

Test Methods for Test Codes 453 and T822:
Using genomic DNA from the submitted specimen, the entire mitochondrial genome is amplified and sequenced using Next Generation sequencing. DNA sequence is assembled and the relevant portion(s) of the sequence is analyzed in comparison with the revised Cambridge Reference Sequence (rCRS GeneBank number NC_012920).

References:

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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)