Full Sequence Analysis and Deletion Testing of the Mitochondrial Genome with Maternal Segregation

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.[2,3,4,5]

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 typically 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]

Test Methods

Using genomic DNA, the entire mitochondrial genome is amplified and sequenced using Next Generation sequencing. DNA sequence is assembled and analyzed in comparison with the revised Cambridge Reference Sequence (rCRS GeneBank number NC_012920) and the reported variants listed in the MITOMAP database (http://www.mitomap.org). Alternative sequencing or other detection methods may be used to

analyze or confirm mtDNA variants. Reportable variants include pathogenic variants, likely pathogenic variants, and variants of uncertain significance. Heteroplasmic likely benign and benign variants, if present, are not routinely reported but are available upon request. If a maternal sample is provided at the time of the proband's sample submission, reportable sequence variants confirmed in the proband by capillary (Sanger) sequencing will be evaluated in the maternal sample by Sanger sequencing of the relevant portion(s) of the mitochondrial genome from genomic DNA. For analysis performed by Sanger sequencing, levels of mutant heteroplasmy 25% or lower may not be detected. Targeted testing of identified mtDNA variants for maternal relatives can be ordered separately.

Test Sensitivity

The combination of full sequence analysis plus deletion testing is expected to identify a mitochondrial DNA variant in approximately 40% of adults and 10-20% of pediatric patients with a primary mitochondrial disorder.[3, 8-10] Next generation sequencing of the mitochondrial genome can detect mtDNA variants as low as 1.5% heteroplasmy and large-scale deletions (2 kb or larger) as low as 5% heteroplasmy. However, for large-scale deletions observed at less than 15% heteroplasmy a quantitative value will not be provided. This test is expected to detect greater than 98% of known pathogenic variants and deletions of the mitochondrial genome.

References

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