Full Sequence Analysis and Deletion Testing of the Mitochondrial Genome

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:

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. 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] Disorders due to nuclear gene variants that affect mitochondrial function may be inherited in an autosomal dominant, autosomal recessive or X-linked manner.

Test Methods:

Using genomic DNA, the entire mitochondrial genome is amplified by long-range PCR and sequenced using Next Generation sequencing that allows sequencing a large number of amplicons in parallel.[7] DNA sequences are assembled and compared to the published mitochondrial genome reference sequences for analysis. The presence of any disease-associated sequence variant is confirmed by conventional dideoxy sequence analysis or other methods. A reference library of more than 6000 samples from different ethnic groups and online databases for mtDNA variations will be used to evaluate variants of unknown clinical significance. In some cases, additional testing may be recommended to elucidate pathogenicity.

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%-5% heteroplasmy and large-scale deletions (2 kb or larger) as low as 2.5%-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.

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