Combined Mito Genome Plus Mito Focused Nuclear Gene Panel

Panel Gene List:

AARS2, ABCB7, ACAD9, ACO2, AFG3L2, AGK, AIFM1, ALAS2, ATP5F1A, ATP5F1E, ATP7B, ATPAF2, AUH, BCS1L, BOLA3, C19orf12, CARS2, CLPB, COA6, COA8, COASY, COQ2, COQ4, COQ6, COQ7, COQ8A, COQ8B, COQ9, COX10, COX15, COX20, COX6A1, COX6B1, CYC1, DARS2, DGUOK, DLAT, DLD, DNA2, DNAJC19, DNM1L, EARS2, ECHS1, ELAC2, ETFA, ETFB, ETFDH, ETHE1, FARS2, FASTKD2, FBXL4, FDX2, FH, FLAD1, FOXRED1, GARS1, GCDH, GFER, GFM1, GFM2, GLRX5, GTPBP3, HARS2, HMGCL, HTRA2, IARS2, IBA57, ISCA2, ISCU, LAMP2, LARS1, LARS2, LIAS, LIPT1, LRPPRC, LYRM4, LYRM7, MARS2, MFF, MFN2, MGME1, MICU1, Mitochondrial Genome*, MPC1, MPV17, MRPL3, MRPL44, MRPS16, MRPS22, MTFMT, MTO1, MTPAP, MTRFR, NARS2, NDUFA1, NDUFA10, NDUFA12, NDUFA2, NDUFA9, NDUFAF1, NDUFAF2, NDUFAF3, NDUFAF4, NDUFAF5, NDUFAF6, NDUFB11, NDUFB3, NDUFS1, NDUFS2, NDUFS3, NDUFS4, NDUFS6, NDUFS7, NDUFS8, NDUFV1, NDUFV2, NFU1, NR2F1, NUBPL, OPA1, OPA3, OTC, PARS2, PC, PCCA, PCCB, PDHA1, PDHB, PDHX, PDP1, PDSS1, PDSS2, PET100, PNPT1, POLG, POLG2, PRKAG2, PUS1, QARS1, RARS1, RARS2, RMND1, RNASEH1, RRM2B, SARS2, SCO1, SCO2, SDHA, SDHAF1, SERAC1, SFXN4, SLC19A2, SLC19A3, SLC22A5, SLC25A26, SLC25A3, SLC25A38, SLC25A4, SLC25A46, SPAST, SPG7, SUCLA2, SUCLG1, SURF1, TACO1, TAFAZZIN, TARS2, TFAM, TIMM8A, TK2, TMEM126A, TMEM126B, TMEM70, TPK1, TRIT1, TRMT10C, TRMU, TRNT1, TSFM, TTC19, TUFM, TWNK, TYMP, UQCC2, UQCRB, UQCRC2, VARS2, WDR45, WFS1, YARS2*

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, individuals with nuclear DNA variants generally present in childhood and those with 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 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. 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]

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 typically arise *de novo*[6]. Each mitochondrion has multiple copies of mtDNA and

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there are hundreds to thousands of mitochondria per cell, dependent on the cell type. Usually, mtDNA variant affect only a fraction of the mtDNA; the coexistence of normal and variant mtDNA is called heteroplasmy. When the percentage of variant 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] Over 300 nuclear genes have reported disease-causing variants associated with a primary mitochondrial disorder.[7,8] 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:

Genomic DNA is extracted directly from the submitted specimen or, if applicable, from cultured fibroblasts. For the nuclear genome, the DNA is enriched for the complete coding regions and splice junctions of the genes on this panel using a proprietary targeted capture system developed by GeneDx for next-generation sequencing with CNV calling (NGS-CNV). The enriched targets are simultaneously sequenced with paired-end reads on an Illumina platform. Bi-directional sequence reads are assembled and aligned to reference sequences based on NCBI RefSeq transcripts and human genome build GRCh37/UCSC hg19. After gene specific filtering, data are analyzed to identify sequence variants and most deletions and duplications involving coding exons at the exon-level; however, technical limitations and inherent sequence properties effectively reduce this resolution for some genes. Alternative sequencing or copy number detection methods are used to analyze or confirm regions with inadequate sequence or copy number data by NGS. Sequence variants are reported according to the Human Genome Variation Society (HGVS) guidelines. Copy number variants are reported based on the probe coordinates, the coordinates of the exons involved, or precise breakpoints when known. The entire mitochondrial genome from the submitted sample 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). Next generation sequencing may not detect large-scale mtDNA deletions present at 5% heteroplasmy or lower or mtDNA point variants present at less than 2% heteroplasmy. Alternative sequencing or other detection methods may be used to analyze or confirm mtDNA variants. Reportable variants in both the nuclear and mitochondrial genome include pathogenic variants, likely pathogenic variants and variants of uncertain significance. Haplogroup and homoplasmic likely benign and benign variants are included in a supplemental table. Nuclear and heteroplasmic likely benign and benign variants, if present, are not routinely reported but are available upon request. Available evidence for variant classification may change over time and the reported variant(s) in nuclear genes may be reclassified according to the ACMG/AMP Standards and Guidelines (PMID: 25741868), while the reported variant(s) in mtDNA may be reclassified according to our mitochondrial variant classification guidelines aligned with the ACMG/AMP Standards and Guidelines which may lead to issuing a revised report. Gene specific exclusions for exon-level deletion/duplication testing for this panel are: SCO2 and SDHA, no copy number testing; COX6A1, GTPBP3, NDUFAF4, NDUFB3, NR2F1, SLC25A26, TAZ, and TYMP genes, only whole gene deletions or duplications may be detected.

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Test Sensitivity:

The combination of full sequence analysis and deletion testing of the mitochondrial genome plus analysis of these nuclear genes is estimated to identify disease-causing variants(s) in approximately 60-80% patients with a primary mitochondrial disorder.[2, 9-11]

References:

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