

MitoXpanded Panel

A Targeted Test for Genetic Causes of Nuclear Mitochondrial Disorders Using a Trio Approach

Overview:

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 chronic progressive external ophthalmoplegia (CPEO) 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. 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 function¹. The prevalence of mitochondrial disorders has been estimated 1/5000 to 1/8500^{2,3,4,5}.

The cause of mitochondrial disorders can be difficult to discern as there are many genes associated with primary mitochondrial disorders and the phenotype of mitochondrial disorders have significant overlap with many other genetic conditions. Moreover, new genes associated with mitochondrial disorders are being discovered regularly, making it challenging for clinical laboratories to keep traditional testing panels up-to-date. Additionally, interpretation of the clinical significance of variants in these newly discovered genes is often difficult in the absence of parental testing to clarify the inheritance of suspicious variants.

The MitoXpanded Panel uses a trio approach that includes concurrent analysis of the affected proband and both parents, which increases the likelihood of identifying a definitive genetic explanation for the suspected mitochondrial disorder in an individual. Depending on the family structure, family history of ataxia, and the availability of both parents, other family members of the affected individual may be evaluated in conjunction with the proband. Please contact GeneDx for prior approval when both parents are not available to submit samples for the MitoXpanded Panel. The MitoXpanded Panel is based on whole exome capture (WEC), NextGeneration sequencing (NGS), and targeted analysis of a comprehensive list of

approximately 1800 genes currently associated with a mitochondrial disorder phenotype. The design of the panel allows for a comprehensive, dynamic gene list that is updated regularly to ensure inclusion of genes recently associated with these conditions.

Inheritance Pattern/Genetics:

Mitochondrial disorders can result from pathogenic variants encoded by genes in the mitochondrial genome the nuclear genome. Approximately 1500 nuclear gene products are involved in maintaining proper mitochondrial respiratory chain function². Disorders due to nuclear gene variants that affect mitochondrial function may be inherited in an autosomal dominant, autosomal recessive or X-linked manner. Pathogenic variants in a particular gene may be associated with a wide range of phenotypes (clinical heterogeneity), and conversely, pathogenic variants in different genes can cause the same phenotype (genetic heterogeneity). In some instances, molecular confirmation of a clinical diagnosis of a mitochondrial disorder may have implications for treatment and management of the specific form of disease.

Test Methods:

Using genomic DNA from the submitted specimen(s), the exonic regions and flanking splice junctions of the genome were captured using a proprietary system developed by GeneDx and sequenced by massively parallel (NextGen) sequencing on an Illumina sequencing system with 100bp or greater paired-end reads. Reads were aligned to human genome build GRCh37/UCSC hg19, and analyzed for sequence variants in the selected genes or regions of interest using a custom-developed analysis tool (Xome Analyzer). Capillary sequencing or another appropriate method was used to confirm all potentially pathogenic variants identified in this individual and relative samples, if submitted. Sequence alterations were reported according to the Human Genome Variation Society (HGVS) nomenclature guidelines. Please note that while the MitoXpanded panel captures and sequences the whole exome, analysis is targeted to the specific phenotype-driven gene list for this panel. The Mito Xpanded Panel gene list includes approximately 1800 genes. The list was developed by searching for genes associated with mitochondrial disorders in multiple sources, including OMIM, HGMD, and Human Phenotype Ontology (HPO) terms. Additionally, genes were added to the list using GeneDx data from clinical whole exome sequencing done on patients with suspected mitochondrial disorders. The gene list is systematically updated at least quarterly. The current gene list is available on our website.

Result Reporting:

The MitoXpanded Panel is performed on the proband and the parental samples (and/or additional relatives) when submitted together for analysis. A single report will be issued on the affected proband in the family. A separate report will not be issued for unaffected parents or other family members who may also have submitted a specimen for the purpose of allowing

better interpretation of the results from the affected individual. If additional reports are requested for other affected family members, extra fees will apply.

The report that is issued for the affected individual will include reportable variants in genes that have been previously associated with a mitochondrial disorder phenotype in the published or emerging literature. The report will include pathogenic or likely pathogenic variants in genes associated or likely associated with the patient's phenotype. In some instances, the report may also include specific variants of uncertain significance (VUS) in genes that are possibly associated with the patient's phenotype. Variants that are considered to be benign or likely benign will not be reported. As the MitoXpanded Panel includes approximately 1800 genes, the report will not include a comprehensive list of all observed variants. If desired, clinicians can request a separate file containing a list of identified variants that will be provided upon completion of testing.

Test Sensitivity:

The clinical sensitivity of the MitoXpanded test depends in part on the patient's clinical phenotype. Previous WES studies have reported identification of a definitive pathogenic variant in approximately 30% of individuals with a suspected mitochondrial disorder with up to 50-60% diagnosis rate reported in highly selected populations^{5,6,7}. The sensitivity of this test is expected to be comparable to trio-based whole exome sequencing since it uses a trio approach to test a comprehensive list of genes known to be associated with mitochondrial disorders. The clinical sensitivity is expected to be significantly lower for singleton testing when only the affected proband is tested⁸.

The average coverage of all genes on the panel is greater than 99% at 10X (with a depth of 10 or more reads), and approximately 96% of the genes on the panel have an average coverage of greater than 99.0% coverage at 10X. Note that these numbers represent the average coverage for the genes on the panel, derived by combining data from a large number of patients. The coverage of each gene on the panel for a specific patient may vary, and the actual coverage for each gene is included in the final report.

Limitations:

Mitochondrial disorders can be caused by genes encoded by the nuclear and mitochondrial genome. Mitochondrial genome sequencing is not performed as part of the MitoXpanded Panel. Additionally, some types of genetic disorders, such as those due to nucleotide repeat expansion/contraction, abnormal DNA methylation, and other mechanisms may not be detectable with this test. Additionally, small sections of a few individual genes have inherent sequence properties that yield suboptimal data and variants in those regions may not be reliably detected.

The scientific knowledge available about the function of all genes in the human genome is incomplete at this time. It is possible that the MitoXpanded Panel may identify the presence of a variant in the sequence of an affected individual, but that we will not recognize it as the cause of their disease due to insufficient knowledge about the gene and its function. Even if the MitoXpanded Panel identifies the underlying genetic cause of a disorder in an affected individual, it is possible that such a diagnosis will not permit an accurate prediction of the prognosis or severity of the disease. While there is a possibility that identifying the genetic cause may help direct management and treatment of the disease, it is also possible that this knowledge will not change management or treatment.

References:

1. Oliveira, G., Diogo, L., Grazina, M., Garcia, P., Ataide, A., Marques, C., Miguel, T., Borges, L., Vicente, A. M., and Oliveira, C. R. (2005) *Dev Med Child Neurol* 47, 185-9.
2. Chinnery, P. F. (1993(updated 2010)) GeneReviews [Internet]. Seattle (WA): University of Washington, Seattle; 1993-.2000 Jun 08 [updated 2010 Sep 16].
3. Tarnopolsky, M. A., and Raha, S. (2005) *Med Sci Sports Exerc* 37, 2086-93.
4. van Adel, B. A., and Tarnopolsky, M. A. (2009) *J Clin Neuromuscul Dis* 10, 97-121.
5. Zhu, X., Peng, X., Guan, M. X., and Yan, Q. (2009) *Acta Biochim Biophys Sin (Shanghai)* 41, 179-87.
6. Bai R, Balog A, Higgs J, Retterer K, Arjona D, Juusola J, Vitazka P, Suchy S, Enns GM, Haas R, Goldstein A, Tarnopolsky M, Parikh S, Copeland WC, Niyazov D, Falk MJ, Chung WK, Bale S, Richard G. WES and WMGS for Molecular Diagnosis of Mitochondrial Disorders: Lessons from 865 Cases (Abstract #119). Presented at the 2015 UMDF Symposium, June 18, 2015, Washington DC
7. Pronicka et al. (2016) *J Transl Med* 14 (1):174 (PMID: 27290639)
8. Taylor et al. (2014) *Jama* 312 (1):68-77 (PMID: 25058219)
9. Retterer et al. (2015) *Genet. Med.*: (PMID: 26633542)