

## Progressive External Ophthalmoplegia (PEO)/ Optic Atrophy Nuclear Gene Panel

### Sequence Analysis and Exon-Level Deletion/Duplication Testing of 44 Nuclear Genes

#### PANEL GENE LIST

*ACO2, AUH, C12orf65, CLPB, DGUOK, DNA2, DNAJC19, DNM1L, EARS2, FH, GYG2, ISCA2, MFF, MFN2, MGME1, MTFMT, MTO1, MTPAP, NARS2, NDUFAF3, NR2F1, OPA1, OPA3, PDHX, PDSS1, POLG, POLG2, RNASEH1, RRM2B, SLC19A2, SLC19A3, SLC25A4, SLC25A46, SPG7, SUCLA2, TACO1, TIMM8A, TK2, TMEM126A, TSFM, TWNK, TYMP, VARS2, WFS1*

#### CLINICAL FEATURES

Autosomal-dominant or autosomal recessive progressive external ophthalmoplegia (adPEO or arPEO) is a genetically heterogeneous group of mitochondrial disorders that are generally characterized by accumulation of multiple mitochondrial DNA (mtDNA) deletions in post-mitotic tissues. Autosomal-dominant optic atrophy (DOA) and Leber hereditary optic neuropathy (LHON) are the two most common inherited optic neuropathies in the general population. Both disorders share striking pathological similarities, marked by the selective loss of retinal ganglion cells (RGCs) and the early involvement of the papillomacular bundle 1. Mitochondrial disorders are clinically heterogeneous and result from dysfunction of the mitochondrial respiratory chain, which can be caused by 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 Hereditary Optic Neuropathy (LHON), KearnsSayre 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 2. The prevalence of mitochondrial disorders has been estimated 1/5000 to 1/8500. 3-5

#### GENETICS

To date, around 200 nuclear genes have reported disease-causing variants associated with a primary mitochondrial disorder. 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 extracted from the submitted specimen, the complete coding regions and splice site junctions of the genes tested are enriched 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; 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. Reportable variants include pathogenic variants, likely pathogenic variants and variants of uncertain significance. Likely benign and benign variants, if present, are not routinely reported but are available upon request.

The technical sensitivity of sequencing is estimated to be >99% at detecting single nucleotide events. It will not reliably detect deletions greater than 20 base pairs, insertions or rearrangements greater than 10 base pairs, or low-level mosaicism. The copy number assessment methods used with this test cannot reliably detect copy number variants of less than 500 base pairs or mosaicism and cannot identify balanced chromosome aberrations. Assessment of exon-level copy number events is dependent on the inherent sequence properties of the targeted regions, including shared homology and exon size. Due to the presence of non-functional pseudogenes, regions of the *GYG2*, *NR2F1*, *PDSS1*, and *TSMF*, and genes are not fully sequenced by this method. For the *RNASEH1*, *SLC25A46*, and *TMEM126B* genes, sequencing but not deletion/duplication analysis, was performed.

#### CLINICAL SENSITIVITY

Variants in seven nuclear genes are associated with adPEO. These seven genes encode both subunits of DNA polymerase gamma (*POLG* and *POLG2*), the DNA helicase Twinkle (*TWINK*), the adenine nucleotide translocator ANT1 (*SLC25A4*), optic atrophy 1 (*OPA1*), ribonucleotide reductase (*RRM2B*), the DNA helicase DNA2 (*DNA2*). Variant(s) in *POLG* and *RRM2B* can cause adPEO or arPEO. Variants in six of these genes (*TWINK*, *OPA1*, *POLG*, *POLG2*, *RRM2B*, *SLC25A4*) account for ~90% of patients with familial PEO.<sup>7,8,9</sup> In DOA, the majority (50-60%) of affected families harbor pathogenic variants in the *OPA1* gene. Variants in 42 other nuclear genes also have reported disease-causing variants associated with syndromic or non-syndromic optic atrophies, and sometimes the phenotypes are clinically indistinguishable from the phenotypes of LHON or DOA<sup>10</sup>. It is estimated that, after excluding the possibility of mtDNA variants, this panel would identify disease-causing variant(s) in ~70-80% of patients with familial optic atrophy and ~50% of patients with sporadic optic atrophy.<sup>10</sup>

#### REFERENCES:

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