Mitochondrial Genetic Testing

Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan’s procedure code list for management requirements.

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What are mitochondrial disorders

Definition

Mitochondrial disorders arise from mutations in both nuclear and mitochondrial (mtDNA) components of the respiratory chain. They comprise a clinically diverse group of diseases that may present at any age and affect a single organ or present as a multi-system condition in which neurologic and myopathic features predominate. Extensive clinical variability and phenotypic overlap exists among the many discrete mitochondrial disorders.

- Mitochondrial disease is suspected in patients with a combination of clinical features in:
  - Muscle: proximal myopathy or cardiomyopathy
  - Nervous system: encephalopathy, seizures, dementia, stroke-like episodes, ataxia and spasticity and migraine
  - Eye: ptosis, ophthalmoparesis, ophthalmoplegia, optic atrophy, pigmentary retinopathy
  - Sensorineural hearing loss
  - Diabetes mellitus
  - Mid or late pregnancy loss

- Mitochondrial disease is not curable. However, in some cases, specific treatment recommendations can be made based on a person’s definitive diagnosis.
Consensus based recommendations have been published by the Mitochondrial Medicine Society for the routine care and management of individuals with mitochondrial disease.\(^1\) Individuals at-risk for mitochondrial conditions may also benefit from clinical assessment to initiate baseline evaluations (neurology, cardiology, ophthalmology, and audiology) and potential intervention prior to exhibiting clinical manifestations.\(^2,3\)

- Mitochondrial conditions caused by nuclear DNA variants can be maternally or paternally inherited and may follow autosomal dominant, autosomal recessive, and X-linked inheritance.

- Mitochondrial conditions caused by mtDNA are always maternally inherited. Pathogenic variants in the mtDNA may be de novo or maternally inherited. This means that a female who carries a mtDNA mutation at high mutation load will typically pass it on to all of her children. However, due to the meiotic bottleneck, the heteroplasmy level may vary significantly between generations. A male who carries the mtDNA mutation will not pass it on to his children.\(^2,4\) mtDNA deletions are rarely transmitted (less than 1% empiric risk).\(^5\) If the mother is symptomatic, then the recurrence risk is approximately 4%.\(^6\)

- For all mtDNA mutations, clinical expressivity depends on the three following factors:\(^2\)
  - The ratio of mutant mtDNA, mutational load (heteroplasmy)
  - The organs and tissues in which the mutant mtDNA is found (tissue distribution), and
  - The vulnerability of each tissue to impaired oxidative metabolism (threshold effect).

- Analysis of an individual’s family history may provide information regarding most likely inheritance patterns for a suspected mitochondrial condition. This may guide decisions to perform mtDNA sequencing, mtDNA deletion/duplication testing, nuclear encoded DNA sequencing, and/or nuclear encoded DNA deletion/duplication testing.

- While genetic test results alone cannot predict the exact course or phenotype of the disease, severity does correlate with mutation load for mitochondrial DNA mutations.\(^4,7\)

- Identification of a pathogenic variant in a proband can allow for informative testing of relatives at risk for diabetes, seizures, hearing loss, optic atrophy, and other findings.

**Test information**

- The investigation and diagnosis of patients with mitochondrial disease often necessitates a combination of techniques including muscle histocytochemistry, biochemical assessment and molecular genetic studies along with clinical
assessment. Any molecular genetic test for a mtDNA mutation should ideally be directed by the clinical phenotype and results of these other investigations.

- While biochemical analyses of an affected tissue may be informative, they are not sensitive or specific enough to definitively diagnose most mitochondrial conditions.

- Due to overlap of clinical findings of mitochondrial conditions and non-mitochondrial conditions, affected individuals are more likely to have multiple tests performed before a molecular genetic cause is identified. If an individual’s clinical findings clearly correlate with a specific mitochondrial condition, then testing can be focused on the most appropriate approach for that condition. However, if the clinical picture strongly suggests a mitochondrial condition but there is uncertainty about which subset of conditions, then larger mtDNA or nuclear DNA testing panels may be appropriate.

- “Approaches to molecular genetic testing of a proband to consider are serial testing of single genes, multi-gene panel testing (simultaneous testing of multiple genes), and/or genomic testing (e.g., sequencing of the entire mitochondrial genome, genome sequencing, or exome sequencing to identify a pathogenic variant in a nuclear gene). In many individuals in whom molecular genetic testing does not yield or confirm a diagnosis, further investigation of suspected mitochondrial disease can involve a range of different clinical tests, including muscle biopsy for respiratory chain function.”

- The efficiency of next generation sequencing (NGS) has led to an increasing number of large, multi-gene testing panels. NGS panels that test several genes at once are particularly well-suited to conditions caused by more than one gene or where there is considerable clinical overlap between conditions making it difficult to reliably narrow down likely causes. As a result, several laboratories have begun to combine genes involved in certain conditions which often have both of those characteristics.

- Mitochondrial Genome Sequencing Panels and Mitochondrial Genome Deletion/Duplication Panels:
  - Whole Mitochondrial Genome Sequencing: NGS testing is capable of simultaneously detecting point mutations, deletions, and point mutation heteroplasmias. Typically, Sanger sequence analysis will miss heteroplasmy below 20%. With suitable depth of coverage, NGS can detect heteroplasmy down to ~1%.

- For some, but not all, mtDNA conditions, such as MERFF, if mtDNA genetic testing is negative in a blood sample in a person with symptoms of the mtDNA condition, testing can be done on other specimens. Typically this is done when the phenotype is highly suggestive of the presence of a mutation associated with a specific gene or set of genes, or when there is a need to assess reproductive risk.
The potential for informativeness versus the invasiveness and procedural costs are factors to consider. For instance, muscle biopsy also allows enzymatic analysis of the electron transport chain, light and ultrastructural microscopy, and mtDNA copy number analysis, which may provide highly useful information for some conditions, such as MERFF.

Genetic testing can also be done on skin fibroblasts, urinary sediment, or buccal mucosa. If cultured fibroblasts are used, measures such as limited passaging and uridine supplementation should be taken to reduce selection against mutant genotypes.

- Nuclear Encoded Mitochondrial Gene Sequencing Panel: A number of large panels are available that sequence numerous nuclear-encoded mitochondrial genes for a broad approach to testing. Multi-gene panel tests, even for similar clinical scenarios, vary considerably laboratory by laboratory in the genes that are included and in technical specifications (e.g. depth of coverage, extent of intron/exon boundary analysis, methodology of large deletion/duplication analysis).

Guidelines and evidence

- No specific evidence-based U.S. testing guidelines were identified.
- The Mitochondrial Medicine Society developed consensus recommendations using the Delphi method and published them in 2015.

Recommendations for DNA testing

- Massively parallel sequencing/NGS of the mtDNA genome is the preferred methodology when testing mtDNA and should be performed in cases of suspected mitochondrial disease instead of testing for a limited number of pathogenic point mutations.
- Patients with a strong likelihood of mitochondrial disease because of a mtDNA mutation and negative testing in blood, should have mtDNA assessed in another tissue to avoid the possibility of missing tissue-specific mutations or low levels of heteroplasmy in blood; tissue-based testing also helps assess the risk of other organ involvement and heterogeneity in family members and guides genetic counseling.
- When considering nuclear gene testing in patients with likely primary mitochondrial disease, NGS methodologies providing complete coverage of known mitochondrial disease genes is preferred. Single-gene testing should usually be avoided because mutations in different genes can produce the same phenotype. If no mutation is identified via known NGS panels, then whole exome sequencing should be considered.

Recommendations for pathology testing
- Biopsy should only be considered when the diagnosis cannot be confirmed with DNA testing of other more accessible tissues. Muscle (and/or liver) biopsies are often not necessary and should be avoided when possible due to their invasive nature, unless other types of analyses such as pathology, enzymology, or mtDNA copy number analyses are required for diagnosis.

- The American College of Medical Genetics and Genomics (ACMG, 2013) states the following regarding testing individuals with isolated autism for mitochondrial disorders:\(^{11}\)
  - “As with metabolic disorders, testing for mitochondrial disorders in persons with ASDs is recommended only if supporting symptoms or laboratory abnormalities are present.”

- The European Federation of Neurological Sciences (2009)\(^ {12}\) provided molecular diagnostic consensus-based guidelines based on literature reviews: “If the phenotype suggests syndromic mitochondrial disease due to mtDNA point mutations (MELAS, MERRF, NARP, LHON) DNA-microarrays using allele-specific oligonucleotide hybridisation, real-time-PCR or single-gene sequencing are indicated.”

- The Clinical Molecular Genetics Society (CMGS) of the United Kingdom (2008)\(^ {13}\) practice-based guidelines for the molecular diagnosis of mitochondrial disease state that: “In cases with strong clinical evidence, testing should begin with checking for the common mutation, m.8344A>G. Subsequent testing for other mutations, such as m.8356T>C, may be indicated in cases with a strong clinical indication of MERRF”. “For routine referrals for NARP, presence of T8993G and T8993C mutations should be investigated.”

- A workshop of the National Institute of Neurological Disorders and Stroke (2008)\(^ {14}\) summarizes:
  - “The diagnosis of mitochondrial diseases is complicated by their heterogeneous presentations and by the lack of screening procedures or diagnostic biomarkers that are both sensitive and specific. The workshop panelists explained that diagnosis is often a lengthy process beginning with a general clinical evaluation followed by metabolic screening and imaging and finally by genetic tests and more invasive biochemical and histological analyses. The identification of known mitochondrial mutations in tissue has greatly aided diagnosis. However, even when clinical features and family history strongly suggest mitochondrial disease, the underlying genetic mutation can elude detection, and there is no current screening procedure that would be practical for all cases of suspected mitochondrial disease.”
Criteria

Whole mtDNA Sequencing

- Pre and post-test counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Member has not had previous whole mtDNA sequencing performed, AND
- Biochemical testing appropriate for the suspected disorder has been performed and is not confirmatory of a diagnosis of a specific mitochondrial condition, AND
- Member has multiple organ system involvement defined as altered function in two or more organ systems, AND
- Member has one or more of the following clinical features: proximal myopathy, cardiomyopathy, encephalopathy, seizures, dementia, stroke-like episodes, ataxia, spasticity, ptosis, ophthalmoparesis, ophthalmoplegia, optic atrophy, pigmentary retinopathy, sensorineural hearing loss, diabetes mellitus, mid- or late pregnancy loss, MRI and/or MRS imaging results consistent with a mitochondrial process, and/or pathology results consistent with a mitochondrial process, AND
- Member’s clinical presentation does not fit a well-described syndrome for which single-gene or targeted panel testing is available (e.g. LHON), AND
- Alternate etiologies have been considered and ruled out when possible (e.g., environmental exposure, injury, infection), AND
- Family history strongly suggests mitochondrial inheritance (e.g. paternal transmission has been ruled out)

Whole mtDNA Deletion/Duplication Analysis

- Pre and post-test counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Member has not had previous whole mtDNA deletion/duplication analysis performed, AND
- Biochemical testing appropriate for the suspected disorder has been performed and is not confirmatory of a diagnosis of a specific mitochondrial condition, AND
- Member has multiple organ system involvement defined as altered function in two or more organ systems, AND
- Member has one or more of the following clinical features: proximal myopathy, cardiomyopathy, encephalopathy, seizures, dementia, stroke-like episodes, ataxia, spasticity, ptosis, ophthalmoparesis, ophthalmoplegia, optic atrophy, pigmentary retinopathy, sensorineural hearing loss, diabetes mellitus, mid- or late pregnancy loss, MRI and/or MRS imaging results consistent with a mitochondrial process, and/or pathology results consistent with a mitochondrial process, AND
• Member’s clinical presentation does not fit a well-described syndrome for which single-gene or targeted panel testing is available (e.g. LHON), AND
• Alternate etiologies have been considered and ruled out when possible (e.g., environmental exposure, injury, infection), AND
• Family history strongly suggests mitochondrial inheritance (e.g. paternal transmission has been ruled out)

**Nuclear Encoded Mitochondrial Gene Sequencing Panel**

• Pre and post-test counseling by an appropriate provider (as deemed by the Health Plan policy), AND
• Member has not had a previous nuclear encoded mitochondrial gene sequencing panel testing performed, AND
• Biochemical testing appropriate for the suspected disorder has been performed and is not confirmatory of a diagnosis of a specific mitochondrial condition, AND
• Member has multiple organ system involvement defined as altered function in two or more organ systems, AND
• Member has one or more of the following clinical features: proximal myopathy, cardiomyopathy, encephalopathy, seizures, dementia, stroke-like episodes, ataxia, spasticity, ptosis, ophthalmoparesis, ophthalmoplegia, optic atrophy, pigmentary retinopathy, sensorineural hearing loss, diabetes mellitus, mid- or late pregnancy loss, MRI and/or MRS imaging results consistent with a mitochondrial process, and/or pathology results consistent with a mitochondrial process, AND
• Member’s clinical presentation does not fit a well-described syndrome for which single-gene or targeted panel testing is available (e.g. LHON), AND
• Alternate etiologies have been considered and ruled out when possible (e.g., environmental exposure, injury, infection), AND
• Family history DOES NOT strongly suggest mitochondrial inheritance (e.g. paternal transmission is observed, autosomal inheritance is likely)

**Exclusions**

• Testing addressed in this guideline applies to patients in whom a mitochondrial disorder is suspected based on a constellation of findings commonly seen in these conditions, while not fitting clearly into one of the discrete mitochondrial syndromes. This guideline is not applicable in the following cases:
  • The patient’s findings fit into a discrete mitochondrial syndrome for which more specific testing is appropriate. Please see one of the following guidelines for information on specific mitochondrial conditions (MELAS, LHON, MNGIE, MERRF, NARP, etc); or
The patient’s findings could be explained nonspecifically by a mitochondrial disorder or other neurological or myopathic condition not related to mitochondrion for which a different genetic test may be considered; or

Individuals who have no increased risk above the general population risk to have inherited a mitochondrial disease and have just one of the following findings in isolation: fatigue; muscle weakness; developmental delay; autism; migraines; abnormal biochemical test results (e.g., elevated lactate); psychiatric symptoms.

Billing and reimbursement considerations

- Whole mtDNA Sequencing will only be considered for coverage when billed under the appropriate panel CPT code: 81460
- Whole mtDNA Deletion/Duplication will only be considered for coverage when billed under the appropriate panel CPT code: 81465
- Nuclear Encoded Mitochondrial Gene Sequencing Panels will only be considered for coverage when billed under the appropriate panel CPT code: 81440
- If the panel will be billed with separate procedure codes for each gene analyzed and the member meets criteria for Whole mtDNA Sequencing, Whole mtDNA Deletion/Duplication, or Nuclear Encoded Mitochondrial Gene Sequencing Panel, the testing will be approved but the laboratory will be redirected to the appropriate CPT code for billing purposes.
- If the panel cannot be redirected to 81460, 81465, or 81440 for any reason, the medical necessity of each billed procedure will be assessed independently.
- If more than one test or procedure code is requested at one time, the member meets criteria for all tests requested, and each test is equally likely based on personal history, clinical findings, and family history, the testing will be tiered in the following order: 81460, 81465, 81440.

References


