Neurogenic Muscle Weakness, Ataxia, and Retinitis Pigmentosa (NARP) Genetic Testing Policy

Procedure(s) addressed by this policy:	Procedure Code(s)
MT-ATP6 Targeted Mutation Analysis	81401
Whole Mitochondrial Genome	81460

What Is NARP?

- **N**eurogenic muscle weakness, **A**taxia, and **R**etinitis **P**igmentosa is a multisystem mitochondrial disease[1]
    - NARP is characterized by proximal neurogenic muscle weakness with sensory neuropathy, ataxia, learning difficulties, and pigmentary retinopathy.[1]
    - Most cases present in childhood with ataxia and learning difficulties. Seizures may also be present.[1]
- NARP is caused by mutations in the mitochondrial DNA (mtDNA) and follows maternal inheritance. This means that a female who carries the mtDNA point 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 point mutation cannot pass it on to his children.[1,2,3]
- For all mtDNA mutations, clinical expressivity depends on the three following factors: [1,2]
    - The relative abundance of mutant mtDNA, or 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).
- No data exists on the prevalence of NARP, but it is expected to be less common than Leigh syndrome, which is noted in about 1/100,000-1/140,000 individuals.[1]
- Management is generally supportive. Regular neurologic, ophthalmologic, and cardiologic screenings are recommended for affected individuals. Anti-epileptic drugs that affect the mitochondrial respiratory chain should be avoided, as they may cause secondary carnitine deficiency or can be used with L-carnitine supplementation.[1]
- At-risk individuals may also benefit from clinical assessment to initiate baseline evaluations (neurology, cardiology, ophthalmology, and audiology) and potential intervention prior to exhibiting clinical manifestations.[1]

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.[2]
- **NARP Targeted Mutation Analysis**
    - m.8993T>G and m.8993T>C in MT-ATP6 cause ~50% of cases of NARP.[1]
- Whole genome sequencing of mitochondrial DNA may detect more rare mutations associated with NARP, but does not significantly increase the detection rate over testing for the common two mutations.[1]
- While genetic test results alone cannot predict the exact course or phenotype of the disease, severity does correlate with mutation load.[1,3] The clinical course for mitochondrial diseases is subject to the concepts of heteroplasmy, tissue distribution, and threshold effect.[1,3]
- Due to its ability to simultaneously sequence the entire mtDNA and measure heteroplasmy at each position, next generation sequencing (NGS) is an attractive option for assessing NARP and overlapping syndromes. However, certain targeted mutation analyses can estimate heteroplasmy. Typically, Sanger sequence analysis will miss heteroplasmy below 20%. With suitable depth of coverage, NGS can detect heteroplasmy down to ~1%.[4,5]
- If genetic testing is negative in a blood sample in a person with symptoms of NARP, testing can be done on other specimens. Typically this is done when the phenotype is highly suggestive of presence of a NARP mutation or when there is a need to assess reproductive risk for offspring with higher mutant load and risk for developing Leigh disease.
    - Muscle may be considered as a secondary tissue, but the invasiveness and procedural costs are factors to consider. However, muscle biopsy also allows enzymatic analysis of the electron transport chain, light and ultrastructural microscopy, and mtDNA copy number analysis—all of which may provide highly useful information.
    - Genetic testing can also be done on skin fibroblasts, urinary sediment, or buccal mucosa.[1] If cultured fibroblasts are used, measures such as limited passaging and uridine supplementation should be taken to reduce selection against mutant genotypes that may lead to skewed heteroplasmy.
- Muscle (and/or liver) biopsies are often not necessary and should be avoided when possible due to their invasive nature. Biopsies should only be considered when the diagnosis cannot be confirmed with DNA testing of other more accessible tissues, for example, when presence of ragged red fibers in muscle is required for diagnosis.
    - T8993G mutation heteroplasmy tends to be comparable in blood and fibroblasts within a patient and relative to other affected individuals.[6]

- Prenatal Testing for At-Risk Pregnancies:
    - Prenatal testing for a known mitochondrial DNA mutation has limited clinical utility. For example, the mother of a child affected with NARP usually exhibits relatively high

heteroplasmy for the causative mutation. Consequently, each subsequent pregnancy is at a high risk to inherit the mutation.[7]

- Published guidelines from the 74th European Neuromuscular Centre International Consensus stated that prenatal testing via CVS in asymptomatic women with family history of NARP (<50% mutant mtDNA) is appropriate, while women with >50% mutant load should consider oocyte donation and pre-implantation genetic screening.[8]
- A retrospective chart review of prenatal samples processed in Europe concluded that prenatal testing for mitochondrial diseases was informative for the select mutations studied. Results of mtDNA heteroplasmy analyses from other family members are helpful in interpreting the prenatal mtDNA result.[9]
- As a result of the issues described above, the availability of prenatal testing for NARP is presently limited.

Guidelines and Evidence

- No specific evidence-based U.S. testing guidelines were identified for NARP.
- The Mitochondrial Medicine Society developed consensus recommendations using the Delphi method and published them in 2015.[10]
  - Recommendations for testing blood, urine, and spinal fluid
    - The initial evaluation in blood for mitochondrial disease should include complete blood count, creatine phosphokinase, transaminases, albumin, lactate and pyruvate, amino acids, and acylcarnitines, along with quantitative or qualitative urinary organic acids. Caution must be taken to ensure that specimens are collected appropriately, especially for lactate and pyruvate measurements.
    - Postprandial lactate levels are more sensitive than fasting specimens and are preferred when possible. *Caution must be taken to not overinterpret small elevations in postprandial lactate.*
    - The lactate/pyruvate ratio in blood or CSF is of value only when the lactate level is elevated.
    - Quantitative 3-methylglutaconic acid (3MG) measurements in plasma and urine should be obtained when possible in addition to urine organic acids in patients being evaluated for mitochondrial disease.
    - Creatine phosphokinase and uric acid should be assessed in patients with muscle symptoms who are suspected of having mitochondrial diseases.
    - Urine amino acid analysis should be obtained in the evaluation of mitochondrial tubulopathy.
    - When CSF is obtained, it should be sent for lactate, pyruvate, amino acid, and 5-methyltetrahydrofolate measurements.
    - Further research is needed regarding other biomarkers such as FGF21, GDF15, glutathione, and CSF neopterin.
  - 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 in these tissues are required for diagnosis.
- Quantitative 3-methylglutaconic acid (3MG) may be useful, if elevations are observed in organic acids.
- Growth differentiation factor 15 (GDF15) has been shown to be a sensitive and specific biomarker for mitochondrial dysfunction. [11]
- A workshop of the **National Institute of Neurological Disorders and Stroke (2008)**[12] 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."
- The **Clinical Molecular Genetics Society (CMGS) of the United Kingdom (2008)**[2] practice-based guidelines for the molecular diagnosis of mitochondrial disease state that: "For routine referrals for NARP, presence of T8993G and T8993C mutations should be investigated."
- The **European Federation of Neurological Sciences (2009)**[13] 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."

Criteria

NARP Known Familial Mutation

- Genetic Counseling:
  - Pre and post-test counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous genetic testing in the individual for NARP*, and
  - NARP pathogenic variant identified in matrilineal relative, AND
- Predictive Testing for Asymptomatic Individual:
  - 18 years of age or older, or
  - Under the age of 18 years, and
    - Screening for learning disabilities, retinitis pigmentosa, and/or ataxia is being considered, OR
- Diagnostic Testing for Symptomatic Individual:
  - Clinical exam and/or biochemical testing suggestive, but not confirmatory, of a diagnosis of NARP, OR
- Prenatal Testing for At-Risk Pregnancies:
  - NARP causing mutation identified in a previous child or in the mother.

NARP Targeted Mutation Analysis

- Genetic Counseling:
  - Pre and post-test counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Testing:
  - No previous genetic testing in the individual for NARP*, and
  - No known NARP pathogenic variants in the family, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Clinical exam and/or biochemical testing suggestive, but not confirmatory, of a diagnosis of NARP, and
  - Genetic testing is needed for one of the following purposes:
    - To confirm the diagnosis, or
    - To offer testing to family members, or
    - For prenatal diagnostic purposes, AND
- No evidence of paternal transmission.

Whole mtDNA Genome Sequencing

- Genetic Counseling:
  - Pre and post-test counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Criteria for NARP Targeted Mutation Analysis is met, AND
- No pathogenic variants identified in the Targeted Mutation Analysis, if performed, AND
- Member has not had previous whole mtDNA sequencing performed*, AND
- No evidence of paternal transmission.

*Genetic testing has rapidly advanced over the last 20 years. Exceptions may be considered if an individual has previously had negative genetic testing, but technical advances in testing demonstrate significant advantages that would support a medical need to re-test.

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