Myoclonic Epilepsy with Ragged Red Fibers (MERRF) Genetic Testing Policy

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

What Is MERRF?

- **M**yoclonic **E**pilepsy with **R**agged **R**ed **F**ibers (MERRF) is a multisystem mitochondrial disease.[1]
  - MERRF typically presents with myoclonus (brief, involuntary spasmodic jerking of a muscle or a group of muscles), followed by generalized epilepsy, ataxia (lack of coordination of muscle movements), weakness, and dementia.[1] Ragged red fibers (RRF) are identified on muscle biopsy pathology.[1]
  - Other common findings include hearing loss, short stature, optic atrophy, and cardiomyopathy with Wolff-Parkinson-White syndrome (a syndrome in which there is extra electrical connection in the heart at birth causing rapid heartbeat). Occasionally pigmentary retinopathy and lipomatosis are observed.[1]
  - Most cases present in childhood after normal early development.[1]
- MERRF 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 a 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]
- Genetic test results alone cannot predict the exact course or phenotype of the disease.[1,3] For all mtDNA mutations, clinical expressivity depends on the three following factors:[1]
  - 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).
- Estimated prevalence is about 0.25-1.5/100,000 individuals.[1]
- Management is usually palliative. Certain antiepileptic drugs, such as valproic acid, 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.[3]
- **MERRF Mutation Panel**: Heteroplasmic mutations in the mtDNA genes, MT-TK, MT-TL1, MT-TF, MT-TI, and MT-TP cause MERRF. Mutations in the mtDNA genes MT-TH, MT-TS1, MT-TS2, cause MELAS/MERRF overlap syndrome.
    - Approximately 90% of cases of MERRF are due to MT-TK mutations. 80% of MERRF cases are the result of a specific genetic change, m.8344A>G (formerly A8344G) in MT-TK.[1,2]
    - Three additional MT-TK mutations, m.8356T>C, m.8363G>A, and m.8361G>A, are present in an additional 10% of affected individuals. These three mutations can also be associated with other mitochondrial or genetic conditions.[1]
    - Detection rate of the four-mutation panel is about 90%.[1]
    - "Sequence analysis /scanning for pathogenic variants is used to detect pathogenic variants throughout mtDNA and is not specific for MERRF. The overall variant detection rate for MERRF by scanning/sequence analysis of mtDNA is 90%-95%."[1]
- 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 MERRF 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 MERRF, testing can be done on other specimens. Typically this is done when the phenotype is highly suggestive of presence of a MERRF mutation or when there is a need to assess reproductive risk.
    - Muscle may be considered as a secondary tissue since it is clinically involved as evidenced by Ragged Red Fibers, 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.
- Prenatal Testing for At-Risk Pregnancies:
    - Prenatal testing for a known mitochondrial mutation has limited clinical utility. For example, the mother of a child affected with MERRF usually exhibits relatively high

heteroplasmy for the causative mutation. Consequently, each subsequent pregnancy is at a high risk to inherit the mutation. Heteroplasmy detected in a fetus is of limited value because the level of heteroplasmy changes over time and between tissues, therefore, it may not be possible to ensure an acceptably low level of postnatal morbidity after detection of a heteroplasmic mutation in the prenatal setting.[1,6]

- Published guidelines from the 74th European Neuromuscular Centre International Consensus stated that prenatal testing via CVS in asymptomatic women with family history of MERFF (>40% mutant mtDNA in blood) is appropriate, while women with high mutant load should consider oocyte donation and pre-implantation genetic screening. For women with a load of <40% mutant mtDNA in blood, severe disease is estimated to be rare in offspring, [7]
- 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.[8]
- As a result of the issues described above, availability of prenatal testing for mitochondrial mutations is presently limited.

Guidelines and Evidence

- No specific evidence-based U.S. testing guidelines were identified.
- Case reports and a limited number of case series are the primary evidence base available for the diagnosis of mitochondrial disease.[9,10,11] There are few prospective studies.
- The **Mitochondrial Medicine Society** developed consensus recommendations using the Delphi method and published them in 2015.[12]
    - 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.
        - Heteroplasmy analysis in urine can selectively be more informative and accurate than testing in blood alone
        - "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 3methylglutaconic (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.[13]
- The clinical utility of genetic testing for MERRF was described by a workshop of the **National Institute of Neurological Disorders and Stroke (2008)**:[14]
    - "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." [14]
    - Initial screening includes testing lactate and CSF protein levels, muscle biopsy, EEG, ECG, and MRI.[1] "It is important to note that biochemical abnormalities may not be present during periods when the mitochondrial disease is quiescent/ dormant." [14]
- The **Clinical Molecular Genetics Society of UK (2008)** Provided practice-based guidelines for the molecular diagnosis of mitochondrial disease:
    - "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."[3]
- The **European Federation of Neurological Sciences (2009)** 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." [15]

Criteria

MERRF 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 MERRF*, and
    - MERRF 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
        - Presymptomatic screening for Wolff-Parkinson-White is being considered, OR
- Diagnostic Testing for Symptomatic Individual:
    - Clinical exam and/or biochemical testing suggestive, but not confirmatory, of a diagnosis of MERRF, OR
- Prenatal Testing for At-Risk Pregnancies:
    - MERFF causing mutation identified in a previous child or in the mother.

MERRF 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 MERRF*, and
    - No known MERRF 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 MERFF, 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 Sequencing

- Genetic Counseling:
    - Pre and post-test counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Criteria for MERRF 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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