

Mitochondrial Encephalomyopathy, Lactic Acidosis, and Stroke-like Episodes (MELAS) Genetic Testing Policy

Procedure(s) addressed by this policy:	Procedure Code(s)
MELAS Known Familial Mutation Analysis	81403
MT-TL1 Targeted Mutation Analysis	81401
MT-ND5 Targeted Mutation Analysis	81401
Whole Mitochondrial Genome	81460

What Is MELAS?

- Mitochondrial Encephalomyopathy, Lactic Acidosis, and Stroke-like Episodes (MELAS) is a progressive, multisystem genetic disease.¹
- The estimated prevalence of MELAS is about 16-18/100,000 individuals.^{2,3}
- MELAS symptoms can present at any age. Most cases present between 2-10 years of age, but some present between 10-40 years of age.¹
- Individuals with MELAS typically experience disease progression that results in death. Median survival time from point of diagnosis is about 16.9 years, with a subgroup of 20.8% who are more severely affected and die within 7.3 years of diagnosis.¹ Overall, children and young adults diagnosed with MELAS who have classical symptoms have a shorter lifespan than older adults with milder symptoms.
- Typical initial clinical presentation includes stroke-like episodes or cortical blindness often occurring with focal seizures or generalized tonic-clonic seizures, and these episodes may be recurrent and associated with altered consciousness. Almost all individuals with MELAS (94%) have lactic acidemia. Individuals may also have recurrent headaches, anorexia, recurrent vomiting, possibly exercise intolerance or proximal limb weakness, Wolff-Parkinson-White syndrome (a syndrome in which there is extra electrical connection in the heart at birth causing rapid heartbeat), and diabetes mellitus. Short stature in children and sensorineural hearing loss in both children and adults is also common.¹
- The natural history of MELAS involves gradual impairment of motor abilities, vision, and cognitive ability by adolescence or young adulthood due to recurring stroke-like episodes.¹
- There is no cure for MELAS. Several types of treatment, however, have demonstrated benefit in affected individuals. The use of oral and intravenous (IV) L-arginine and citrulline has shown reduction of frequency and/or severity of stroke-like episodes.^{2,4-8} Both endurance and resistance exercise have been studied and shown to increase mitochondrial metabolism.⁶ Vitamin and cofactor supplementation including CoQ10, alpha lipoic acid, and riboflavin should



be offered, and addition of folinic acid and L-carnitine should be considered, especially if there is documented deficiency.⁴

- Designer endonucleases (TALENs and CRISPR-CAS9) are under active investigation as tools for reducing mutation heteroplasmy below the phenotypic threshold.⁹
- At-risk individuals may also benefit from assessment to initiate baseline evaluations (neurology, cardiology, ophthalmology, and audiology) and potential intervention prior to exhibiting clinical manifestations.⁵ Screening for diabetes mellitus by fasting serum glucose concentration and glucose tolerance test is recommended.¹
- Diagnosis of MELAS is based on a combination of clinical findings and genetic testing.^{1,10}
- MELAS 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 will not pass it on to his children.^{1,10,11}
- Mutations in the mtDNA gene, MT-TL1, cause MELAS. A majority of affected individuals with classic symptoms, about 80%, have a specific mutation, m.3243A>G.^{1,10,11} Other rare mtDNA mutations in the MT-TL1 gene, m.3271T>C and m.3252A>G, and in 9 other mtDNA genes are also associated with MELAS.^{1,10}
- Genetic test results alone cannot predict the exact course or phenotype of the disease.^{1,10} For all mtDNA mutations, clinical expressivity depends on the three following factors:¹
 - The relative abundance 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).
- There is suggested clinical utility with the use of genetic testing for MELAS at the present time. Each patient and family is unique therefore it necessary to consider the case to determine the clinical utility in regards to impactful management.¹⁰ This may include changes to stroke treatment, treatment during illness, the use of anesthesia, the use of exercise as treatment, and the use of vitamin and xenobiotics.⁶

Test Information

- The investigation and diagnosis of patients with mitochondrial respiratory chain 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.¹⁰
- Targeted mutation testing for MELAS is available at many laboratories. The specific mutations included in these targeted tests can vary by laboratory; however, they typically include the most common pathogenic variant found in MELAS, m.3243A>G.
- The common MELAS mutations are also included on a number of more general mitochondrial targeted mutation panels (in conjunction with genes for LHON, MERFF and Leigh syndrome).
- Full sequencing of the entire mitochondrial genome can be done to identify the remaining rare mtDNA mutation in individuals affected with MELAS. Since the mitochondrial genome is highly polymorphic, this is not routinely offered unless clinical suspicion is very high and there is no

evidence of paternal transmission.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 MELAS 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%.^{12,13}

- A number of large panels sequence the mitochondrial genome in conjunction with nuclear-encoded mitochondrial genes for a broad approach to testing.
- DNA testing can be performed on a blood specimen. Muscle biopsy is generally not necessary, but some labs accept blood, saliva and muscle samples.
- A muscle biopsy or heteroplasmy analysis in urine may be recommended for testing of m.3243A>G variant in cases with a clinical presentation of classic MELAS and where the variant is not detected on blood specimens.¹ If the status of heteroplasmy is of concern, next generation testing with high read depth may be preferable, however certain targeted mutation analysis can detect low level heteroplasmy.
- The m.3243A>G variant heteroplasmic level in blood samples has been shown to decrease with age.^{14,15,16} Fibroblasts, muscle, urine sediment, and hair follicle samples typically have a higher level of heteroplasmy compared to blood.¹⁷
- 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 MELAS 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.^{1,18}
 - Published guidelines from the 74th European Neuromuscular Centre International Consensus stated that women known or suspected of carrying the m.3243A>G mutation have a significant risk for affected offspring. Preimplantation genetic diagnosis and CVS probably predict m.3243A>G mutation load in offspring. However, careful counseling is recommended because severity is less clearly related to mutant load. Mutant DNA may be undetectable in women capable of transmitting the disorder; the level of mutant mtDNA in blood falls with time. Sampling of other tissues, including oocytes, may be of value.¹⁹
 - 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.²⁰
 - 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 for MELAS.
- The **Mitochondrial Medicine Society**⁴ developed consensus recommendations for the diagnosis and management of mitochondrial disease using the Delphi method and published them in 2015. Testing strategies, including strategies for genetic testing, were discussed.
 - 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 to guide genetic counseling.
 - Heteroplasmy analysis in urine can selectively be more informative and accurate than testing in blood alone, especially in cases of MELAS due to the common m.3243A>G mutation.
 - “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
 - Muscle (and/or liver) biopsies should be performed in the routine analysis for mitochondrial disease when the diagnosis cannot be confirmed with DNA testing of other more accessible tissues.”
- Quantitative 3-methylglutaconic (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.²¹
- The **European Federation of Neurological Sciences (EFNS, 2009)** provided molecular diagnostic consensus-based guidelines based on literature reviews:²²
 - "If the phenotype suggests syndromic mitochondrial disease due to mtDNA point mutations (MELAS, MERRF, NARP, LHON) DNA-microarrays using allele-specific oligonucleotide hybridization, real-time-PCR or single-gene sequencing are indicated."⁵
- The clinical utility of genetic testing for MELAS was described by a workshop of the **National Institute of Neurological Disorders and Stroke (2008)**:²³
 - "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."
 - Initial screening includes testing for blood lactate, urine amino acids, acyl-carnitine profile, and MRI. "It is important to note that biochemical abnormalities may not be present during periods when the mitochondrial disease is quiescent/dormant."
- The **Clinical Molecular Genetics Society (CMGS) of UK (2008)** provided practice-based guidelines for the molecular diagnosis of mitochondrial disease:¹⁰
 - In cases with strong clinical evidence, testing should begin with checking for the common m.3243A>G mutation. Testing for the rare mutations including m.3271T>C and m.3252A>G is not routinely indicated unless there is strong clinical diagnosis of MELAS testing.

Criteria

MELAS 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 MELAS*, and
 - MELAS pathogenic variant identified in 1st degree biological maternal relative, AND
- Predictive Testing for Asymptomatic Individual:
 - 18 years of age or older, or
 - Under the age of 18 years, and
 - Presymptomatic screening for diabetes mellitus is being considered, OR
- Diagnostic Testing for Symptomatic Individual:
 - Clinical exam and/or biochemical testing suggestive, but not confirmatory, of a diagnosis of MELAS, OR
- Prenatal Testing for At-Risk Pregnancies:
 - MELAS causing pathogenic variant in a previous child or in the mother.

MELAS Targeted Mutation Analysis (m.3243A>G)

- 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 for MELAS*, and
 - No known MELAS 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 MELAS by one or more of the following:
 - Lactic acidosis both in blood and in the CSF,¹ and/or
 - Muscle biopsy showing ragged red fibers,¹ and/or
 - Respiratory chain enzyme studies that are consistent with a diagnosis of MELAS,¹ and/or
 - Stroke-like episodes before the age of 40 years,¹ and/or
 - Encephalopathy with seizures and/or dementia,¹ 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.

MELAS Targeted Mutation Analysis (m.13513G>A, m.3271T>C, and m.3252A>G)

- Genetic Counseling:
 - Pre and post-test counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Criteria for MELAS targeted mutation analysis (m.3243A>G) is met, AND
- No pathogenic variants identified in the targeted mutation analysis (m.3243A>G).

Whole mtDNA Sequencing

- Genetic Counseling:
 - Pre and post-test counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Criteria for MELAS Targeted Mutation Analysis is met, AND
- No pathogenic variants identified in the Targeted Mutation Analysis (m.3243A>G , m.13513G>A, m.3271T>C, and m.3252A>G) 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.

References

1. DiMauro S, Hirano M. MELAS. 2001 Feb 27 [Updated 2013 Nov 21]. In: Pagon RA, Adam MP, Ardinger HH, et al., editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2016. Available from: <http://www.ncbi.nlm.nih.gov/books/NBK1233/>
2. Majamaa K, Moilanen JS, Uimonen S, Remes AM, Salmela PI, Kärppä M, Majamaa-Voltti KA, Rusanen H, Sorri M, Peuhkurinen KJ, Hassinen IE. Epidemiology of A3243G, the mutation for mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes: prevalence of the mutation in an adult population. *Am J Hum Genet.* 1998;63:447–454.
3. Uusimaa J, Moilanen JS, Vainionpää L, Tapanainen P, Lindholm P, Nuutinen M, Löppönen T, Mäki-Torkko E, Rantala H, Majamaa K. Prevalence, segregation, and phenotype of the mitochondrial DNA 3243ANG mutation in children. *Ann Neurol.* 2007;62:278–287.
4. Parikh S, Goldstein A, Koenig MK, Scaglia F, Enns G, Saneto R, Anselm I, Cohen B, Falk M, Greene C, Gropman A, Haas R, Hirano M, Morgan P, Sims K, Tanopolsky M, Van Hove JLK, Wolfe L, DiMauro S. Diagnosis and management of mitochondrial disease: a consensus statement from the Mitochondrial Medicine Society. *Gen in Med.* 2015; (17):689-701.
5. Koga Y, Akita Y, Nishioka J, Yatsuga S, Povalko N, Tanabe Y, Fujimoto S, Matsuishi T. L-Arginine improves the symptoms of stroke-like episodes in MELAS. *Neurology.* 2005;64:710–712.
6. Koga Y, Akita Y, Nishioka J, Yatsuga S, Povalko N, Katayama K, Matsuishi T. MELAS and L-arginine therapy. *Mitochondrion* 2007;7:133–139.
7. El-Hattab AW, Hsu JW, Emrick LT, Wong LJ, Craigen WJ, Jahoor F, Scaglia F. Restoration of impaired nitric oxide production in MELAS syndrome with citrulline and arginine supplementation. *Mol Genet Metab.* 2012;105:607–614.

8. El-Hattab AW, Emrick LT, Craigen WJ, Scaglia F. Citrulline and arginine utility in treating nitric oxide deficiency in mitochondrial disorders. *Mol. Genet. Metab.* 2012;107:247–252.
9. Pereira CV, Moraes CT. Current strategies towards therapeutic manipulation of mtDNA heteroplasmy. *Front Biosci (Landmark Ed).* 2017;22:991-1010.
10. Clinical Molecular Genetics Society Guidelines (CMGS), UK. Practice guidelines for the molecular diagnosis of mitochondrial diseases. July 2008. Available at http://www.acgs.uk.com/media/774654/mito_2008.pdf
11. Mao C, Holt I. Clinical and Molecular Aspects of Diseases of Mitochondrial DNA Instability. *Chang Gung Med J.* 2009;32:354-69.
12. Zhang W, Cui H, Wong LJ. Comprehensive one-step molecular analyses of mitochondrial genome by massively parallel sequencing. *Clin Chem.* 2012;58(9):1322-31.
13. Cui H, Li F, Chen D, et al. Comprehensive next-generation sequence analyses of the entire mitochondrial genome reveal new insights into the molecular diagnosis of mitochondrial DNA disorders. *Genet Med.* 2013;15(5):388-94.
14. Frederiksen AL, Andersen PH, Kyvik KO, Jeppesen TD, Vissing J, Schwartz M. Tissue specific distribution of the 3243A->G mtDNA mutation. *J Med Genet.* 2006;43:671–7.
15. Hammans SR, Sweeney MG, Hanna MG, Brockington M, Morgan-Hughes JA, Harding AE. The mitochondrial DNA transfer RNA^{Leu}(UUR) A->G(3243) mutation. A clinical and genetic study. *Brain* 1995;118(Pt 3):721–34.
16. Rahman S, Poulton J, Marchington D, Suomalainen A. Decrease of 3243 A->G mtDNA mutation from blood in MELAS syndrome: a longitudinal study. *Am J Hum Genet.* 2001;68:238–40.
17. Chin J, Marotta R, Chiotis M, Allan EH, Collins SJ. Detection rates and phenotypic spectrum of m.3243A>G in the MT-TL1 gene: a molecular diagnostic laboratory perspective. *Mitochondrion.* 2014;17:34–41.
18. Bredenoord AL, Pennings G, Smeets HJ, de Wert G. Dealing with uncertainties: ethics of prenatal diagnosis and preimplantation genetic diagnosis to prevent mitochondrial disorders. *Hum Reprod. Update.* 2008;14(1):83-94.
19. Poulton J, Turnbull, DM. 74th ENMC international workshop: Mitochondrial diseases 19–20 November 1999, Naarden, The Netherlands, *Neuromuscular Disorders.* 2000;10(6):460–462. doi: 10.1016/S0960-8966(00)00101-2.
20. Nesbitt V, Alston CL, Blakely EL, Fratter C, Feeney CL, Poulton J, Brown GK, Turnbull DM, Taylor RW, McFarland R. A national perspective on prenatal testing for mitochondrial disease. 2014; 22(11).
21. Montero et al. GDF-15 Is Elevated in Children with Mitochondrial Diseases and Is Induced by Mitochondrial Dysfunction. *PLOS One.* 2016;Feb 11;11(2).
22. Finsterer J, Harbo HF, Baets J, et al. EFNS guidelines on the molecular diagnosis of mitochondrial disorders. *Eur J Neurol.* 2009;16(12):1255-64.
23. National Institute of Neurological Disorders and Stroke (NINDS, NIH). Available at: http://www.ninds.nih.gov/news_and_events/proceedings/20090629_mitochondrial.htm.

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