Mitochondrial Genome Sequencing, Deletion/Duplication, and/or Nuclear Gene Panel

I. Mitochondrial genome sequencing, deletion/duplication, and/or nuclear genes analysis to establish or confirm a diagnosis of a primary mitochondrial disorder is considered **medically necessary** when:

A. The member has a classic phenotype of one of the maternally inherited syndromes (e.g., Leber hereditary optic neuropathy, mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes [MELAS], myoclonic epilepsy with ragged red fibers [MERRF], maternally inherited deafness and diabetes [MIDD], neuropathy, ataxia, retinitis pigmentosa [NARP], Kearns-Sayre syndrome/CPEO); or of a nuclear DNA mitochondrial disorder (e.g., mitochondrial neurogastrointestinal encephalopathy [MNGIE]); **OR**

B. The member has non-specific clinical features suggestive of a primary mitochondrial disorder and meets **ALL** of the following:

1. Clinical findings of at least two of the following:

a) Ptosis, **OR**

b) External ophthalmoplegia, **OR**

c) Proximal myopathy, **OR**

d) Exercise intolerance, **OR**

e) Cardiomyopathy, **OR**

f) Sensorineural deafness, **OR**

g) Optic atrophy, **OR**

h) Pigmentary retinopathy, **OR**

i) Diabetes mellitus, **OR**

j) Fluctuating encephalopathy, **OR**

k) Seizures, **OR**

l) Dementia, **OR**

m) Migraine, **OR**

n) Stroke-like episodes, **OR**

o) Ataxia, **OR**

p) Spasticity, **OR**

q) Chorea, **OR**

r) Multiple late term pregnancy loss, **AND**

2. Conventional biochemical laboratory studies have been completed and are non-diagnostic, including at least: plasma or CSF lactic acid concentration, ketone bodies, plasma acylcarnitines, and urinary organic acids, **AND**

3. Additional diagnostic testing indicated by the member's clinical presentation (e.g., fasting blood glucose, electrocardiography, neuroimaging, electromyography, echocardiography, audiology, thyroid testing, electroencephalography, exercise testing) have been completed and are non-diagnostic.

II. Mitochondrial genome sequencing, deletion/duplication, and/or nuclear genes analysis to establish or confirm a diagnosis of a primary mitochondrial disorder is considered **investigational** for all other indications.

RATIONALE AND REFERENCES

Mitochondrial Genome Sequencing, Deletion/Duplication, and/or Nuclear Gene Panel

Mitochondrial Medicine Society

In 2015, the Mitochondrial Medicine Society published the following consensus recommendations for DNA testing for mitochondrial disorders:

- 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.
- mtDNA deletion and duplication testing should be performed in cases of suspected mitochondrial disease via NGS of the mtDNA genome, especially in all patients undergoing a diagnostic tissue biopsy.
    - If a single small deletion is identified using polymerase chain reaction–based analysis, then one should be cautious in associating these findings with a primary mitochondrial disorder.
    - When multiple mtDNA deletions are noted, sequencing of nuclear genes involved in mtDNA biosynthesis is recommended.
- When a tissue specimen is obtained for mitochondrial studies, mtDNA content (copy number) testing via real-time quantitative polymerase chain reaction should strongly be considered for mtDNA depletion analysis because mtDNA depletion may not be detected in blood.

    - mtDNA proliferation is a nonspecific compensatory finding that can be seen in primary mitochondrial disease, secondary mitochondrial dysfunction, myopathy, hypotonia, and as a by-product of regular, intense exercise.
- 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 known mutation is identified via known NGS gene panels, then whole exome sequencing should be considered (p. 692-693).

Parikh S, Goldstein A, Koenig MK, et al. Diagnosis and management of mitochondrial disease: a consensus statement from the Mitochondrial Medicine Society. Genet Med. 2015;17(9):689-701. doi:10.1038/gim.2014.177

GeneReviews: Primary Mitochondrial Disorders Overview

GeneReviews is an expert-authored review of current literature on a genetic disease, and goes through a rigorous editing and peer review process before being published online.

Their recommendations are as follows:

Common clinical features of mitochondrial disorders include:

- ptosis
- external ophthalmoplegia
- proximal myopathy
- exercise intolerance
- cardiomyopathy
- sensorineural deafness
- optic atrophy
- pigmentary retinopathy
- diabetes mellitus
- fluctuating encephalopathy
- seizures
- dementia
- migraine

- stroke-like episodes
- ataxia
- spasticity
- chorea
- high incidence of mid- and late-pregnancy loss

When a patient's clinical picture is nonspecific but highly suggestive of a mitochondrial disorder, the clinician should start with measurement of plasma or CSF lactic acid concentration, ketone bodies, plasma acylcarnitines, and urinary organic acids.

Traditionally, the diagnosis of mitochondrial disorders has been based on demonstrating mitochondrial dysfunction in a relevant tissue biopsy (e.g., a skeletal muscle or liver biopsy, or skin fibroblasts), with the particular pattern of biochemical abnormality being used to direct targeted molecular genetic testing of mtDNA, specific nuclear genes, or both.

However, the more widespread availability of molecular diagnostic techniques and the advent of exome and genome sequencing has changed the diagnostic approach.

One important caveat arises from the fact that many mtDNA pathogenic variants are heteroplasmic, and the proportion of mutated mtDNA in blood may be undetectable. This can be circumvented by analyzing mtDNA from another tissue – typically skeletal muscle or urinary epithelium – in which the level of heteroplasmy tends to be higher. Some common mtDNA pathogenic variants (e.g., large-scale deletions causing CPEO) may only be detected in skeletal muscle.

In individuals with a specific clinical phenotype (e.g., MELAS, LHON, POLG-related disorders), it may be possible to reach a diagnosis with targeted analysis of specific mtDNA pathogenic variants or single-gene testing of a nuclear gene.

A mitochondrial disorder multigene panel is most likely to identify the genetic cause of the condition while limiting identification of variants of uncertain significance and pathogenic variants in genes that do not explain the underlying phenotype.

Comprehensive genomic testing does not require the clinician to determine which gene is likely involved. Such testing includes exome sequencing, genome sequencing, and mitochondrial sequencing which can simultaneously analyze nuclear DNA and mtDNA.

Chinnery PF. Primary Mitochondrial Disorders Overview. 2000 Jun 8 [Updated 2021 Jul 29]. In: Adam MP, Ardinger HH, Pagon RA, et al., editors. GeneReviews [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2025. Available from: https://www.ncbi.nlm.nih.gov/books/NBK1224/

DEFINITIONS

1. **Autism spectrum disorder** is defined in the DSM V as persistent deficits in social communication and social interaction across multiple contexts, as manifested by the following, currently or by history:

    a. Deficits in social-emotional reciprocity, ranging, for example, from abnormal social approach and failure of normal back-and-forth conversation; to reduced sharing of interests, emotions, or affect; to failure to initiate or respond to social interactions.

    b. Deficits in nonverbal communicative behaviors used for social interaction, ranging, for example, from poorly integrated verbal and nonverbal communication; to abnormalities in eye contact and body language or deficits in understanding and use of gestures; to a total lack of facial expressions and nonverbal communication.

    c. Deficits in developing, maintaining, and understanding relationships, ranging, for example, from difficulties adjusting behavior to suit various social contexts; to difficulties in sharing imaginative play or in making friends; to absence of interest in peers.

2. **Close relatives** include first, second, and third-degree blood relatives:

    a. **First-degree relatives** are parents, siblings, and children

    b. **Second-degree relatives** are grandparents, aunts, uncles, nieces, nephews, grandchildren, and half siblings

    c. **Third-degree relatives** are great grandparents, great aunts, great uncles, great grandchildren, and first cousins

3. **Congenital anomalies** (according to ACMG) are anomalies not specific to a well-delineated genetic syndrome. These are structural or functional abnormalities requiring medical intervention that are usually evident at birth, or shortly thereafter, and are consequential to an individual's life expectancy, health status, or physical/social functioning.

4. **Developmental delay** (DD) is defined as slow-to-meet or not reaching milestones in one or more of the areas of development (communication, motor, cognition, social-emotional, or adaptive skills) in the expected way for a child's age.

5. **Dissection** refers to a tear in the inner layer of a main artery (aorta).

    a. **Type A aortic dissections** occur at the ascending part of the aorta, just as it branches off of the heart.

    b. **Type B aortic dissections** occur at the descending part of the aorta, and may extend into the abdomen.

6. **Exome Sequencing** (ES) is a genomic technique for sequencing all of the protein-coding regions of genes in the genome (also known as the exome).

7. **Genome Sequencing** (GS) is a genomic technique for sequencing the complete DNA sequence, which includes protein coding as well as non-coding DNA elements.

8. **Global developmental delay** is diagnosed when a child under age 5 is slow-to-meet or not reaching milestones in the expected way for their age in at least two areas of development (communication, gross/fine motor, cognition, social-emotional, or adaptive skills). Examples include (but are not limited to): not sitting independently by 9 months; not crawling or rolling over by a year; not walking by 18 months (based on CDC Developmental milestones).

9. **Intellectual disability (ID)** is defined by the DSM V as an individual age 5 or older with either an IQ score of 70 or below, OR with a clinical diagnosis of intellectual disability per the DSM V, which includes all of the following:

a. Deficits in intellectual functions, such as reasoning, problem solving, planning, abstract thinking, judgment, academic learning, and learning from experience, confirmed by both clinical assessment and individualized, standardized intelligence testing.

b. Deficits in adaptive functioning that result in failure to meet developmental and sociocultural standards for personal independence and social responsibility. Without ongoing support, the adaptive deficits limit functioning in one or more activities of daily life, such as communication, social participation, and independent living, across multiple environments, such as home, school, work, and community.

c. Onset of intellectual and adaptive deficits during the developmental period.

10. **Mitochondrial disorder** refers to a heterogenous group of disorders caused by dysfunctional mitochondria, the organelles responsible for oxidative phosphorylation within the cell.

11. **Reanalysis of exome sequencing (ES) (aka exome sequencing reanalysis) or genome sequencing (GS) (aka genome sequencing reanalysis)** involves a bioinformatic re-review of both reported and unreported variants detected by the original assay. This is typically performed when (1) the patient's phenotype has changed and the changes are not explainable by the original result or (2) the original test was not diagnostic and the clinician or laboratory suspect that advances in variant classification or analysis pipelines may result in a diagnosis. Reanalysis may not be possible or useful in some situations due to changes in bioinformatic pipeline compatibility or new information regarding the genetic etiology of a condition that could explain the patient's clinical features but would not have been captured by previous ES or GS sequencing methods. **Exome sequencing reanalysis** or **Reanalysis of exome** may not be possible in some situations. Sequencing platforms may have changed substantially enough that the performing lab can no longer use the data from the original ES in their pipeline. Specifically, ES reanalysis may not be possible if there have been improvements in technology/chemistry (e.g., new methods for DNA capture and/or sequencing), bioinformatics advancements, or there is new information

regarding the genetic etiology of a condition that could explain the patient's clinical features and would not have been able to be detected by the previous exome sequencing.

12. **Trio Testing** is testing of the child and both biological/genetic parents, which increases the chances of finding a definitive diagnosis while reducing false-positive findings.