Mitochondrial Disorders Genetic Testing

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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.

Procedures addressed by this guideline	Procedure codes
Genomic Unity Comprehensive Mitochondrial Disorders Analysis	0417U
Mitochondrial Disorder Known Familial Mutation Analysis	81403
MT-ATP6 Targeted Mutation Analysis	81401
MT-ND1 Targeted Mutation Analysis	81479
MT-ND4, MT-ND6 Targeted Mutation Analysis	81401
MT-ND5 Targeted Mutation Analysis	81401
MT-TK Targeted Mutation Analysis	81401
MT-TL1 Targeted Mutation Analysis	81401
Nuclear Encoded Mitochondrial Gene Sequencing Panel	81440
TYMP Sequencing	81405
Whole Mitochondrial Genome Sequencing	81460
Whole Mitochondrial Genome Deletion/Duplication Analysis	81465

Criteria

Known Familial Mutation Testing

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

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- Previous Genetic Testing:
  - No previous genetic testing inclusive of the known familial mutation, and
  - Disease causing mutation(s) identified in 1st degree biological relative, and
  - Member is at risk to have the familial mutation based on inheritance pattern of the disorder in question, AND
- Predictive Testing for Asymptomatic Individuals:
  - 18 years of age or older, or
  - Under the age of 18 years, and
    - Test results are needed for treatment or medical screening, OR
- Diagnostic Testing for Symptomatic Individuals:
  - Clinical examination and/or biochemical results are suggestive, but not confirmatory, of the familial diagnosis, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

Targeted Mutation Analysis or Single Gene Analysis

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Genetic Testing:
  - No previous genetic testing for the mitochondrial disorder to be targeted, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Clinical examination and/or biochemical results are suggestive, but not confirmatory, of the targeted disorder (see table titled *Select Mitochondrial Disorders)*, and
  - Inheritance pattern is consistent with the targeted mitochondrial disorder, AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

Whole Mitochondrial DNA (mtDNA) Sequencing

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Testing:

- Member has not had previous whole mtDNA sequencing performed, and
- Targeted mitochondrial testing, if performed, was negative, and
- Biochemical testing appropriate for the suspected disorder has been performed and is not confirmatory of a diagnosis of a specific mitochondrial condition, AND

- Diagnostic Testing for Symptomatic Individuals:
  - Member has multiple organ system involvement defined as altered function in two or more organ systems, suggestive of a mitochondrial disorder, 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, brain magnetic resonance imaging (MRI) or magnetic resonance spectroscopy (MRS) results consistent with a mitochondrial process, and/or pathology results consistent with a mitochondrial process, and
  - Targeted mutation analysis is not feasible because of one of the following:
    - Member's clinical presentation does not fit a well-described syndrome for which single-gene or targeted panel testing is available (see table titled *Select Mitochondrial Disorders*), or
    - Member's clinical presentation fits a well-described syndrome and applicable single-gene or targeted mutation analysis was negative, 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., no evidence of paternal transmission), AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

Whole Mitochondrial DNA (mtDNA) Deletion/Duplication Analysis

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Testing:
  - Member has not had previous whole mtDNA deletion/duplication analysis performed, and
  - Targeted mitochondrial deletion testing, if performed, was negative , and
  - Biochemical testing appropriate for the suspected disorder has been performed and is not confirmatory of a diagnosis of a specific mitochondrial condition, AND

- Diagnostic Testing for Symptomatic Individuals:
  - Member has multiple organ system involvement defined as altered function in two or more organ systems, suggestive of a mitochondrial disorder, 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, brain magnetic resonance imaging (MRI) or magnetic resonance spectroscopy (MRS) results consistent with a mitochondrial process, and/or pathology results consistent with a mitochondrial process, and
  - Targeted mutation analysis is not feasible because of one of the following:
    - Member's clinical presentation does not fit a well-described syndrome for which single-gene or targeted panel testing is available (see table titled *Select Mitochondrial Disorders*), or
    - Member's clinical presentation fits a well-described syndrome and applicable single-gene or targeted mutation analysis was negative, 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., no evidence of paternal transmission), AND
- Rendering laboratory is a qualified provider of service per the Health Plan policy.

Nuclear Encoded Mitochondrial Gene Sequencing Panel

- Genetic Counseling:
  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Previous Testing:
  - Member has not had a previous nuclear encoded mitochondrial gene sequencing panel testing performed, and
  - Targeted nuclear-encoded mitochondrial gene testing (e.g., TYMP or POLG analysis), if performed, was negative, and
  - Biochemical testing appropriate for the suspected disorder has been performed and is not confirmatory of a diagnosis of a specific mitochondrial condition, AND
- Diagnostic Testing for Symptomatic Individuals:
  - Member has multiple organ system involvement defined as altered function in two or more organ systems, suggestive of a mitochondrial disorder, 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, brain magnetic resonance imaging (MRI) or magnetic resonance spectroscopy (MRS) results consistent with a mitochondrial process, and/or pathology results consistent with a mitochondrial process, and
- Targeted mutation analysis is not feasible because of one of the following:
  - Member's clinical presentation does not fit a well-described syndrome for which single-gene or targeted panel testing is available (see table titled *Select Mitochondrial Disorders*), or
  - Member's clinical presentation fits a well-described syndrome and applicable single-gene or targeted mutation analysis was negative, 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), AND

- Rendering laboratory is a qualified provider of service per the Health Plan policy.

Other considerations

- For information on POLG-related disorders, please refer to the guideline *Polymerase Gamma (POLG) Related Disorders Genetic Testing*, as this testing is not addressed here.
- Testing addressed in this guideline applies to individuals in whom a mitochondrial disorder is suspected based on a constellation of findings commonly seen in these conditions. Mitochondrial genetic testing is not considered medically necessary in the following cases:
  - The individual'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.

Table: Select Mitochondrial Disorders

Disorder, genes, CPT code, symptoms

Mitochondrial Disorder	Associated Genes / Mitochondrial DNA Mutations	CPT Code(s)	Symptoms
Leber Hereditary Optic Neuropathy (LHON)	MT-ND1, MT-ND4, MT-ND6	81401, 81479	Bilateral painless subacute vision loss that begins in the second and third decades of life, central or cecocentral scotomas, and/or impaired color vision
Mitochondrial Encephalopathy, Lactic Acidosis, and Stroke-like Episodes (MELAS)	MT-TL1, MT-ND5	81401	Stroke-like episodes, encephalopathy with seizures, and/or dementia, muscle weakness and exercise intolerance, recurrent headaches, recurrent vomiting, hearing impairment, peripheral neuropathy, learning disability, and/or short stature
Mitochondrial Epilepsy with Ragged Red Fibers (MERRF)	MT-TK	81401	Myoclonus, generalized epilepsy, ataxia, weakness, dementia, and/or ragged red fibers on muscle biopsy

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Mitochondrial Disorder	Associated Genes / Mitochondrial DNA Mutations	CPT Code(s)	Symptoms
Mitochondrial Neurogastrointestinal Encephalopathy (MNGIE)	TYMP	81405	Progressive gastrointestinal dysmotility (possibly presenting as nausea, dysphagia, reflux, early satiety, vomiting after a meal, episodic abdominal pain, bloating, and/or diarrhea), cachexia, ptosis, ophthalmoplegia, leukoencephalopathy, and/or peripheral neuropathy
Neurogenic Muscle Weakness, Ataxia, and Retinitis Pigmentosa (NARP)	MT-ATP6	81401	Proximal neurogenic muscle weakness with sensory neuropathy, ataxia, learning difficulties, and/or pigmentary retinopathy

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Mitochondrial Disorder	Associated Genes / Mitochondrial DNA Mutations	CPT Code(s)	Symptoms
POLG-Related Disorders (Alpers-Huttenlocher syndrome (AHS), Childhood Myocerebrohepatopathy Spectrum (MCHS), Myoclonic Epilepsy Myopathy Sensory Ataxia (MEMSA), Ataxia Neuropathy Spectrum (ANS), Autosomal Dominant or Autosomal Recessive Progressive External Ophthalmoplegia (adPEO/arPEO))	POLG	81406	Please refer to the guideline *Polymerase Gamma (POLG) Related Disorders Genetic Testing*
Mitochondrial Nonsyndromic Hearing Loss and Deafness	MT-RNR1, MT-TS1	81401, 81403	Please refer to the guideline *Nonsyndromic Hearing Loss and Deafness Genetic Testing*
mtDNA Deletion Syndromes (Kearns-Sayre Syndrome (KSS), Pearson syndrome, Progressive External Ophthalmoplegia (PEO))	Full mtDNA Deletion Analysis	81465	KSS: childhood onset of pigmentary retinopathy and/or progressive external ophthalmoplegia Pearson syndrome: sideroblastic anemia and/or exocrine pancreas dysfunction PEO: ptosis

Billing and Reimbursement

Introduction

This section outlines the billing requirements for tests addressed in this guideline. These requirements will be enforced during the case review process whenever appropriate. Examples of requirements may include specific coding scenarios, limits on allowable test combinations or frequency and/or information that must be provided on a claim for automated processing. Any claims submitted without the necessary information to allow for automated processing (e.g., ICD code, place of service, etc.) will not be reimbursable as billed. Any claim may require submission of medical records for post service review.

- When otherwise reimbursable, the following limitations apply:
  - Any individual gene or multi-gene panel is only reimbursable once per lifetime.
  - When a whole mtDNA analysis or a panel is being performed, it is only reimbursable when billed with a single, appropriate panel procedure code (e.g., 81460 for Whole mtDNA Sequencing, 81465 for Whole mtDNA Deletion/Duplication, and 81440 for Nuclear Encoded Mitochondrial Gene Sequencing Panels)*.
  - When use of a panel code is not possible, each billed component procedure will be assessed independently.
  - In general, only a limited number of panel components that are most likely to explain the member's presentation will be reimbursable. The remaining panel components will not be reimbursable.
  - If more than one test or procedure code is requested at one time, the member meets criteria for all tests requested, and mtDNA and nuclear DNA mutations (or causes) are equally likely based on personal history, clinical findings, and family history, the testing will be tiered in the following order: 81460, 81465, 81440.
  - If a single panel code is requested that includes testing of both mtDNA and nuclear DNA (e.g., 0417U), the member meets criteria for all tests described by the requested code, and mtDNA and nuclear DNA mutations (or causes) are equally likely based on personal history, clinical findings, and family history, the code will be reimbursable.

Note *The panel code(s) listed here may not be all-inclusive. For further discussion of what is considered an appropriate panel code, please refer to the guideline *Genetic Testing by Multigene Panels*.

For general coding requirements, please refer to the guideline *Laboratory Procedure Code Requirements*.

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What are mitochondrial disorders?

Definition

Mitochondrial disorders are conditions resulting from mutations in the nuclear (nDNA) or mitochondrial (mtDNA) genes that are involved in the production, function, maintenance, or transmission of mitochondria.

Incidence

Mitochondrial disorders have an estimated minimum incidence of 1 in 5,000.[1]

Symptoms

Mitochondrial disorders are 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.[2,3]

Mitochondrial disease is suspected in individuals with a combination of clinical features which can include any of the following:

- 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
- Gastrointestinal: recurrent vomiting, anorexia
- Sensorineural hearing loss
- Diabetes mellitus
- Growth: failure to thrive, short stature
- Mid- or late-pregnancy loss

Several mitochondrial disorders, due to mutations in the mtDNA, are characterized by a cluster of clinical features or syndromic presentation. These disorders are described in the table titled *Select Mitochondrial Disorders.*

Cause

Mitochondrial disorders result from dysfunction of the mitochondrial respiratory chain due to abnormality of the production, function, maintenance, or transmission of mitochondria.[2] They can be caused by mutations in either mitochondrial or nuclear DNA.

Underlying nDNA and mtDNA causes are frequently indistinguishable based on this symptomology. Diagnosis of the majority of mitochondrial conditions is based on a combination of clinical findings and genetic testing.[4,5]

For all mtDNA mutations, clinical expressivity depends on the three following factors:[2]

- The ratio of mutant mtDNA to normal mtDNA (mutational load or 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).

Inheritance

Mitochondrial conditions due to mutations in the mtDNA are maternally inherited or may be de novo. Mitochondrial conditions caused by mutations in the nuclear DNA can be maternally or paternally inherited and may follow autosomal dominant, autosomal recessive, or X-linked inheritance.

Mitochondrial Inheritance

MtDNA mutations may be de novo (not inherited) or follow maternal inheritance. This means that a female who carries the mtDNA 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 mutation cannot pass it on to his children. Clinical expressivity of mtDNA mutations depends on the degree of heteroplasmy and the organs and tissues most affected by the mutation.

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.[4,6] mtDNA deletions are rarely transmitted (less than 1% empiric risk).[2] If the mother is symptomatic, then the recurrence risk is approximately 4%. A male who carries the mtDNA mutation will not pass it on to his children.[4,6,7]

Autosomal dominant inheritance

In autosomal dominant inheritance, individuals have 2 copies of the gene and only one mutation is required to cause disease. When a parent has a mutation, each offspring has a 50% risk of inheriting the mutation. Males and females are equally likely to be affected.

Autosomal recessive inheritance

In autosomal recessive inheritance, individuals have 2 copies of the gene and an individual typically inherits a gene mutation from both parents. Usually only siblings are at risk for also being affected. Males and females are equally affected. Individuals who inherit only one mutation are called carriers. Carriers do not

typically show symptoms of the disease, but have a 50% chance, with each pregnancy, of passing on the mutation to their children. If both parents are carriers of a mutation, the risk for each pregnancy to be affected is 1 in 4, or 25%.

X-Linked Inheritance

In X-linked inheritance, the mutation is carried on the X chromosome. Females have two X chromosomes, and males have one. Males typically have more severe symptoms than females. A female with a mutation has a 50% chance to pass that mutation to her children. A male with a mutation cannot pass the mutation to any sons, but will pass it to all daughters. A process called X-inactivation in females results in random inactivation of expression of one X-chromosome in each cell of the body. For females with one mutation, the percentage and distribution of cells with expression of the X chromosome carrying the mutation can influence the degree of severity.

Identification of a mutation in a proband may allow for informative testing of relatives at risk for diabetes, seizures, hearing loss, optic atrophy, and other findings in the corresponding phenotypic range.

Diagnosis

Clinical findings may point to a specific, well-described mitochondrial disorder, and the clinical diagnosis is often confirmed with molecular testing.[8]

The investigation and diagnosis of individuals with mitochondrial disease often necessitate a combination of techniques including clinical assessment and biochemical assessment, neuroimaging, molecular genetic studies, and sometimes muscle biopsy.

Biochemical assessment includes measurement of plasma or CSF lactate and pyruvate, glucose, creatine kinase (CK), transaminases (AST, ALT), ketone bodies, plasma acylcarnitines, and urinary organic acids. Normal plasma or CSF lactic acid concentration does not exclude the presence of a mitochondrial disorder.[2,6]

Brain magnetic resonance imaging (MRI) is recommended if CNS symptoms are present. Brain magnetic resonance spectroscopy (MRS) for elevated lactate is also useful. Neuroimaging results are not confirmatory, but may aid in the diagnosis of a mitochondrial disorder if other clinical features are present.

Molecular genetic testing for a mtDNA mutation should ideally be directed by the clinical phenotype and results of these other investigations.[2]

If a specific disorder is not evident, 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.

Management

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.[1,4,9]

Survival

Mitochondrial disorders are clinically heterogeneous with a wide range of severity and age of onset, depending upon the specific disorder.[1] While genetic test results alone cannot predict the exact course or phenotype of the disease, severity does correlate with mutation load for mtDNA mutations.[6,10]

Test information

Introduction

Testing for mitochondrial diseases may include known familial mutation analysis, targeted mutation analysis, mitochondrial genome sequencing, deletion/duplication analysis, and NGS panels.

Known Familial Mutation (KFM) Testing

Known familial mutations analysis is performed when a causative mutation has been identified in a close biological relative of the individual requesting testing. Analysis for known familial mutations typically includes only the single mutation. However, if available, a targeted mutation panel that includes the familial mutation may be performed.

Targeted Mutation Analysis

Targeted mutation analysis uses hybridization, single nucleotide extension, select exon sequencing, or similar methodologies to assess a set of disease-causing mutations. This analysis identifies common and/or recurring mutations. Targeted mutation panels or select exon sequencing may have differing clinical sensitivities dependent upon ethnicity, phenotypic presentation, or other case-specific characteristics.

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. "False negative rates vary by genomic region; therefore, genomic testing may not be as accurate as targeted single gene testing or multigene molecular genetic testing panels." [2]

Whole Mitochondrial Genome Sequencing

Full sequencing of the entire mitochondrial genome by next generation sequencing (NGS) is capable of simultaneously detecting point mutations, deletions, and point mutation heteroplasmies in the assessment of a number of overlapping mitochondrial syndromes. Since the mitochondrial genome is highly polymorphic, this is not routinely offered unless clinical suspicion is high and there is no evidence of paternal transmission. DNA testing can be performed on a blood specimen. Muscle biopsy is generally not necessary, but some labs accept blood, saliva, and muscle samples.

Deletion and Duplication Analysis

Analysis for deletions and duplications can be performed using a variety of technical platforms including exon array, Multiplex ligation-dependent probe amplification (MLPA), and NGS data analysis. These assays detect gains and losses too large to be identified through standard sequence analysis, often single or multiple exons or whole genes.

Multi-Gene Testing Panels

The efficiency of 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. Additionally, tests should be chosen to maximize the likelihood of identifying mutations in the genes of interest, contribute to alterations in management for an individual, and/or minimize the chance of finding variants of uncertain clinical significance.

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).

NGS testing is capable of simultaneously detecting point mutations, deletions, and point mutation heteroplasmies. Typically, Sanger sequence analysis will miss heteroplasmy below 20%. With suitable depth of coverage, NGS can detect heteroplasmy down to ~1%.[11,12]

Test Strategy

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.

“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.” [2]

Testing of alternative tissues by biochemical and/or molecular analysis may be required, especially if blood testing is negative and 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.

Guidelines and evidence

American College of Medical Genetics and Genomics

The American College of Medical Genetics and Genomics (ACMG, 2013) states the following regarding testing individuals with isolated autism for mitochondrial disorders:[13]

- "As with metabolic disorders, testing for mitochondrial disorders in persons with ASDs is recommended only if supporting symptoms or laboratory abnormalities are present."

European Federation of Neurological Sciences

The European Federation of Neurological Sciences (EFNS, 2009)[5] provided molecular diagnostic consensus-based guidelines based on literature reviews: "If the phenotype suggests syndromic MID [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."

International Consensus Statement on Leber Hereditary Optic Neuropathy

An international consensus conference (2017) with a panel of experts from Europe and North America made the following statements regarding the clinical and therapeutic management of LHON.[14]

- "LHON primarily is a clinical diagnosis…. A definitive diagnosis of LHON is rapidly obtained by the molecular identification of one of the 3 common mtDNA mutations (m.11778G>A/MT-ND4, m.3460G>A/MT-ND1, m.14484T>C/MTND6), accounting for about 90% of cases. If this primary screen is negative and there is a high index of clinical suspicion supported by a maternal mode of inheritance in a patient with a family history, sequencing the entire mtDNA is advisable to identify other, but rare, mtDNA mutations."
- "The diagnosis of LHON should be based on a careful history, evaluation of key structural and functional visual parameters, and on a molecular confirmation of a pathogenic mtDNA mutation. The management of LHON includes genetic counseling, informing the patient about potentially preventable lifestyle risk factors and, for subacute and dynamic cases, the use of idebenone at the currently approved dose. Idebenone should be discontinued in nonresponder patients and is currently not recommended in patients in the chronic stages of the disease. These guidelines and recommendations are based on a consensus developed on the current state of the literature. Further investigations and clinical trials are needed to

lead to better disease-modifying treatments and to improve the management of patients with LHON."

Mitochondrial Medicine Society

The Mitochondrial Medicine Society (MMS, 2015) developed consensus recommendations using the Delphi method.[15]

- 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."
  - "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.

United Kingdom Best Practices Guideline

A working group of Clinical Scientists from the NHS Highly Specialised Service, in collaboration with national laboratory consultation, published (2023) best practice guidelines for genetic testing for mitochondrial disease.[16] The guidelines summarize current recommended technologies and methodologies for analysis of mtDNA and nuclear-encoded genes in patients with suspected mitochondrial disease, as well as genetic testing strategies for diagnosis. The guidelines outline two main alternative strategies:[16]

- "Targeted testing of 'common' mtDNA variants and/or targeted nuclear testing, followed by more comprehensive testing if required and if resources allow."

- "NGS of the mitochondrial genome and/or nuclear genes, e.g., by whole exome sequencing (WES) or whole genome sequencing (WGS)."

Targeted testing can be appropriate for routine referrals where there is not an urgent clinical need to obtain a diagnosis, for clinical presentations which are highly suggestive of a particular variant or gene, and/or where resources are limited.

Comprehensive NGS-based testing can be used for all referral indications but is particularly appropriate for more complex phenotypes and/or for urgent referrals. Simultaneous testing of both mtDNA and nDNA is recommended, as both account for a significant proportion of childhood-onset and adult-onset mitochondrial disorders.

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