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| <b>Medical Benefit</b>  |     | <b>Effective Date:</b> 10/01/17                 | <b>Next Review Date:</b> 07/18 |
| <b>Preauthorization</b> | Yes | <b>Review Dates:</b> 07/14, 07/15, 07/16, 07/17 |                                |

**Preauthorization is required.**

*The following protocol contains medical necessity criteria that apply for this service. The criteria are also applicable to services provided in the local Medicare Advantage operating area for those members, unless separate Medicare Advantage criteria are indicated. If the criteria are not met, reimbursement will be denied and the patient cannot be billed. Please note that payment for covered services is subject to eligibility and the limitations noted in the patient's contract at the time the services are rendered.*

| Populations  | Interventions  | Comparators   | Outcomes  |
|--|--|---|---|
| Individuals: <ul style="list-style-type: none"> <li>• With signs and/or symptoms of a mitochondrial disorder</li> </ul>  | Interventions of interest are: <ul style="list-style-type: none"> <li>• Genetic testing</li> </ul>                   | Comparators of interest are: <ul style="list-style-type: none"> <li>• Standard clinical workup without genetic testing</li> </ul> | Relevant outcomes include: <ul style="list-style-type: none"> <li>• Test accuracy</li> <li>• Test validity</li> <li>• Other test performance measures</li> <li>• Symptoms</li> <li>• Functional outcomes</li> <li>• Health status measures</li> <li>• Quality of life</li> </ul>  |
| Individuals: <ul style="list-style-type: none"> <li>• Who are asymptomatic with a close relative with a mitochondrial disorder and a known pathogenic variant</li> </ul> | Interventions of interest are: <ul style="list-style-type: none"> <li>• Targeted familial variant testing</li> </ul> | Comparators of interest are: <ul style="list-style-type: none"> <li>• Standard risk assessment without genetic testing</li> </ul> | Relevant outcomes include: <ul style="list-style-type: none"> <li>• Test accuracy</li> <li>• Test validity</li> <li>• Other test performance measures</li> <li>• Changes in reproductive decision making</li> <li>• Symptoms</li> <li>• Functional outcomes</li> <li>• Health status measures</li> <li>• Quality of life</li> </ul> |

### Description

Mitochondrial disorders are multisystem diseases that arise from dysfunction in the mitochondrial protein complexes involved in oxidative metabolism. There are many related but distinct syndromes, and some patients have overlapping syndromes. As a result these disorders can be difficult to diagnose. Genetic testing has the potential to improve the accuracy of diagnosis for mitochondrial disorders. Genetic testing also has the potential to determine future risk of disease in individuals who have a close relative with a pathogenic variant.

### Summary of Evidence

For individuals who have signs and/or symptoms of a mitochondrial disorder who receive genetic testing, the evidence includes case series and cohort studies. Relevant outcomes are test accuracy and validity, other test performance measures, symptoms, functional outcomes, health status measures, and quality of life. There is a

lack of published data on analytic validity. Commercial testing sites claim analytic validity approaches 100% and describe testing methods expected to have high analytic validity. There is some evidence on clinical validity that varies by the patient population and testing strategy. Studies reporting diagnostic yield for known pathogenic variants using next-generation sequencing (NGS) panels tend to report rates ranging from 15% to 25%. Clinical specificity is unknown, but population-based studies have reported that the prevalence of certain variants exceeds the prevalence of clinical disease, suggesting that the variant will be found in some people without clinical disease (false positives). Clinical utility is relatively high for confirming the diagnosis of mitochondrial disorders in people who have signs and symptoms of disease. In these patients, a positive result on genetic testing can avoid a muscle biopsy and eliminate the need for further clinical workup. The evidence is sufficient to determine qualitatively that the technology results in a meaningful improvement in the net health outcome.

For individuals who are symptomatic with a close relative with a mitochondrial disorder and a known pathogenic variant and who receive targeted familial variant testing, the evidence includes case series and cohort studies. Relevant outcomes are test accuracy and validity, other test performance measures, changes in reproductive decision making, symptoms, functional outcomes, health status measures, and quality of life. There is a lack of published data on analytic validity. Commercial testing sites claim analytic validity approaching 100% and describe testing methods expected to have high analytic validity. Clinical validity is expected to be high for targeted testing of a known familial variant, assuming sufficient analytic validity. Clinical utility can be demonstrated for testing of at-risk family members who have a close relative with a pathogenic variant. When a specific mitochondrial disease is present in the family that is severe enough to cause impairment and/or disability, genetic testing may impact reproductive decision making. The evidence is sufficient to determine qualitatively that the technology results in a meaningful improvement in the net health outcome.

### Policy

Genetic testing to establish a genetic diagnosis of a mitochondrial disorder may be considered **medically necessary** when signs and symptoms of a mitochondrial disorder are present and genetic testing may eliminate the need for muscle biopsy.

Targeted genetic testing for a known familial variant of at-risk relatives may be considered **medically necessary** as preconceptual carrier testing under the following conditions:

- There is a defined mitochondrial disorder in the family of sufficient severity to cause impairment of quality of life or functional status; AND
- A variant that is known to be pathogenic for that specific mitochondrial disorder has been identified in the index case.

Genetic testing for mitochondrial disorders is considered **investigational** in all other situations when the criteria for medical necessity are not met.

### Policy Guidelines

Mitochondrial disorders can be caused by variants in mitochondrial DNA (mtDNA) or nuclear DNA (nDNA). A three-generation family history may suggest a mode of inheritance. A family history in which affected women transmit the disease to male and female children and affected men do not transmit the disease to their children suggests the familial variant(s) is in the mtDNA. A family history consistent with Mendelian autosomal dominant or autosomal recessive inheritance or with X-linked inheritance suggests the familial variant(s) is in the nDNA. De novo pathogenic variants are also possible.

### Testing Strategy

#### Individuals With a Suspected Mitochondrial Disorder

If the phenotype is highly suggestive of a specific disorder that is supported by the inheritance pattern noted in the family history, it would be reasonable to begin genetic testing with single genes or targeted multigene panels that test for pathogenic variants specific for that disorder.

If a mitochondrial disorder is suspected, but the phenotype is nonspecific, broader genetic testing is appropriate under the guidance of a clinical geneticist and genetics counselor. For patients in whom the family history is suggestive of a disorder due to pathogenic variant(s) in mtDNA, multi-gene panels or sequencing of the mitochondrial genome may be appropriate. If multiple mtDNA deletions are noted, or the family history is suggestive of a disorder due to variants in nDNA, then multigene panels covering known nuclear genes associated with mitochondrial disease may be appropriate. Testing using whole exome sequencing is reviewed in the Whole Exome and Whole Genome Sequencing for Diagnosis of Genetic Disorders Protocol.

#### Individuals With a Family Member With a Mitochondrial Disorder and Known Familial Variant

Targeted testing for a known familial variant in at-risk relatives as part of preconceptional carrier testing is appropriate. At-risk relatives include only female relatives if the familial pathogenic variant is in the mtDNA but includes both male and female relatives if the familial pathogenic variant is in the nDNA.

### Genetics Nomenclature Update

Human Genome Variation Society (HGVS) nomenclature is used to report information on variants found in DNA and serves as an international standard in DNA diagnostics. It is being implemented for genetic testing medical evidence review updates starting in 2017 (see Table PG1). HGVS nomenclature is recommended by HGVS, the Human Variome Project, and the HUMAN Genome Organization (HUGO).

The American College of Medical Genetics and Genomics (ACMG) and Association for Molecular Pathology (AMP) standards and guidelines for interpretation of sequence variants represent expert opinion from ACMG, AMP, and the College of American Pathologists. These recommendations primarily apply to genetic tests used in clinical laboratories, including genotyping, single genes, panels, exomes, and genomes. Table PG2 shows the recommended standard terminology—"pathogenic," "likely pathogenic," "uncertain significance," "likely benign," and "benign"—to describe variants identified that cause Mendelian disorders.

Table PG1. Nomenclature to Report on Variants Found in DNA

| Previous | Updated                    | Definition  |
|----------|----------------------------|---|
| Mutation | Disease-associated variant | Disease-associated change in the DNA sequence   |
|          | Variant                    | Change in the DNA sequence  |
|          | Familial variant           | Disease-associated variant identified in a proband for use in subsequent targeted genetic testing in first-degree relatives |

Table PG2. ACMG-AMP Standards and Guidelines for Variant Classification

| Variant Classification            | Definition   |
|-----------------------------------|--|
| Pathogenic                        | Disease-causing change in the DNA sequence               |
| Likely pathogenic                 | Likely disease-causing change in the DNA sequence        |
| Variant of uncertain significance | Change in DNA sequence with uncertain effects on disease |
| Likely benign                     | Likely benign change in the DNA sequence                 |
| Benign                            | Benign change in the DNA sequence                        |

ACMG: American College of Medical Genetics and Genomics; AMP: Association for Molecular Pathology.

### Genetic Counseling

Genetic counseling is primarily aimed at patients who are at risk for inherited disorders, and experts recommend formal genetic counseling in most cases when genetic testing for an inherited condition is considered. The inter-

pretation of the results of genetic tests and the understanding of risk factors can be very difficult and complex. Therefore, genetic counseling will assist individuals in understanding the possible benefits and harms of genetic testing, including the possible impact of the information on the individual's family. Genetic counseling may alter the utilization of genetic testing substantially and may reduce inappropriate testing. Genetic counseling should be performed by an individual with experience and expertise in genetic medicine and genetic testing methods.

### Medicare Advantage

For Medicare Advantage genetic testing for mitochondrial disorders is considered **not medically necessary** if the above criteria are not met.

### Background

#### *Mitochondrial DNA*

Mitochondria are organelles within each cell that contain their own set of DNA, distinct from the nuclear DNA that makes up most of the human genome. Human mitochondrial DNA (mtDNA) consists of 37 genes. Thirteen genes code for protein subunits of the mitochondrial oxidative phosphorylation complex, and the remaining 24 genes are responsible for proteins involved in the translation and/or assembly of the mitochondrial complex.<sup>1</sup> In addition, there are over 1000 nuclear genes that code for proteins that support mitochondrial function.<sup>2</sup> The protein products from these genes are produced in the nucleus and later migrate to the mitochondria.

Mitochondrial DNA differs from nuclear DNA in several important ways. Inheritance of mtDNA does not follow traditional Mendelian patterns. Rather, mtDNA is inherited only from maternal DNA so that disorders that result from variants in mtDNA can only be passed on by the mother. Also, there are thousands of copies of each *mtDNA* gene in each cell, as opposed to nuclear DNA, which contains only one copy per cell. Because there are many copies of each gene, variants may be present in some copies of the gene but not others. This phenomenon is called heteroplasmy. Heteroplasmy can be expressed as a percentage of genes that have the variant, ranging from 0% to 100%. Clinical expression of the variant will generally depend on a threshold effect (i.e., clinical symptoms will begin to appear when the percentage of mutated genes exceeds a threshold amount).<sup>3</sup>

#### *Mitochondrial Disorders*

Primary mitochondrial disorders arise from dysfunction of the mitochondrial respiratory chain. The mitochondrial respiratory chain is responsible for aerobic metabolism, and dysfunction therefore affects a wide variety of physiologic pathways dependent on aerobic metabolism. Organs with a high energy requirement, such as the central nervous system, cardiovascular system, and skeletal muscle, are preferentially affected by mitochondrial dysfunction.

The prevalence of these disorders has risen over the last two decades as the pathophysiology and clinical manifestations have been better characterized. It is currently estimated that the minimum prevalence of primary mitochondrial disorders is at least one in 5000.<sup>1,4</sup>

Some of the specific mitochondrial disorders are listed next:

- Mitochondrial encephalopathy with lactic acidosis and stroke-like episodes (MELAS) syndrome;
- Myoclonic epilepsy with ragged-red fibers (MERRF) syndrome;
- Kearns-Sayre syndrome (KSS);
- Leigh syndrome;
- Chronic progressive external ophthalmoplegia (CPEO);

- Leber hereditary optic neuropathy;
- Neurogenic weakness with ataxia and retinitis pigmentosa.

Most of these disorders are characterized by multisystem dysfunction, which generally includes myopathies and neurologic dysfunction and may involve multiple other organs. Each of the defined mitochondrial disorders has a characteristic set of signs or symptoms. The severity of illness is heterogeneous and can vary markedly. Some patients will have only mild symptoms for which they never require medical care, while other patients have severe symptoms, a large burden of morbidity, and a shortened life expectancy.

### Diagnosis

The diagnosis of mitochondrial disorders can be difficult. The individual symptoms are nonspecific and symptom patterns can overlap considerably. As a result, a patient often cannot be easily classified into one particular syndrome.<sup>5</sup> Biochemical testing is indicated for patients who do not have a clear clinical picture of one specific disorder. Measurement of serum lactic acid is often used as a screening test, but the test is neither sensitive nor specific for mitochondrial disorders.<sup>2</sup>

A muscle biopsy can be performed if the diagnosis is uncertain after biochemical workup. However, this is an invasive test and is not definitive in all cases. The presence of “ragged red fibers” on histologic analysis is consistent with a mitochondrial disorder. Ragged red fibers represent a proliferation of defective mitochondrial.<sup>1</sup> This characteristic finding may not be present in all types of mitochondrial disorders, and also may be absent early in the course of disease.<sup>2</sup>

### Treatment

Treatment of mitochondrial disease is largely supportive, because there are no specific therapies that impact the natural history of the disorder.<sup>5</sup> Identification of complications such as diabetes and cardiac dysfunction is important for early treatment of these conditions. A number of vitamins and cofactors (e.g., coenzyme Q, riboflavin) have been used, but empirical evidence of benefit is lacking.<sup>6</sup> Exercise therapy for myopathy is often prescribed, but the effect on clinical outcomes is uncertain.<sup>5</sup> The possibility of gene transfer therapy is under consideration, but is at an early stage of development and has not yet been tested in clinical trials.

### *Genetic Testing for Mitochondrial Disorders*

Mitochondrial disorders can be caused by pathogenic variants in the maternally inherited mtDNA or one of many nuclear nDNA genes. Genetic testing for mitochondrial disorders may involve testing for point mutations, deletion/duplication analysis, and/or whole exome sequencing of nuclear or mitochondrial DNA. The type of testing done depends on the specific disorder being considered. For some primary mitochondrial disorders such as MELAS and MERFF, most variants are point mutations, and there are a finite number of variants associated with the disorder. When testing for one of these disorders, known pathogenic variants can be tested for with polymerase chain reaction, or sequence analysis can be performed on the particular gene. For other mitochondrial disorders such as CPEO and KSS, the most common variants are deletions, and therefore duplication/deletion analysis would be the first test when these disorders are suspected. Table 3 shows examples of clinical symptoms and particular genetic variants in mtDNA or nDNA associated with particular mitochondrial syndromes.<sup>5,7</sup> A repository of published and unpublished data on polymorphisms and variants in human mitochondrial DNA is available in the MITOMAP database.<sup>8</sup> Lists of mtDNA and nDNA genes that may lead to mitochondrial disorders and testing laboratories in the United States are provided at the GeneTests website funded by BioReference Laboratories and Genetic Testing Registry of the National Center for Biotechnology Information website<sup>9,10</sup>.

Table 3. Examples of Mitochondrial Disorders, Clinical Manifestations, and Associated Pathogenic Genes

| Syndrome              | Main Clinical Manifestations   | Major Genes Involved  |
|-----------------------|--|---|
| MELAS                 | <ul style="list-style-type: none"> <li>Stroke-like episodes at age &lt; 40 y</li> <li>Seizures and/or dementia</li> <li>Pigmentary retinopathy</li> <li>Lactic acidosis</li> </ul> | <ul style="list-style-type: none"> <li><i>MT-TL1, MT-ND5</i> (&gt; 95%)</li> <li><i>MT-TF, MT-TH, MT-TK, MT-TQ, MT-TS<sub>1</sub>, MT-TS<sub>2</sub>, MT-ND1, MT-ND6</i> (rare)</li> </ul>  |
| MERFF                 | <ul style="list-style-type: none"> <li>Myoclonus</li> <li>Seizures</li> <li>Cerebellar ataxia</li> <li>Myopathy</li> </ul>   | <ul style="list-style-type: none"> <li><i>MT-TK</i> (&gt; 80%)</li> <li><i>MT-TF, MT-TP</i> (rare)</li> </ul>   |
| CPEO                  | <ul style="list-style-type: none"> <li>External ophthalmoplegia</li> <li>Bilateral ptosis</li> </ul>   | <ul style="list-style-type: none"> <li>Various deletions of MT-DNA</li> </ul>   |
| Kearns-Sayre syndrome | <ul style="list-style-type: none"> <li>External ophthalmoplegia &lt; 20 y</li> <li>Pigmentary retinopathy</li> <li>Cerebellar ataxia</li> <li>Heart block</li> </ul>               | <ul style="list-style-type: none"> <li>Various deletions of MT-DNA</li> </ul>   |
| Leigh syndrome        | <ul style="list-style-type: none"> <li>Subacute relapsing encephalopathy</li> <li>Infantile onset</li> <li>Cerebellar/brain stem dysfunction</li> </ul>                            | <ul style="list-style-type: none"> <li><i>MT-ATP6, MT-TL1, MT-TK, MT-TW, MT-TV, MT-ND1, MT-ND2, MT-ND3, MT-ND4, MT-ND5, MT-ND6, MT-CO3</i></li> <li>MT-DNA deletions (rare)</li> <li><i>SUCLA2, NDUSF<sub>x</sub>, NDFV<sub>x</sub>, SDHA, BCS1L, SURF1, SCO2, COX15</i></li> </ul> |
| LHON                  | <ul style="list-style-type: none"> <li>Painless bilateral visual failure</li> <li>Male predominance</li> <li>Dystonia</li> <li>Cardiac pre-excitation syndromes</li> </ul>         | <ul style="list-style-type: none"> <li><i>MT-ND1, MT-ND4, MT-ND6</i></li> </ul>   |
| NARP                  | <ul style="list-style-type: none"> <li>Peripheral neuropathy</li> <li>Ataxia</li> <li>Pigmentary retinopathy</li> </ul>  | <ul style="list-style-type: none"> <li><i>MT-ATP6</i></li> </ul>  |
| MNGIE                 | <ul style="list-style-type: none"> <li>Intestinal malabsorption</li> <li>Cachexia</li> <li>External ophthalmoplegia</li> <li>Neuropathy</li> </ul>                                 | <ul style="list-style-type: none"> <li><i>TP</i></li> </ul>   |
| IOSCA                 | <ul style="list-style-type: none"> <li>Ataxia</li> <li>Hypotonia</li> <li>Athetosis</li> <li>Ophthalmoplegia</li> <li>Seizures</li> </ul>  | <ul style="list-style-type: none"> <li><i>TWINKLE</i></li> </ul>  |
| SANDO                 | <ul style="list-style-type: none"> <li>Ataxic neuropathy</li> <li>Dysarthria</li> <li>Ophthalmoparesis</li> </ul>  | <ul style="list-style-type: none"> <li><i>POLG</i></li> </ul>   |
| Alpers syndrome       | <ul style="list-style-type: none"> <li>Intractable epilepsy</li> <li>Psychomotor regression</li> <li>Liver disease</li> </ul>  | <ul style="list-style-type: none"> <li><i>POLG, DGUOK, MPV17</i></li> </ul>   |
| GRACILE               | <ul style="list-style-type: none"> <li>Growth retardation</li> <li>Aminoaciduria</li> <li>Cholestasis</li> <li>Iron overload</li> <li>Lactic acidosis</li> </ul>                   | <ul style="list-style-type: none"> <li><i>NDUSF<sub>x</sub></i></li> </ul>  |

| Syndrome                            | Main Clinical Manifestations   | Major Genes Involved   |
|-------------------------------------|--|--|
| Coenzyme Q <sub>10</sub> deficiency | <ul style="list-style-type: none"> <li>Encephalopathy</li> <li>Steroid-resistant nephrotic syndrome</li> <li>Hypertrophic cardiomyopathy</li> <li>Retinopathy</li> <li>Hearing loss</li> </ul> | <ul style="list-style-type: none"> <li>COQ2</li> <li>COQ9</li> <li>CABC1</li> <li>ETFDH</li> </ul> |

Adapted from Chinnery et al (2014)<sup>5</sup> and Angelini et al (2009)<sup>7</sup>

CPEO: chronic progressive external ophthalmoplegia; GRACILE: growth retardation, aminoaciduria, cholestasis, iron overload, early death; IOSCA: infantile onset spinal cerebellar atrophy; LHON: Leber hereditary optic neuropathy; MELAS: mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes; MERFF: myoclonic epilepsy with ragged-red fibers; MNGIE: mitochondrial neurogastrointestinal encephalopathy; NARP: neuropathy, ataxia, and retinitis pigmentosa; SANDO: sensory ataxia, neuropathy, dysarthria and ophthalmoplegia

Table 4. Examples of Commercially Available Panels Simultaneously Testing for Multiple for Mitochondrial Disorders

| Laboratory                                     | Test Name                                      | No. of Genes Included on Panel |
|--|--|--------------------------------|
| Gene Dx® (Gaithersburg, MD)                    | Comprehensive Mitochondrial Nuclear Gene Panel | 319                            |
| Transgenomic® (New Haven, CT)                  | Complete Mitochondrial Evaluation              | 485                            |
| Courtagen® (Woburn, MA)                        | nucSEEK® Comprehensive                         | 1189                           |
|  | nucSEEK® Focus                                 | 181                            |
|  | mtSEEK®  | 40                             |
| ARUP® (Salt Lake City, UT)                     | Mitochondrial Disorders Panel                  | 121                            |
|  | Mitochondrial Disorders (mtDNA) Sequencing     | 33                             |
| Baylor® Genetics Laboratory (Houston, TX)      | BCM-MitomeNGSSM                                | 201                            |
| MEDomics® (Azusa, CA)                          | MitoMED1204™                                   | > 1200                         |
| Emory Genetics Laboratory (Tucker, GA)         | Mitochondrial Diseases: Sequencing Panel       | 44                             |
| Knight Diagnostics Laboratories (Portland, OR) | Comprehensive Mitochondrial Metabolic Panel    | 196                            |

### Regulatory Status

Clinical laboratories may develop and validate tests in-house and market them as a laboratory service; laboratory-developed tests (LDTs) must meet the general regulatory standards of the Clinical Laboratory Improvement Amendments (CLIA). Genetic testing for mitochondrial disorders is under the auspices of CLIA. Laboratories that offer LDTs must be licensed by CLIA for high-complexity testing. To date, the U.S. Food and Drug Administration has chosen not to require any regulatory review of this test.

### Related Protocols

General Approach to Evaluating the Utility of Genetic Panels

General Approach to Genetic Testing

Services that are the subject of a clinical trial do not meet our Technology Assessment Protocol criteria and are

considered investigational. *For explanation of experimental and investigational, please refer to the Technology Assessment Protocol.*

It is expected that only appropriate and medically necessary services will be rendered. We reserve the right to conduct prepayment and postpayment reviews to assess the medical appropriateness of the above-referenced procedures. **Some of this protocol may not pertain to the patients you provide care to, as it may relate to products that are not available in your geographic area.**

## References

We are not responsible for the continuing viability of web site addresses that may be listed in any references below.

1. Schon EA, DiMauro S, Hirano M. Human mitochondrial DNA: roles of inherited and somatic mutations. *Nat Rev Genet.* Dec 2012; 13(12):878-890. PMID 23154810
2. Wong LJ. Diagnostic challenges of mitochondrial DNA disorders. *Mitochondrion.* Feb-Apr 2007; 7(1-2):45-52. PMID 17276740
3. DiMauro S, Schon EA. Mitochondrial DNA mutations in human disease. *Am J Med Genet.* Spring 2001; 106(1):18-26. PMID 11579421
4. Falk MJ, Sondheimer N. Mitochondrial genetic diseases. *Curr Opin Pediatr.* Dec 2010; 22(6):711-716. PMID 21045694
5. Chinnery PF. Mitochondrial Disorders Overview. In: Pagon RA, Adam MP, Ardinger HH, et al., eds. *GeneReviews(R)*. Seattle, WA: University of Washington; 2014.
6. Chinnery P, Majamaa K, Turnbull D, et al. Treatment for mitochondrial disorders. *Cochrane Database Syst Rev.* Jan 25 2006(1):CD004426. PMID 16437486
7. Angelini C, Bello L, Spinazzi M, et al. Mitochondrial disorders of the nuclear genome. *Acta Myol.* Jul 2009; 28(1):16-23. PMID 19772191
8. FOSWIKI. MITOMAP: a human mitochondrial genome database. 2017; <http://www.mitomap.org/MITOMAP>. Accessed May 3, 2017.
9. GeneTestsTM. <https://www.genetests.org/>. Accessed May 08, 2017.
10. National Center for Biotechnology Information. GTR: Genetic Testing Registry. n.d.; <https://www.ncbi.nlm.nih.gov/gtr/>. Accessed May 5, 2017.
11. Transgenomic Web Site. Mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS). 2014; <http://www.transgenomic.com/labs/neurology/melas>. Accessed March, 2015.
12. Courtagen Web site. Physician Overview. 2014; <http://www.courtagen.com/professionals-overview.htm>. Accessed March, 2015.
13. Legati A, Reyes A, Nasca A, et al. New genes and pathomechanisms in mitochondrial disorders unraveled by NGS technologies. *Biochim Biophys Acta.* Aug 2016; 1857(8):1326-1335. PMID 26968897
14. Pronicka E, Piekutowska-Abramczuk D, Ciara E, et al. New perspective in diagnostics of mitochondrial disorders: two years' experience with whole-exome sequencing at a national paediatric centre. *J Transl Med.* Jun 12 2016; 14(1):174. PMID 27290639
15. Kohda M, Tokuzawa Y, Kishita Y, et al. A comprehensive genomic analysis reveals the genetic landscape of mitochondrial respiratory chain complex deficiencies. *PLoS Genet.* Jan 2016; 12(1):e1005679. PMID 26741492
16. Wortmann SB, Koolen DA, Smeitink JA, et al. Whole exome sequencing of suspected mitochondrial patients in clinical practice. *J Inherit Metab Dis.* May 2015; 38(3):437-443. PMID 25735936
17. Ohtake A, Murayama K, Mori M, et al. Diagnosis and molecular basis of mitochondrial respiratory chain disorders: exome sequencing for disease gene identification. *Biochim Biophys Acta.* Apr 2014; 1840(4):1355-1359. PMID 24462578



18. Taylor RW, Pyle A, Griffin H, et al. Use of whole-exome sequencing to determine the genetic basis of multiple mitochondrial respiratory chain complex deficiencies. *JAMA*. Jul 2 2014; 312(1):68-77. PMID 25058219
19. Lieber DS, Calvo SE, Shanahan K, et al. Targeted exome sequencing of suspected mitochondrial disorders. *Neurology*. May 7, 2013; 80(19):1762-1770. PMID 23596069
20. DaRe JT, Vasta V, Penn J, et al. Targeted exome sequencing for mitochondrial disorders reveals high genetic heterogeneity. *BMC Med Genet*. Nov 11 2013; 14:118. PMID 24215330
21. McCormick E, Place E, Falk MJ. Molecular genetic testing for mitochondrial disease: from one generation to the next. *Neurotherapeutics*. Apr 2013; 10(2):251-261. PMID 23269497
22. Calvo SE, Compton AG, Hershman SG, et al. Molecular diagnosis of infantile mitochondrial disease with targeted next-generation sequencing. *Sci Transl Med*. Jan 25 2012; 4(118):118ra110. PMID 22277967
23. Qi Y, Zhang Y, Wang Z, et al. Screening of common mitochondrial mutations in Chinese patients with mitochondrial encephalomyopathies. *Mitochondrion*. Feb-Apr 2007; 7(1-2):147-150. PMID 17276742
24. Deschauer M, Krasnianski A, Zierz S, et al. False-positive diagnosis of a single, large-scale mitochondrial DNA deletion by Southern blot analysis: the role of neutral polymorphisms. *Genet Test*. Winter 2004; 8(4):395-399. PMID 15684869
25. Elliott HR, Samuels DC, Eden JA, et al. Pathogenic mitochondrial DNA mutations are common in the general population. *Am J Hum Genet*. Aug 2008; 83(2):254-260. PMID 18674747
26. Majamaa K, Moilanen JS, Uimonen S, et al. Epidemiology of A3243G, the mutation for mitochondrial encephalomyopathy, lactic acidosis, and strokelike episodes: prevalence of the mutation in an adult population. *Am J Hum Genet*. Aug 1998; 63(2):447-454. PMID 9683591
27. DiMauro S, Hirano M. Melas. In: Pagon RA, Adam MP, Ardinger HH, et al., eds. *GeneReviews*(R). Seattle, WA: University of Washington; 2013.
28. Jean-Francois MJ, Lertrit P, Berkovic SF, et al. Heterogeneity in the phenotypic expression of the mutation in the mitochondrial tRNA(Leu) (UUR) gene generally associated with the MELAS subset of mitochondrial encephalomyopathies. *Aust N Z J Med*. Apr 1994; 24(2):188-193. PMID 8042948
29. Foundation for Mitochondrial Medicine. Mitochondrial Disease: Overview of Mitochondrial Disease. 2013; <http://mitochondrialdiseases.org/mitochondrial-disease/> and <http://mitochondrialdiseases.org/mitochondrial-disease/diagnosis-and-treatments/>. Accessed June 13, 2016.
30. Parikh S, Goldstein A, Koenig MK, et al. Diagnosis and management of mitochondrial disease: a consensus statement from the Mitochondrial Medicine Society. *Genet Med*. Sep 2015; 17(9):689-701. PMID 25503498