Cardiovascular Involvement in mtDNA Disease

Diagnosis, Management, and Therapeutic Options

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KEYWORDS

• Mitochondrial diseases • MELAS syndrome • mtDNA • Hypertrophic cardiomyopathy

KEY POINTS

- Mitochondrial diseases include an heterogenous group of systemic disorders caused by sporadic or inherited mutations in nuclear or mitochondrial DNA (mtDNA), causing impairment of oxidative phosphorylation system.
- Hypertrophic cardiomyopathy is the dominant pattern of cardiomyopathy in all forms of mtDNA diseases (mtDNA-D), being observed in almost 40% of the patients.
- The diagnosis of mtDNA-D is challenging because of wide clinical and genetic heterogeneity and requires a multisystemic approach.

INTRODUCTION

Mitochondrial diseases (MD) include an heterogenous group of systemic disorders caused by sporadic or inherited mutations in nuclear (nDNA) or mitochondrial DNA (mtDNA), causing impairment of oxidative phosphorylation system (OXPHOS) and subsequent reduction in adenosine triphosphate production, leading to different phenotypes, depending on the tissue involved, type of pathogenic mutations, and heteroplasmy level.[1–3] MD affect preferentially tissue with high energy

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demands and show multiorgan involvement, resulting in complex multisystemic diseases mainly characterized by neurologic, ophthalmologic, audiological, endocrine, and cardiovascular disease.[4,5] In particular, myocardial involvement is present in up to 60% of patients with MD and represents an independent predictor of morbidity and early mortality.[6,7] Although the exact prevalence of MD is unknown, population-based studies report a prevalence of 9.2/100000 among adults younger than 65 years; such data are not completely accurate because of the lack of standardized criteria for diagnosis and may reflect an underestimation of the true prevalence of MD.[8,9] Currently, more than 250 nuclear genes over 37 mitochondrial genes are associated with OXPHOS defects.

Before the advent of next-generation sequencing (NGS) technology, few of the genes and mutations were known to be pathogenic or likely pathogenic for MD. In particular, the m.3243A>G mutation has been detected in up to 1/300 of the general population, and although many individuals are asymptomatic carriers of low levels of mutation, its clinical prevalence is estimated to be of 1/5000 in the general population.[9]

In this article, the authors discuss the current clinical knowledge on MD, focusing on diagnosis and management of MD caused by mtDNA mutations (mtDNA-D).

CLINICAL PRESENTATION AND RED FLAGS

The clinical spectrum of mtDNA disease (mtDNA-D) is heterogeneous and can range from oligosymptomatic patients to severe multisystemic involvement.[10] Organs with high aerobic metabolic demands as brain tissue, heart, or skeletal muscle are more severely affected in patients with mtDNA-D.[11] Lactic acidosis may be present, but its absence should not be used to rule out mtDNA-D.[12]

Patients with neuromuscular mtDNA-D often present with increased creatine kinase levels and symptoms of skeletal myopathy; rarely nervous conduction tests show axonal sensorimotor neuropathy.[3,13–15] Almost 10% of the patients have abnormal liver function tests; gastrointestinal symptoms such as constipation, dysphagia, and chronic intestinal pseudoobstruction are common.[16,17] Renal involvement is characterized by proximal tubulopathy, Fanconi syndrome, and tubulointerstitial nephritis, causing a progressive reduction in glomerular filtration rate.[14,18] Endocrine disorders may include diabetes mellitus, hypothyroidism, hypoparathyroidism, diabetes insipidus, and hypogonadism.[16] Short stature has been reported in up to 20% of the cases.[13] Ophthalmologic manifestations include retinitis pigmentosa, palpebral ptosis, external ophthalmoplegia, cataract, and optic atrophy.[3,11,13]

Central nervous system involvement is a major determinant of adverse events in patients with mtDNA-D and common clinical features such as encephalopathy, strokelike episodes, cognitive dysfunction, ataxia, seizures, migraine, or depression should raise the suspicion of an underlying mtDNA disorder.[7,11] Bilateral sensorineural deafness occurs in 7% to 26% of the patients, and its prevalence increases with age.[13,19]

Although there are no consensus statements evaluating the performance of clinical “red flags” for the diagnosis of mitochondrial cardiomyopathies, the presence of a maternal inheritance pattern or the identification of extra cardiac features of mtDNA-D should guide further investigations in order to exclude an underlying mitochondrial disorder.[20] Clinical clues for the diagnosis of mtDNA-D are listed in **Table 1**.

CARDIOVASCULAR INVOLVEMENT IN MITOCHONDRIAL DISEASE

Cardiovascular involvement in MD is progressive, and it is an independent predictor of morbidity and mortality,[6,21] both in pediatric and adult patients. In a cohort study including 113 children with mtDNA-D, survival rate to age 16 years was 18% in patients with cardiovascular involvement compared with 92% in patients without evidence of heart disease.[21]

In a large, retrospective study enrolling 260 adult patients, most of them with mtDNA-D, cardiovascular involvement was present in almost 31% of the patients at baseline. Over a 7-year follow-up, hypertrophic cardiomyopathy (HCM) was observed in 18% of the patients, mostly carriers of the m.3243A>G mutation.[7] Major adverse clinical events, including sudden death, death or hospitalization due to heart failure, resuscitated cardiac arrest, high-grade atrioventricular block, or cardiac transplantation, were observed in 10% of the patients; multivariate analysis showed hypertrophic phenotype to be an independent predictor of MACE (hazard ratio 2.5; 95% confidence interval: 1.1–5.8).[7]

HCM is the dominant pattern of cardiomyopathy in all forms of mtDNA-D, being observed in almost 40% of the patients.[6,22–24] Although different mtDNA mutations may have heterogeneous phenotypical expression, cross-sectional studies seem to show recurrent patterns of genotype-phenotype correlation.[24,25] Cardiomyopathies

Table 1
Clinical red flags for the suspicion of mitochondrial disorders

Organ System	
Neurologic	Hypotonia + metabolic acidosis Encephalopathy Myoclonus Ataxia Axonal neuropathy Skeletal myopathy
Ophthalmologic	Retinitis pigmentosa Palpebral ptosis External ophthalmoplegia Cataract Optic atrophy
ENT	Bilateral sensorineural deafness
Endocrine	Diabetes mellitus Hypothyroidism Diabetes insipidus Hypogonadism
Renal	Fanconi syndrome Proximal tubulopathy
Cardiovascular	HCM Ventricular preexcitation Left ventricular noncompaction Conduction system disease
Biomarkers	Increased CK enzyme Lactic acidosis Lactate/pyruvate ratio>20 Leukocytopenia (Barth syndrome)
Brain magnetic resonance	Diffuse, fluctuating strokelike lesion in nonvascular distribution pattern, with elevated T2 and ECV signal and normal DWI Symmetric abnormalities of deep gray matter, with high T2 and FLAIR and low T1 signal Delayed myelination pattern

Abbreviations: CK, creatine kinase; DWI, diffusion-weighted imaging; ECV, extracellular volume.

with hypertrophic remodeling seem to be associated to mt-tRNA mutations, whereas single, large-scale deletions are more often associated to conduction disturbances, such as atrioventricular (AV) block in Kearns-Sayre syndrome (KSS).[6,26,27] According to recent studies, the echocardiographic prevalence of left ventricular hypertrophy (LVH) ranges from 38% to 56% in carriers of m3243A>G mutation; noteworthy mutation load seemed to predict the severity of hypertrophy[23,24] and heart failure. Of note, LVH is less common in association with mt-rRNA gene and protein-coding-genes mutations.[28–31]

The natural history of mitochondrial cardiomyopathies shows many important differences with sarcomeric HCM: left ventricular outflow tract obstruction is less common in mtDNA-D, and the risk of progression to end-stage clinical variants, characterized by LV chamber dilatation and systolic dysfunction, is higher than in sarcomeric forms.[10,23] In patients with mtDNA-D, dilated cardiomyopathy often results from the end-stage progression of HCM rather than being the initial pattern of clinical presentation and has been reported in carriers of m.8344A>G, m.3243A>G, m4317A>G, and m.4269A>G gene mutations.[2,25,26,29,32]

Recently, left ventricular noncompaction has been described in mtDNA-D, especially in pediatric patients with multisystemic involvement.[21,33] Restrictive cardiomyopathy is rare in mtDNA-D, but it has been associated to diabetes and inherited bilateral deafness in patients with m3243A>G mutation and in otherwise healthy carriers of the m1555A>G gene mutation.[34,35]

Conduction system disease commonly occurs in patients with mtDNA-D. Conduction disturbances represent a major diagnostic criterion in patients with KSS, among which high-grade AV block has a reported prevalence of 84%.[36] Bradyarrhythmias can present as syncope, Adam-Stokes syndrome, or sudden death; noteworthy, their onset usually occurs after the development of ophthalmoplegia and retinopathy.[11,36–38] Therefore, close clinical monitoring with 24-ECG Holter or internal loop recorder is recommended in patients with KSS after the development of ophthalmic involvement. Albeit less commonly, AV conduction disturbances have been reported in other mtDNA-D, with a prevalence that ranges up to 10%, in association with m.3243A>G and m.8344A>G mutations.[7,24] The risk of progression of conduction disease to high-grade AV block is often unpredictable in patients with mtDNA-D, particularly among patients with large-scale deletions who carry a significantly higher risk of SCD and sudden AV block; therefore, prophylactic pacing should be considered only in this subgroup of patients.[39]

Ventricular preexcitation and Wolff Parkinson White syndrome were first described in association with Leber hereditary optic neuropathy, with a prevalence of 10% and 8% among affected individuals and their maternal relatives, respectively, compared with 1.6% among paternal relatives.[40]

Case series and cohort studies have reported a prevalence of manifest preexcitation pattern ranging from 3% to 27% among carriers of m.3243A>G and m.8433A>G gene mutations; interestingly, Wolff-Parkinson-White preceded the manifestations of mitochondrial encephalomyopathy with lactic acidosis and strokelike episodes (MELAS) syndrome in a subgroup of patients.[7,22,24,26,41]

DIAGNOSIS OF mtDNA DISEASE

The diagnosis of mtDNA-D is challenging because of wide clinical and genetic heterogeneity and requires a multisystemic approach including extensive medical, laboratory, and neuroimaging investigations. Cardiologists should be aware of the complexity of diagnosis and management in MD; an integrated approach constituting of assessment by a multidisciplinary specialist team is recommended.

Two clinical scenarios are possible: (1) patients with confirmed mtDNA-D being periodically evaluated for cardiovascular involvement and (2) patients with cardiovascular disease and red flags for the suspicion of mtDNA-D. The diagnostic algorithm in patients with cardiovascular involvement and suspected mtDNA-D is shown in Fig. 1.

Patients with Suspected mtDNA Disease

A comprehensive evaluation by a specialized team with expertise in mtDNA-D, including cardiologists, neurologists, and pathologists, is advised.

Patients with suspected mtDNA-D should undergo a complete diagnostic workup: a pedigree up to third-generation relatives should be collected to identify the inheritance pattern (matrilinear vs recessive).[42] Clinicians should evaluate the extent of organ involvement and detect specific clues for differential diagnosis at physical

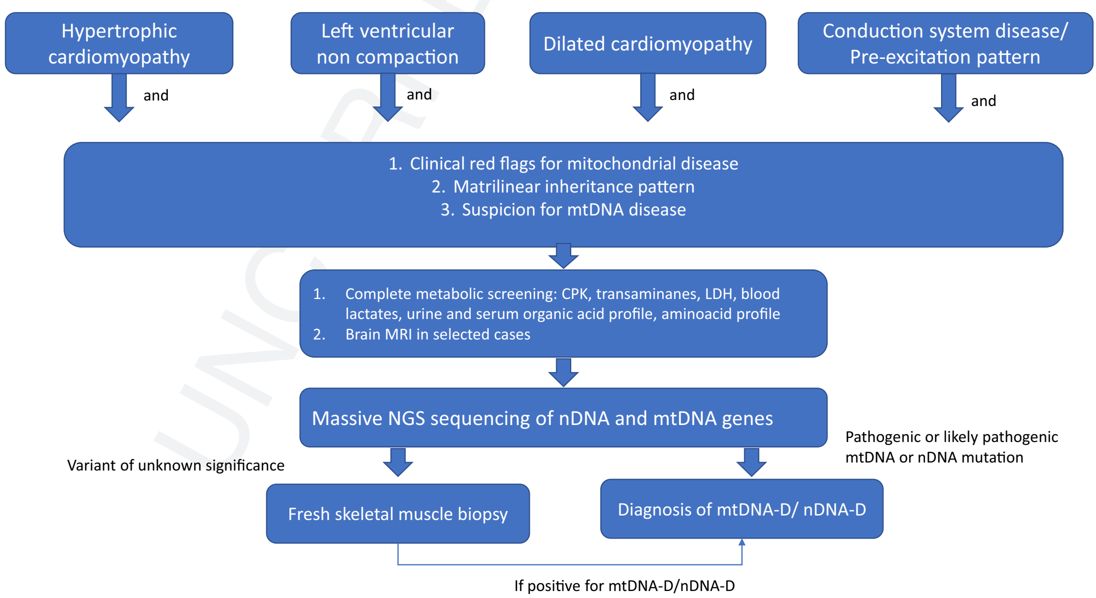

Fig. 1. Proposed algorithm for the diagnosis of nuclear (nDNA-D) and mtDNA disease (mtDNA-D).

examination.[43] Although most of the brain lesions in mtDNA-D are not specific, their pattern of distribution can be suggestive. A global delay in cortical myelination is common; strokelike lesions in a nonvascular distribution, symmetric signal abnormalities of deep gray matter presenting with hyperintensity on T2 and FLAIR images, and hypointensity on T1 images are common findings in mtDNA-D.[44,45] Diffusion-weighted sequences can be useful to differentiate strokelike lesions in MELAS syndrome from acute ischemic foci: noteworthy strokelike foci are fluctuating and they lack the reduction in diffusion coefficient usually found in vascular lesions.[44]

Laboratory investigations of suspected mtDNA-D are complex, and recently, the Mitochondrial Medicine Society has provided consensus recommendations on the diagnosis and management of mtDNA-D.[46]

The baseline evaluation should include complete blood count and determination of glycated hemoglobin, iron status, creatine kinase, plasma proteins and transaminases, serum, and urine amino acids; the presence of lactic acidosis should be systematically assessed.[42] An increased postprandial lactate to pyruvate molar ratio (>20), although not specific of MD, could help in the differential diagnosis of congenital lactic acidosis.[47]

A urine organic acid panel is recommended in patients with suspected mtDNA-D and could be diagnostic in case of propionic and methylmalonic aciduria or show a 3-methylglutaconic aciduria. Although acylcarnitine profile may be normal in absence of metabolic stress, plasma and urine carnitine and acylcarnitine levels should be dosed in patients with suspected mtDNA-D to detect fatty acid beta oxidation or carnitine uptake defects.[43,48]

When the metabolic screening or the clinical evaluation can suggest a specific diagnosis, molecular testing with mtDNA or nDNA sequencing of target genes should be considered.[10,43]

Growing evidence support screening in lymphocytes or urine samples for mtDNA mutations in specific clinical scenarios.[10,39] Among patients with unexplained LVH and suspected mtDNA-D if biochemical panel is inconclusive, a first-level molecular screening for the most common mtDNA mutations (such as m.3243A>G or m.4300A>G) is recommended to rule out an OXPHOS deficiency.[10,43,49–51]

Fresh skeletal muscle biopsy is considered the gold standard for the diagnosis of mtDNA-D.[52,53] Under light microscopy, affected muscles contain peripheral and interfibrillar accumulation of abnormal mitochondria, nonetheless the hallmark of mtDNA-D is represented by red ragged fibers that can be demonstrated using modified trichrome Gomori stains.[5,54] Tissue samples should undergo biochemical and spectrophotometrical analysis to measure enzymatic activity of each OXPHOS complex.[55,56] Blue native polyacrylamide gel electrophoresis allows assessment of proper assembly of the 5 OXPHOS complexes, whereas spectrophotometric assays may be used to assess the enzymatic activity of each complex, to quantify ATP production and proper oxidation of substrate. Recently, new high-resolution respirometry techniques have been proposed to assess oxidative chain function in frozen samples.[57]

Skeletal muscle biopsy is a low-risk procedure, and it is recommended as first-line approach in patients requiring definitive diagnosis. However, in patients with isolated heart involvement or in case of mutations with tissue specificity, endomyocardial biopsy may represent another diagnostic option.[58,59] In consideration of the relatively low rate of serious complications, opportunistic assessment of cardiac tissue obtained during other cardiac procedures should be considered.[60] Molecular diagnostic testing performed by Sanger or next-generation sequencing is recommended to assess the presence of single missense or nonsense point mutations and large rearrangements, both in nuclear genes and mtDNA to confirm diagnosis.[61–63]

Molecular Diagnosis of mtDNA Disease

The use of molecular analysis in the evaluation and prevention of cardiovascular risk, with particular emphasis on sudden cardiac death risk stratification, in patients, healthy subjects, and athletes is an important issue in clinical practice.[64]

Traditionally, the genetic diagnosis of mitochondrial cardiomyopathies provides analysis of "candidate genes." This approach is achieved by Sanger sequencing of known genes in order to assess the presence of single missense or nonsense point mutations.[61–63] In fact, the mitochondrial genome is often initially sequenced in patients with a clinical diagnosis and a strongly suggestive family history of MD, to exclude a primary defect of mtDNA.[65] However, due to the complexity of genetic testing, many patients still lack a molecular diagnosis.

To date, the widespread application of NGS technology has allowed to investigate, at the same time, both the entire mitochondrial genome and different nuclear genes.

In this scenario, the distinction between rare or novel pathogenetic variants from nonpathogenic polymorphisms in known/unknown genes represents the main difficulty.[66] Although the correct classification of these variants needs functional studies, requiring specific laboratory competences, in silico evaluations using several bioinformatics tools are, currently, used to predict the likelihood of pathogenicity of missense and splicing variants. Furthermore, Guidelines from the American College of Medical Genetics allows to classify variants by using different criteria, also including in silico analysis.[67]

Cardiac Investigations in Patients with Confirmed mtDNA Disease

Cardiac involvement in mtDNA-D may remain silent until an advanced state, because of the limitation in the exercise capacity in this subset of patients.[10]

A complete cardiovascular assessment, comprehensive of patient's history, 12-lead ECG, and transthoracic echocardiogram should be performed in patients with mtDNA-D and in carriers of a mitochondrial mutation at initial evaluation and should be repeated at annual interval and with the development of new symptoms, if cardiovascular disease is present. In patients without cardiovascular involvement, repeated assessment at 3-year intervals should be considered, with the exclusion of patients with KSS, proximal myopathy, or large-scale deletions, for whom an annual ECG could be useful.[68–71]

Electrocardiogram and 24-hour holter monitoring

A 12-lead ECG can show prolongation of the PR interval and signs of AV block, a Wolff-Parkinson-White pattern, or repolarization abnormalities such as inverted T waves and prolonged corrected QT interval (>440 msec); an abnormal ECG represents the most common cardiovascular manifestation in mtDNA disease.[6,68,72]

Cardiac conduction abnormalities with an unpredictable course are frequent in mtDNA-D, with a higher prevalence in Kearns-Sayre syndrome and in carriers of m.3243A>G mutations.[7] Patients with large mtDNA deletions or carriers of m.3243A<G mutation, diabetes, intraventricular conduction blocks, LVH, or evidence of premature ventricular complexes seem to carry the highest risk for sudden AV block.[7] A 24-hour ECG Holter monitoring should be performed in patients with mtDNA-D and asymptomatic carriers of mtDNA mutations at initial evaluation and repeated at 1-year intervals.[69] In selected patients with risk factors for sudden AV block, Holter monitoring should be performed more frequently.[7,25,69]

Echocardiography

Transthoracic echocardiography is recommended in patients with mtDNA-D to assess cardiovascular involvement and should be performed at initial diagnosis and with the development of new symptoms and repeated on annual basis.

Of note, hypertrophic cardiomyopathy and LVH seem to be more prevalent in patients with point missense or nonsense mutation (such as m.3243A>G "MELAS" mutation or in myoclonic epilepsy with ragged red fibers syndrome), whereas patients with KSS or mitochondrial myopathy display a bradyarrhythmic phenotype, with lower prevalence of LVH.[7,10,25,42,68]

Cardiac magnetic resonance

Cardiac magnetic resonance has unique tissue-characterizing features and should be considered in patients with suspected mtDNA disease when transthoracic echocardiography is inconclusive, for the quantification of chamber volumes and left ventricular mass, and to exclude other differential diagnoses.[73,74] Specific patterns of late gadolinium enhancement have been described: compared with controls, patients with Kearns Sayre Syndrome and Chronic Progressive External Ophthalmoplegia show a higher prevalence of late gadolinium enhancement (LGE) confined to an intramural pattern in the inferolateral wall; a predominantly focal, patchy LGE with homogeneous distribution among left ventricular segments and severe concentric LVH is common in MELAS-like patients.[75–77]

Extracellular volume imaging and quantitative T2 mapping can be useful in patients without LGE, showing an expanded extracellular volume and diffuse increase in T2 signal.[78]

Cardiopulmonary exercise test

Cardiopulmonary exercise test in mtDNA-D often shows a reduced peak VO_2 and early lactic acidosis during exercise, because of increased reliance on anaerobic metabolism. Respiratory exchange ratio is increased in MD (often >1.5), reflecting a high rate of bicarbonate buffer. Other specific findings of mtDNA-D include a large increment in VE/VCO2 between the nadir and peak exercise and reduced mean arteriovenous oxygen difference.[79,80]

MANAGEMENT OF mtDNA DISORDERS

General Measures

To date, there is no drug treatment that has shown clinical benefit in mtDNA-D.[81] When possible, patients with mtDNA-D should avoid medications that could interfere with respiratory chain and precipitate acute metabolic crisis, such as metformin;

statins; valproic acid; high-dose acetaminophen; and antibiotics, including aminoglycosides, linezolid, tetracycline, and macrolides.[46] Patients affected by mtDNA-D should prevent entering catabolism and avoid prolonged fasting.[82] Intravenous dextrose-containing solutions may be considered for caloric supplementation in patients undergoing surgery or medical procedures, or in acute clinical settings, unless contraindicated. Patients with pyruvate metabolism disorders, ketogenic diet, or glucose intolerance should undergo careful nutritional supplementation during acute illness.[46]

There is growing evidence demonstrating the benefits of endurance exercise in patients with mtDNA-D.[83–88] Individuals with mtDNA-D undergoing exercise training showed improved exercise tolerance and reduced postexercise blood lactates,[86] recruitment of satellite cells in muscle fibers, and increased peripheral muscle strength.

Bradyarrhythmias

According to current 2018 AHA/ACC/HRS Guidelines,[89] permanent pacemaker implantation is recommended in patients with neuromuscular disease, including mtDNA-D, with evidence of second-degree AV block, third-degree AV block, or an HV interval of 70 ms or greater, regardless of symptoms. Pacemaker implantation has been proposed at earlier stage in patients with mtDNA-D than general population, as this subgroup of patients show an unpredictable rate of progression to high-grade AV block, mainly in carriers of single large-scale deletions. Thus, according to current guidelines[89] permanent pacemaker implantation may be considered in patients with MD with a PR interval greater than 240 ms, a QRS duration greater than 120 ms, or any grade of fascicular block.

Supraventricular Tachyarrhythmias

Current treatment options for the management of supraventricular tachyarrhythmias can be used in patients with mtDNA-D. In case of symptomatic ventricular preexcitation with recurrent episodes of AVNRT, accessory pathway ablation has been successfully performed in patients with mtDNA-D.[40,41,90,91] In asymptomatic patients with intermittent ventricular preexcitation, the role of electrophysiological study (EPS) and catheter accessory pathway ablation is more controversial. Although data are lacking, there is general consensus that catheter ablation of accessory pathway should be performed in asymptomatic patients in whom invasive EPS risk stratification identifies high-risk properties, according to current guidelines.[92]

CLINICS CARE POINTS

- The clinical spectrum of mtDNA-D is heterogeneous and can range from oligosymptomatic patients to severe multisystemic involvement.
- The presence of a maternal inheritance pattern or the identification of extra cardiac features of mtDNA-D should guide further investigations in order to exclude an underlying mitochondrial disorder.
- Patients with neuromuscular mtDNA-D often present with increased creatine kinase levels and symptoms of skeletal myopathy.
- In the suspicion of mtDNA-D, the baseline evaluation should include complete blood count and determination of glycated hemoglobin, iron status, creatine kinase, plasma proteins, transaminases, serum and urine aminoacids, and blood lactates.
- When the metabolic screening or the clinical evaluation can suggest a specific diagnosis, molecular testing with mtDNA or nDNA sequencing of target genes should be considered.
- Skeletal muscle biopsy is considered the gold standard for the diagnosis of mtDNA-D.

DISCLOSURE

The authors declare that they have no conflict of interest.

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