Review

A clinical approach to diagnosis and management of mitochondrial myopathies

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ARTICLE INFO

ABSTRACT

This paper provides an overview of the different types of mitochondrial myopathies (MM), associated phenotypes, genotypes as well as a practical clinical approach towards disease diagnosis, surveillance, and management. nDNA-related MM are more common in pediatric-onset disease whilst mtDNA-related MMs are more frequent in adults. Genotype-phenotype correlation in MM is challenging due to clinical and genetic heterogeneity. The multisystemic nature of many MMs adds to the diagnostic challenge. Diagnostic approaches utilizing genetic sequencing with next generation sequencing approaches such as gene panel, exome and genome sequencing are available. This aids molecular diagnosis, heteroplasmy detection in MM patients and furthers knowledge of known mitochondrial genes. Precise disease diagnosis can end the diagnostic odyssey for patients, avoid unnecessary testing, provide prognosis, facilitate anticipatory management, and enable access to available therapies or clinical trials. Adjunctive tests such as functional and exercise testing could aid surveillance of MM patients. Management requires a multi-disciplinary approach, systemic screening for comorbidities, cofactor supplementation, avoidance of substances that inhibit the respiratory chain and exercise training. This update of the current understanding on MMs provides practical perspectives on current diagnostic and management approaches for this complex group of disorders.

Introduction

Mitochondrial myopathies (MM) are a genetically diverse group of mitochondrial diseases that affect skeletal muscles. They are caused by mutations in mitochondrial or nuclear genes encoding proteins related to mitochondrial oxidative metabolism resulting in the inability of mitochondria to sustain cellular energy demand. Body systems relying heavily on oxidative metabolism such as muscle, heart, nervous system, kidneys, and endocrine organs often manifest pathology most frequently. Myopathy is a common manifestation of mitochondrial disorders because the skeletal muscles have a high and constant cellular energy demand. However, individuals with MM often have dysfunction in other organ systems as well and it is relatively rare to present with isolated mitochondrial myopathy. Recognizing MM requires the clinician to be alert to symptoms of progressive worsening of muscle weakness, episodic symptoms of exercise intolerance, cramps, and fatigue as well as presence of other organ dysfunction.

MM has been reported with a frequency of about 5–15/10000 [1]. The estimated prevalence of mitochondrial disorders across populations is approximately 1/5000 [2]. The real prevalence is likely underestimated as mitochondrial disorders are often undiagnosed due to the diagnostic complexity of disease presentation as most mitochondrial disorders present with multisystemic symptoms with variable age of onset ranging from infancy to adulthood.

Mitochondrial DNA (mtDNA) mutations are maternally-inherited whereas nuclear DNA (nDNA) mutations follow Mendelian inheritance principles (autosomal dominant, recessive or X-linked). Each cell contains multiple copies of mtDNA and variable load of wild-type and pathogenic mtDNA (mitochondrial heteroplasmy) and therefore disease manifestation may occur only when the mutation load crosses a threshold for respiratory chain dysfunction to occur. Mitochondrial heteroplasmy often contributes to phenotypic variability and variable disease expression even within the same family. Most mtDNA mutations impact structural protein subunits of the respiratory chain while nDNA mutations impact structural

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and ancillary protein subunits of the respiratory chain as well as elements of mtDNA maintenance and expression. Ultimately, making sense of the heterogenous presentation of mitochondrial disorders also requires an appreciation of the underlying disease mechanisms as well as identification of specific clues and red flags that may help in the diagnostic approach to narrow down the differential diagnoses and allow more targeted testing. In this review, we discuss a rational diagnostic approach to MM, which is one of the common presentations of mitochondrial diseases.

Pediatric and adult phenotypes

MM can present at any age. Patients with pediatric-onset MM typically have more significant generalized muscle and systemic involvement [2,3]. Isolated myopathy is uncommon in children and the more typical finding is that of myopathy being one of the many features of a multisystemic disease.

Individuals with adult-onset MM often have milder phenotypes, occasionally with presentation confined to specific muscles [3,4]. The most common presentation in adults is Chronic Progressive External Ophthalmoplegia (CPEO) and represents up to 20% of adult-onset mitochondrial disorders [2,5]. Interestingly, Kearns Sayre Syndrome (KSS) may resemble some cases of CPEO that are caused by single large-scale mitochondrial deletions, and is a clinical subtype of CPEO [5]. KSS emerges before the age of 20 years, with similar symptoms of ptosis and progressive external ophthalmoplegia but with additional multisystemic features of pigmentary retinopathy, cardiac conduction block, sensorineural hearing loss, ataxia and endocrine manifestations. This underlines the typically more severe pediatric phenotype for mitochondrial disorders and the greater burden of disease.

MtDNA disorders versus nDNA disorders

Genotype-phenotype correlation in mitochondrial disorders is challenging due to phenotypic variability and genetic heterogeneity. nDNA-related mitochondrial disorders are generally more common in paediatric-onset mitochondrial diseases while mtDNA-related diseases are more frequently observed in adults [6]. Yet it is not uncommon for disorders such as MELAS (Mitochondrial Encephalopathy, Lactic Acidosis and Stroke-like episodes) related to the common A3243G mutation to appear even in the neonatal and infant age group and therefore genetic evaluation in all age groups should include both mtDNA and nDNA testing [7].

Disorders of mtDNA can be classified according to the type of genetic defect: large-scale rearrangements, such as mitochondrial deletions or duplications, or point mutations. Large-scale rearrangements like deletions affecting protein synthesis genes may result in KSS, Pearson syndrome and CPEO and are generally sporadic in inheritance. Point mutations may occur in protein synthesis genes such as the tRNA genes causing MELAS (tRNA$^{Leu(UUR)}$ A3243G) or MERRF (Myoclonic Epilepsy with Ragged Red Fibers) (tRNA Lys A8344G) or in protein coding genes like ATP6 subunit T8993G variant causing NARP (Neuropathy, Ataxia, Retinitis Pigmentosa) or MILS (Maternally Inherited Leigh Syndrome) and are maternally-inherited.

nDNA-encoded mitochondrial disorders are often tissue-specific, severe, and frequently fatal.

Underlying pathogenic mechanisms for nDNA disorders include.

(1) nuclear gene mutations resulting in OXPHOS subunit deficiency such as *ACAD9* related to complex I deficiency with a clinical spectrum including infantile encephalomyopathy, hypertrophic cardiomyopathy, myopathy with exercise intolerance [8];
(2) nuclear genes involved in mtDNA maintenance and replication such as *TK2, RRM2B, MGME1* influencing myopathy related to mitochondrial depletion [9–11];

(3) nuclear genes influencing mitochondrial translation such as *YARS2* and *PUS1* and its associated mitochondrial myopathy, lactic acidosis and sideroblastic anemia (MLASA) [12,13];
(4) nuclear genes influencing mitochondrial fusion and fission such as *MIEF2* [14];
(5) nuclear genes encoding proteins involved in the assembly of iron-sulfur clusters which are important components of respiratory complexes, such as *ISCU* and associated congenital myopathy and *FDX1L* and associated childhood onset proximal myopathy [15, 16]; and
(6) nuclear genes influencing lipid metabolism and fatty oxidation such as *HADHA* or *HADHB* and Long Chain 3-Hydroxyacyl-CoA Dehydrogenase (LCHAD) deficiency [17], *ETFDH* and myopathy related to coenzyme Q10 deficiency [18], *CPT2* and carnitine transport disorder [19] and *ACADVL*-associated Very Long Chain Acyl-CoA Dehydrogenase deficiency (VLCAD) [20].

Mitochondrial myopathy syndromes

Many MM disorders are part of general multisystemic mitochondrial disorders with a conglomeration of symptom clusters with recognizable presentations (Table 1). Recognizing these clinical syndromes is helpful to narrow down the possible disorder and, in some cases, may even allow limited and specific testing e.g. mtDNA sequencing or gene panel for MELAS.

Certain pharmacological agents can contribute towards inhibition of the respiratory chain and are known to aggravate myopathy symptoms. Such medications can sometimes be described to result in iatrogenic MM and should be avoided in patients with MM [23]. Examples include statins which has been linked to mitochondrial complex III inhibition [24], metformin which inhibits mitochondria and can trigger lactic acidosis [25], linezolid which inhibits mitochondrial ribosomal protein synthesis [26], and sodium valproate which is known to inhibit oxidative phosphorylation and beta-oxidation, exacerbating disease especially in individuals with *POLG* disease [23].

It is imperative to obtain a specific diagnosis for patients to end their diagnostic odyssey, avoid unnecessary testing, provide prognosis, and facilitate anticipatory management of systemic risks. This also enables access to specific therapies (e.g. coenzyme Q10 supplementation for CoQ10 deficiency), novel therapies or clinical trials. Screening of at-risk relatives is also imperative, and their pre-symptomatic diagnosis could facilitate earlier treatment with potentially better outcomes.

Diagnostic algorithm for mitochondrial myopathy

A strong index of suspicion is required in approaching MM - for the very young, delayed motor milestones are common and children are often described to be less athletic than their peers. The most common presentation across the ages is limb weakness and fatigue followed by sudden onset of respiratory failure, ptosis, myalgia, limb swelling and tremor [9]. Creatine kinase and lactic acid levels are often high but could fluctuate in severity between stable state and metabolic crises.

Because of the high energy demands of metabolically active organs, there can be a puzzling multisystemic presentation that can present a challenge for the diagnostician. It is often the combination of seemingly unrelated pathologies in a patient that when put together, suggest a mitochondrial pathology [27]. This emphasizes the importance of a thorough review of the patient's medical history and detailed history taking. Features that should arouse the suspicion of MM include exercise intolerance, acquired ptosis, ophthalmoplegia, pigmentary retinopathy, sideroblastic anemia, stroke-like episodes, epilepsy, multisystemic involvement (e.g. hearing loss, cardiac arrhythmia, cardiomyopathy), elevated plasma or CSF lactate, elevated plasma alanine, and presence of urinary 3-methylglutaconic acid [28].

Table 1
Genotype and phenotype of common multisystemic mitochondrial "syndromes" presenting with mitochondrial myopathy.

Disease	mtDNA variation	nDNA genes	Inheritance	Age of onset	Key clinical symptoms	Distinctive features	Prognosis
Chronic Progressive External Ophthalmoplegia (CPEO)	*MT-TL1* m.3243A>G or large-scale deletion of mtDNA	*POLG* (95%), *C10orf2, RRM2B, SLC25A4, POLG2, DGUOK, SPG7*	Autosomal dominant, autosomal recessive, mitochondrial maternal inheritance	20–40s	Ptosis, ophthalmo-plegia, hearing loss, mild muscle weakness, dysphagia, cataracts	Pigmentary retinopathy ("salt and pepper" pigmentation)	Slowly progressive
Mitochondrial Encephalomyopathy, Lactic Acidosis and Stroke-like episodes (MELAS)	*MT-TL1* m.3243A>G (80%), m.3271T>C, *MT-TQ, MT-TH, MT-TK, MT-TS1, MT-ND1, MT-ND5, MT-ND6, MT-TS2*		Mitochondrial maternal inheritance	Childhood to 40s	Stroke-like episodes, hemiparesis, seizures, hearing loss, muscle weakness, vision, renal impairment, mitochondrial diabetes	Lactic acidosis; strokes that do not conform to vascular territories	Prognosis depends on level of organ involvement
Kearn-Sayre syndrome (KSS)	Large-scale deletions of mtDNA		Usually sporadic	Childhood, <20y	Progressive external ophthalmoplegia, pigmentary retinopathy, cardiac conduction block, cerebellar ataxia, deafness, short stature	CSF protein >100mg/dL Severe combined defects of mitochondrial complexes especially cytochrome C oxidase	Progressive disorder, prognosis depends on level of organ involvement
Myoclonus Epilepsy with Ragged Red Fibers (MERRF)	*MT-TK* m.8344A>G (80%), m.8356T>C, m,8363G>A, m.8361G>A		Sporadic or mitochondrial maternal inheritance	Childhood, <30y	Myoclonus, epilepsy, ataxia, myopathy, optic atrophy, deafness, peripheral neuropathy, cardiomyopathy with WPW syndrome	Myoclonus	Prognosis depends on level of organ involvement
Primary Coenzyme Q10 Deficiency [21]		*COQ2, COQ7, COQ8A, COQ8B COQ9*	Autosomal recessive	Infantile, childhood to adult onset	Predominant myopathy associated with encephalopathy, ataxia and retinopathy		Late onset disease shows better response to high dose CoQ10 supplementation
TK2 Deficiency		*TK2*	Autosomal recessive	Infantile, Childhood and adult onset	Muscle weakness, hypotonia, bulbar dysarthria and dysphagia	Elevated creatine kinase (5-10x upper limit of normal), Severe reduction in mtDNA content in affected tissues and organs	Poor
Reversible Infantile Respiratory Chain Deficiency myopathy (RIRCD) [22]	mt-tRNAGlu m.14674T>C (homoplasmic)	Associated with nDNA genes interacting with mt-tRNA Glu e.g. *EARS2, TRMU*		Infancy	Profound muscle weakness, hypotonia, feeding difficulties, Reversble transaminitis during periods of severe metabolic crises	Muscle biopsy in neonatal period shows numerous RRF and COX negative fibres, accumulation of lipids and glycogen which later resolve	Spontaneous improvement by 1 year, mostly asymptomatic by 2–3 years old

Family history should be reviewed in detail, where features that should arouse interest include a history of conditions like diabetes, epilepsy, myopathy, hearing loss, blindness in the maternal family line. The absence of any suggestive family history should not exclude the possibility of MM, as some conditions can be sporadic or arise *de novo* in the affected individual. A diagnostic algorithm is suggested (Fig. 1) based on the symptoms, some of which may cluster into a recognizable syndrome.

Testing strategy

Testing of patients with MM includes both diagnostic testing as well as disease severity evaluation and monitoring (Fig. 2).

Biochemistry

Lactate and creatine kinase (CK) levels have been demonstrated to be elevated in only a proportion of patients with specific genotypes [29,30]. Normal levels do not exclude the diagnosis of MM. For example, only 40% of individuals with the common m.3243A>G patients and MM had elevated lactate levels [31]. Plasma amino acids may be helpful as elevated alanine may be seen especially during periods of metabolic decompensation. Urine organic acids may show increased excretion of TCA cycle intermediates such as ethylmalonic acid and 3-methyl-glutaconic acid or dicarbolic aciduria [32].

Biochemical markers like growth differentiation factor 15 (GDF-15) and fibroblast growth factor 21 (FGF-21) were significantly elevated in patients with mitochondrial myopathies compared with a matched control population, with no correlation with severity of disease [31]. However it is important to note that GDF-15 can also be elevated in individuals with other neuromuscular diseases of non-mitochondrial origin such as muscular dystrophy or spinal muscular atrophy and hence it is not specific [33].

Molecular genetics

Genetic testing plays a crucial role in the diagnosis of MM, and broad coverage approaches using next generation sequencing (NGS) techniques such as whole exome sequencing (WES) or whole genome sequencing (WGS) with mtDNA sequencing coverage is recommended as the first line test in individuals suspected with a mitochondrial disease [34–36]. These could include individuals with a myopathy phenotype and with a personal or family history of multisystem involvement (e.g. diabetes, deafness, developmental delay, seizures). It is important to consider analysis of both nDNA and mtDNA as MM can be caused by both. This approach has multiple advantages, including reducing the need for invasive muscle biopsy, enabling a rapid diagnosis, and parallel evaluation of nDNA and mtDNA variants, as well as for other rare genetic diagnoses with similar phenotypes which can present as phenocopies.

WES has coverage of the exome, or protein-coding regions of the genome. 80% of disease-variants are reported to be found in the exome [37]. WGS covers exonic and intronic regions of the genome and has better sensitivity and identification of exonic single nucleotide variants (SNV) or copy number variants (CNV). WGS is, however, more costly than WES and thus potentially less accessible to patients. Additionally, many, though not all, laboratories include mtDNA analysis with WES/WGS analysis and hence this must be included if MM is suspected.

Multiple options of genetic testing exist. Targeted variant or gene testing has largely been phased out in favor of broad scale analysis due to relative affordability and coverage of NGS panels, WES and WGS. Individuals with a myopathy phenotype may be offered testing with a neuromuscular gene panel due to cost considerations. However, If MM is suspected, it is imperative to ascertain that the test selected has adequate coverage of relevant nDNA genes and mtDNA or to consider more detailed testing using WES or WGS with mtDNA coverage instead.
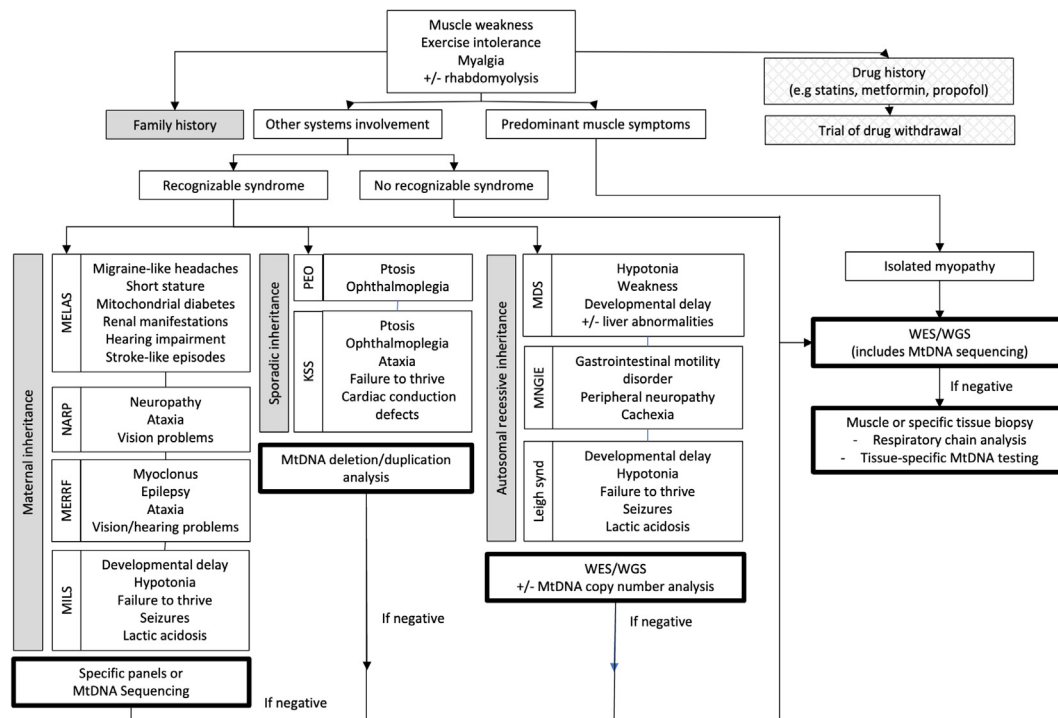
Fig. 1. Diagnostic algorithm for mitochondrial myopathies. (KSS - Kearns Sayre Syndrome; MDS - Mitochondrial Depletion Syndrome; MELAS - Mitochondrial Encephalopathy, Lactic Acidosis and Stroke-like episodes; MERFF - Myoclonic Epilepsy and Ragged Red Fibers; MILS - Maternally Inherited Leigh Syndrome; MNGIE - Mitochondrial Neuro-Gastro-Intestinal Encephalopathy; NARP - Neuropathy, Ataxia and Retinitis Pigmentosa, PEO - Progressive External Ophthalmoplegia; WES - Whole Exome Sequencing; WGS - Whole Genome Sequencing).
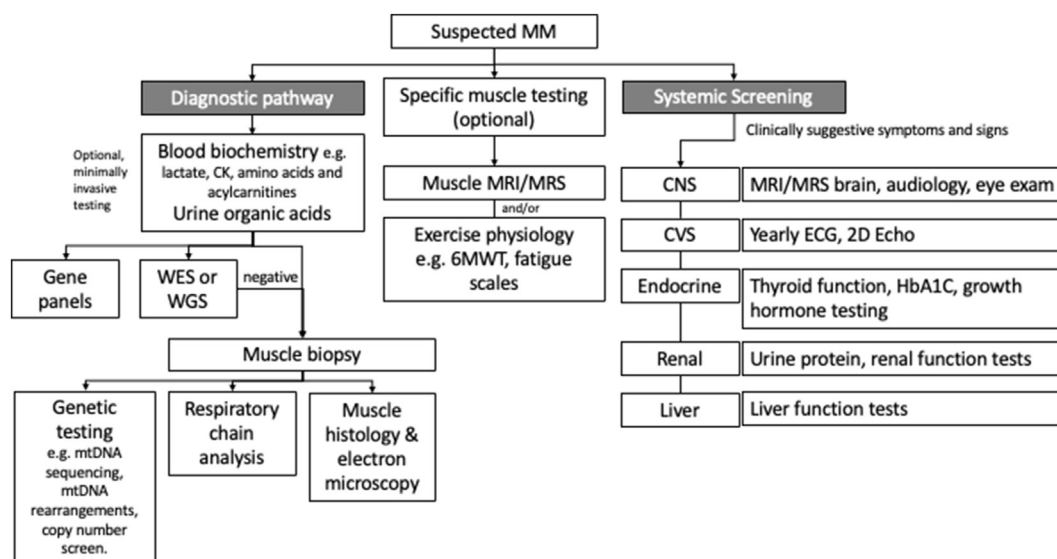
Fig. 2. Testing strategy and systemic screening for mitochondrial myopathies for diagnosis as well as evaluation of disease severity. (CK - creatine kinase; WES - whole exome sequencing; WGS - whole genome sequencing; MRI - magnetic resonance imaging; MRS - magnetic resonance spectroscopy; 6MWT - 6 minute walk test; CNS - central nervous system; CVS - cardiovascular system; ECG - electrocardiogram; 2D Echo - echocardiogram).

Analysis of the genetic findings is best undertaken with specialists familiar with clinical interpretation of genomic test results. Adoption of the American College of Medical Genetics and the Association of Molecular Pathology (ACMG-AMP) standards and guidelines for variant interpretation and specifications for mtDNA variant interpretation has aided the standardization of analysis but discrepancies can still occur, particularly in the analysis of novel variants where variant-level information may be limited [38,39].

Genetic testing is usually performed with whole blood DNA with alternatives like hair follicle DNA, saliva/buccal swab DNA. On occasion, the mtDNA variant may not be detectable on blood samples due to tissue-specific heteroplasmy. In addition, mtDNA deletions, depletion and rearrangements are often only detectable in muscle DNA and may be missed on analysis of blood specimens. In such instances, negative results from blood samples require follow up genetic testing on other tissue samples such as muscle (see section on Biopsy).

MtDNA findings are usually identified heteroplasmic although there are disease-causing homoplasmic mtDNA mutations. Measuring the level of heteroplasmy in blood samples has limited efficacy as this often does not correlate with disease severity or prognosis. This could be due to different distribution of heteroplasmy among different tissue types and variable heteroplasmy with age due to mitotic division throughout a person's lifetime [40–42]. One study has suggested that the level of heteroplasmy in the urine correlates better than blood as urinary samples include post-mitotic cells derived from the urinary system [43,44].

In addition, transcriptome analysis via RNA-seq may enable genetic diagnosis in patients with uncertain splice or null variants or who remain unsolved by current DNA sequencing approaches [45,46]. Emerging methods like real-time and long read sequencing can enhance diagnosis with the ease of detection of mtDNA deletions, other structural variants, tandem repeats, epigenetic modifications and cis/trans phasing of compound heterozygote variants for patients suspected with MM [47,48].

Tissue biopsies

Muscle biopsies have traditionally been done as part of MM diagnostic work up and includes mtDNA sequencing, histopathology, and respiratory chain enzyme analysis. However, with the facility of NGS technologies, muscle biopsies now form the next layer of testing when molecular genetic testing is negative or if there is a suspicion of tissue-specific heteroplasmy or mtDNA large scale rearrangements. It has also been shown that atypical MELAS variants encoding mtDNA-encoded complex subunits had mutation loads that were very low in blood but were high in muscle tissues [49]. Similarly, other mtDNA copy number variants may be missed on NGS in blood as well. Such findings are assessed for in muscle tissue specimens via Sanger sequencing, long range PCR, quantitative PCR, digital droplet PCR, or deep sequencing [50,51].

Muscle histopathology findings for MM include 1) ragged-red fibers (RRF) on modified Gomori trichrome stain, 2) proliferation of mitochondria observed with histochemical stain for succinate dehydrogenase (SDH) often described as ragged-blue fibers, 3) cytochrome *c* oxidase (COX or complex IV) deficient or negative fibers, and on electron microscopy 4) subsarcolemmal accumulation of ultrastructurally abnormal mitochondria, often with paracrystalline inclusions [51]. Such findings help to distinguish MM from other muscle disorders like congenital myopathies, muscular dystrophies, or metabolic myopathies. Certain features such as RRF may however not be seen in young children as there may not have been adequate time for muscle degeneration and sub-sarcolemmal mitochondrial accumulation to occur.

Muscle respiratory chain (RC) enzyme (complexes I-IV) analysis is done spectrophotometrically or in native gels while western blot, native gel, or two-dimensional gel assays and can assess levels of oxidative phosphorylation proteins on blood, skin, muscle, liver samples [51]. Abnormalities of several complex activities may be seen in disorders of mtDNA maintenance, transcription, translation, or nucleotide synthesis. On the other hand, defects in a single complex could indicate a deficit in a structural subunit or an assembly protein [52]. More recently, this can also be assessed via a quadruple immunofluorescent technique enabling the quantification of key RC subunits of complexes I and IV which gives precise and objective quantification of protein abundance in large numbers of individual muscle fibres [53]. Limitations of RC analysis include the fact that RC defects can be secondary to other metabolic or neuromuscular diseases.

Imaging

There are limited studies on the utility of muscle imaging in MM. Magnetic resonance spectroscopy (MRS) of muscle has detected high lactate in patient with CPEO and Fourier transform infrared spectroscopy (FTIR) has been shown to be able to distinguish CPEO from other muscle disorders but these biomarkers may not be in common clinical use as molecular genetics often suffice for diagnostic purposes [54,55].

MRI brain structural imaging has been used to identify specific mitochondrial syndromes such as MELAS where patients may have strokes that cross vascular territories. In general however, MRI brain findings tend to be non-specific although specific features such as symmetrical signal abnormality of the deep gray structures showing high signal on T2 and FLAIR images and hypointensity on T1 images may be very suggestive of a mitochondrial neurological disorder. MRS changes may also be seen when impaired RC function shifts metabolism from the tricarboxylic acid cycle to glycolysis [56]. ^{1}H-MRS has also been helpful especially if areas of abnormality are compared with normal regions of the brain and demonstrate a double lactate peak and corresponding decreased NAA peak.

Positron emission tomography (PET) imaging measures metabolic flux and is able to directly evaluate subtle biological changes like the redox status of the brain. Several radioisotopically labeled metabolites have been used to study mitochondrial disorders and include ^{15}O, 2-deoxy-2 ^{18}F-fluoro-D-glucose (FDG) and ^{11}C pyruvate. PET study of tissue-specific bioenergetics has shown both region and global impairment of cerebral oxygen metabolic rate in patients with mitochondrial disease with neurological phenotype e.g. MELAS A3243G variant. However, patients with predominant muscle phenotype may not demonstrate as robust PET imaging changes as patients with neurological involvement [57].

Functional testing

Functional testing aids with definition of the extent and severity of muscle weakness but is unlikely to provide a specific molecular diagnosis. These tests may be useful in the clinical setting to evaluate disease progression as well as responses to specific interventions.

Exercise tests like the 6-Minute Walk Test (6MWT), Timed Up and Go test (3TUG), Five Times Sit to Stand Test (5XSST) and Test of Masticating and Swallowing Solids (TOMASS) have been demonstrated to display a significant difference in patients with mitochondrial myopathies versus normative controls at time of diagnosis [31]. These scores were not shown to correlate with heteroplasmy levels.

Patient-reported outcome measures such as the Fatigue Severity Scale (FSS) and West Haven-Yale Multidimentional Pain Inventory (WHYMPI) also had differential scores in MM patients with significant statistical differences, and more prominent in females [31].

Mitochondrial disease-specific scales such as the Newcastle Mitochondrial Disease Adult Scale (NMDAS) and the International Pediatric Mitochondrial Disease Scale (IPMDS) are often used for natural disease evaluation of patients with multiple system involvement and may be less useful for patients with an isolated or predominant muscle phenotype [58,59]. Specific muscle scales such as the Primary Mitochondrial Myopathy Symptom Assessment (PMMSA) scale may however be useful in adult patients with predominant myopathy symptoms and may also identify degree of fatigue that may affect daily functioning [60].

Exercise testing

Exercise testing has a limited role in evaluating patients with MM, although it may be helpful in patients who are oligosymptomatic who experience exercise intolerance with fatigue. Challenges to exercise testing would be an individual's exercise capacity due to cardiovascular fitness, intellectual ability to follow commands and presence of other physical disabilities [28].

Whole body maximal exercise testing measuring maximum oxygen uptake (VO_{2max}) has been shown to correlate with mutation load and is reflective of the oxidative capacity of skeletal muscle in MM [61]. Measuring VO_{2max} with cycle ergometry is therefore an attractive non-invasive method of evaluating oxidative capacity of skeletal muscle. However, patients with other forms of myopathy may also have poorer VO_{2max} and so diagnostic utility requires a combination of VO_{2max} testing with other parameters such as ventilation response or serum biomarkers such as serum growth and differentiation factor-15 (GDF-15). A short duration maximal exercise test has been found to show 5-fold increase in GDF-15 levels in patients with MM compared to healthy controls or patients with metabolic myopathy.

Novel biomarkers

Development of biomarkers requires prioritization as new therapies are being evaluated. Biomarkers include.

1. Metabolomic markers measured with nuclear magnetic resonance (NMR) spectrometry or mass spectrometry (MS) methods. Specific diseases such as Leber's Hereditary Optic Neuropathy (LHON) with distinctive signatures such as altered sphingomyelins and phosphatidylcholines may be studied with MS methods [62].
2. Serum markers such as fibroblast growth factor-21 (FGF-21) and growth and differentiation factor-15 (GDF-15) are cytokines that were studied in mitochondrial disease mouse models but have yet to be used clinically as biomarkers in humans. Although FGF-21 is produced in skeletal muscle, and both FGF-21 and GDF-15 have been found to be elevated in patients with mitochondrial diseases, specificity is low as other diseases such as renal failure, hepatic disease, malignancy, diabetes, and obesity may also be associated with elevated levels. Nonetheless, these cytokine biomarkers may be useful in evaluating mitochondrial diseases especially those affecting mitochondrial translation and mtDNA maintenance [63].
3. MicroRNAs are small, highly conserved non-coding RNA regions that regulate gene expression. Distinctive microRNA patterns have been found in A3243G cybrid cell lines and may potentially be useful in cell-based screening of potential mitochondrial disease treatments.
4. Small molecule reporters are tailor-made probes that can be targeted to a substrate of interest and delivered to accumulate within the mitochondria within an organ of interest. This may allow *in vivo* measurement of mitochondrial function and generation of reactive oxygen species. This is an emerging technology that has not been used in human studies yet.

Treatment of mitochondrial myopathies

General management of patient with MM

Clinical goals of managing a patient with MM are to evaluate and screen for comorbidities, mitigate symptoms and minimize or slow down disease progression [64,65]. Management of a patient with MM requires a multidisciplinary approach, including involvement of the physiotherapist and occupational therapist for optimization of strength and motor function, speech therapist for dysphagia management, dietician for optimization of nutrition and avoidance of rapid weight loss and catabolism, and clinical specialists such as the neurologist, ophthalmologist, geneticist, respiratory specialist and other relevant specialists depending on the patient's symptoms and manifestations [65]. Appropriate post-test genetic counseling and support is imperative after diagnosis to help the patient and family cope with acceptance of a challenging rare disease diagnosis.

Regular and periodic screening for systemic involvement should be considered [65]. This can vary depending on the genotype identified on genetic testing if there are known genotype-phenotype correlations. Such screens can include testing for hearing loss, arrhythmia or cardiomyopathy, endocrinological problems like diabetes or hypothyroidism, pulmonary function, ophthalmic involvement, or renal disease, Medications inhibiting respiratory chain function with the potential to exacerbate clinical symptoms should be avoided as discussed above [65,66]. These include statins, metformin, valproate, linezolid acid, aminoglycosides, and neuromuscular blocking agents. A comprehensive list of medications considered safe and unsafe can be found at https://www.mitopatients.org/mitodisease/list-of-medicines [66]. The patient's drug chart should

Table 2
New and experimental treatments.

Type of therapy	Mechanism of action	Compound/drug	Evidence	References
Drug Therapy	Increase cellular concentration of mitochondrial NAD+	KL1333	KL1333 has been shown to improve mitochondrial biogenesis and function in fibroblast line derived from a MELAS patient. No in vivo studies yet.	[82]
	Increase cellular concentration of mitochondria concentration	Omaveloxolone	Well tolerated and improved lowering heart rate and lactate levels during submaximal exercise, did not significantly change peak exercise workload in MM.	[83]
		REN001	PPAR β/δ agonist shown to improve fatigue and function in patients with fatty acid oxidation defects. Phase II trials in MM ongoing.	[84]
		Bezafibrate	Modest improvement in cardiac function and reduction in immunodeficient muscle fibers in MM patients	[85]
		Acipimox	Acipimox has been shown to improve mitochondria expression in vitro. Phase I clinical trials in adult MM patients ongoing.	[86,87]
	Protecting mitochondria from damage	Elamipretide	Shown to be associated with clinical and functional improvements in children and adults with MM.	[88–93]
	Restoring mitochondrial homeostasis	Deoxynucleoside therapy	Use in patients with TK2 deficiency showed improved motor and respiratory function	[94–96]
	Enzyme replacement	Erythrocyte Encapsulated Thymidine Phosphorylase (EE-TP)	Use of patients with MNGIE showed clinical improvement and reductions in thymidine, and deoxyuridine.	[97–99]
Dietary supplementation	Correct taurine modification defect at the first anticodon nucleotide of mitochondrial tRNA$^{Leu(UUR)}$	High dose taurine	Use in MELAS patients was shown to reduce frequency of stroke-like episodes and improved taurine modification of mitochondrial tRNA$^{Leu(UUR)}$ from peripheral blood leukocytes	[100]
	Improve systemic NAD+ deficiency	Niacin	Oral niacin supplement increased blood NAD+ up to 8-fold and muscle NAD+ up to level of controls	[101,102]
	Influencing glutamate-glutamine cycle and glutamine transporters in the blood-brain barrier	High dose glutamine	Significant reduction in CSF glutamate and increment of CSF glutamine level in MELAS patients	[103]
	Stimulate mitochondrial function	Resveratrol	In vitro studies suggest improvements in mitochondrial fatty oxidation. However in vivo studies demonstrate lack of improvement in exercise capacity in adults with MM.	[104,105]
Dietary manipulation	Stimulate mitochondrial function	Ketogenic diet	Positive impact on mitochondrial bioenergetics, mitochondrial ROS/redox metabolism and mitochondrial dynamics	[106]
Exercise therapy	Improve oxidative capacity and activity tolerance	Aerobic training	Aerobic training improves mitochondrial volume. Uncertain effect on muscle strength, effort tolerance and quality of life.	[61,61,107,108]
Device	Reduce oxidative stress	Near-infrared light-emitting diode	In vitro evidence as an effective antioxidant therapy	[109,110]
	Modulate cortical and subcortical functional abnormalities	Transcranial direct current stimulation	Improved mitochondrial function and attenuated mitochondrial damage in mouse models. Aided improved clinical outcomes in autism, dyslexia and attention deficit.	[111,112]
Surgery	Alleviate symptoms due to ptosis-related impairment of visual axis and head posture	Ptosis surgery in CPEO	Ptosis surgery (levator resection or frontalis silicone sling surgery) in patients with CPEO showed statistically significant improvement in marginal-to-reflex distance (MDRI) and chin-up posture.	[113]
Gene therapy	tRNA modification		MTO1 overexpression fully restored 5-taurinomethyluridine frequency and partially increased the aminoacylation efficiency of MELAS tRNA, leading to the upregulation of mitochondrial protein synthesis and respiratory activity in MELAS myoblasts in vitro.	[114]
	AAV gene delivery		Administration of human *NDUFS4* coding sequence by AAV2/9 and/or AAV-PHP.B vectors improved clinical phenotype and prolonged the lifespan in Leigh syndrome mouse models	[115–117]
			AAV9 delivery of human *TK2* cDNA delaying disease onset and extending lifespan in mouse models.	[96]
	Mitochondrial targeting with recombinant oligoribonucleotides		In vitro studies showed improved heteroplasmy proportions of mutant mtDNA in cultured cells with KSS mtDNA deletion and with mtDNA ND5 point mutation.	[118,119]
	CRISPR-Cas9-mediated mitochondrial genome editing		In vitro studies in human cell lines and zebrafish has shown ability for this to target and reduce mtDNA copy number.	[120,121]
	CRISPR-free base editing		In vitro studies have shown application for mitochondrial base editing in human cell lines, mice, zebrafish and plants.	[122–124]

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be reviewed and unsuitable medications adjusted to suitable alternatives, for example statins should be switched to drugs like ezetimibe or alirocumab for treatment of hyperlipidemia in MM patients [67].

Prevention of illnesses which can induce catabolism and metabolic decompensation is important and the patient should be informed accordingly. This should be done by vaccination for preventable infections and typical infection control measures including avoidance of sick individuals, mask-wearing, and hand hygiene. Vaccination safety in MM has not been specifically investigated but expert consensus is that the risk of mitochondrial disease worsening due to an infectious trigger is greater than the theoretical risk of metabolic decompensation directly related to vaccination [66]. Aggressive treatment of fever, seizures, hyperlacticaemia and electrolyte and hormonal abnormalities is necessary to get the patient into a state of balanced homeostasis and decreased risk of metabolic decompensation.

Cofactor supplementation is recommended in MM with established cofactor deficiency known to respond to supplementation such as coenzyme Q10 (CoQ10) in primary or secondary CoQ10 deficiency [64]. The use of mitochondrial medication regimens including carnitine, CoQ10, arginine and others could optimize residual respiratory chain function, reduce oxidative stress, support alternative pathways for energy production and remove toxic metabolites where relevant [64]. Such regimens are colloquially termed as "mito-cocktails". There is a paucity of randomized controlled trials and difficulty in measuring clinical outcomes to evaluate the efficacy of such regimens. For example, a systematic review of 37 articles on the use of l-arginine in MELAS showed no demonstrable clinical efficacy, but the studies were assessed to be of poor methodologic quality [68]. Nonetheless, such regimens have good safety profiles and reasonable acceptance and relatively easy applicability.

Management of acute metabolic crises

Patients with MM are susceptible to metabolic decompensation and worsening of symptoms during intercurrent illnesses or other catabolic stresses. Upon diagnosis, patients should be well-educated on their risks, emergency measures and to carry a memo stating their diagnosis and risks/precautions [65]. Prompt attention and intervention by a care team familiar with management of MM will aid in mitigating risks. Acute crises include severe lactic acidosis, which may require the use of dichloroacetate which can acutely reduce blood and CSF lactate but is not known to improve overall long term mortality from MM [69], or acute neurological deficits with metabolic stroke in MELAS requiring urgent administration of IV L-arginine [70]. Supportive intensive care for patients with MM should include surveillance for systemic risks related to MM including cardiac arrhythmia, cardiomyopathy, respiratory decline requiring ventilatory support and electrolyte disturbances due to tubulopathy or adrenal dysfunction [65].

Exercise training

In the absence of specific treatments for MM, efforts have been focused on exercise training to improve muscle function for patients with MM. Exercise training increases oxidative capacity of the muscles by increasing mitochondrial mass but may also increase mutation load of the muscles [3]. Nonetheless, exercise training has been shown to be generally beneficial to patients and a gradually progressive program of alternating aerobic and resistance training is recommended, avoiding exercise on days where the patients may have fever, illness, significant muscle pain or fasting [71].

Special considerations

High altitude travel including air travel should be carefully considered in patients with MM due to a theoretical risk of medical worsening. Hypoxia associated with altitude can downregulate respiratory chain

capacity and reduce muscle mitochondria content [72]. Early recognition of medical deterioration and appropriate care, as well as need for oxygen saturation monitoring and supplemental oxygen in those with cardiomyopathy or respiratory weakness should be considered [65].

When undergoing general anesthesia, care must be taken to minimize the metabolic stress of surgery to prevent metabolic decompensation [73]. Certain medications should be used with caution such as propofol, known to inhibit the respiratory chain with its use associated with propofol infusion syndrome [74]. Use of depolarizing neuromuscular blockers such as suxamethonium should be avoided due to risk of exaggerated hyperkalemic response [75]. Avoidance of intraoperative hypoglycemia, hypotension, hypoxia and hypothermia, monitoring of intraoperative lactate as a marker of metabolic stress and avoidance of lactate-containing solutions is also recommended [76].

New and experimental treatments

Novel therapies targeted at improving mitochondrial function, patient's functional status and replacing defective genes have been studied in recent years [77]. Recent developments have been summarized in Table 2.

Family planning

Risk of recurrence and inheritance of MM depend on the cause of the condition. nDNA-related MM tend to obey Mendelian inheritance, where risk to offspring depends on the mode of inheritance of the gene in question. Prenatal genetic testing and preimplantation genetic testing for the familial variant. mtDNA-related MM follows a maternal inheritance pattern. Prenatal testing is complex in mtDNA variation as factors such as mutant heteroplasmy levels, threshold levels, phenotypic expression need to be considered and may not accurately predict offspring phenotype [78]. Genetic counseling is indicated to aid such families to understand their risks and options. Mitochondrial replacement therapy and assisted reproductive technology offers promise to such families to have unaffected children but has associated ethical considerations that may hinder implementation [79–81].

MM is a common presentation for mitochondrial disorders and may appear in isolation or in tandem with other organ presentations. Diagnosis and management are challenging because of the heterogeneity of disease presentations but an understanding of disease mechanisms as well as a reasoned approach to recognizing the cluster of disease symptomatology may aid in narrowing down the differential diagnoses. Genetic testing in suspected individuals including parallel analysis of nDNA and mtDNA should be considered to enable rapid diagnosis and reduce the need for other investigations. This facilitates prognostication and management of the MM patient. Strides have been made in the development of novel biomarkers and targeted therapies that show future promise to aid and improve the clinical outcomes of an otherwise potentially devastating diagnosis.

Author contribution statement

H.L.C, L.P.S and S.K.H.T contributed to the conception, design and writing of this manuscript.

Declaration of competing interest

H.L.C. is a shareholder in Alamya Health. The other co-authors do not have any conflicts of interests to declare in relation to this manuscript.

Acknowledgements

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