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REVIEW

Diagnosis, management, and follow-up of mitochondrial disorders in childhood: a personalized medicine in the new era of genome sequence

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Abstract

Primary mitochondrial disorders are highly variable in clinical presentation, biochemistry, and molecular etiology. Mitochondrial disorders can be caused by genetic defects in the mitochondrial, in nuclear genome, or in the interplay between the two genomes. Biochemical screening tests may be inconclusive or misleading since patients, with confirmed mitochondrial disorders specially in pediatric age, may exhibit normal routine biochemistry, muscle histology, or enzymatic analysis of the mitochondrial respiratory chain. Diagnosis is often challenging even with combination of multiple criteria (clinical, biochemical, histological, and functional), as innumerous conditions cause secondary mitochondrial dysfunction. Nowadays, a definite diagnosis is only possible by genetic confirmation since no single score system is satisfactorily accurate, being sensitive but not specific.

Conclusion: Awareness between physicians is of major importance considering that clinical suspicion may not be obvious regarding the heterogenicity in presentation and biochemical features of mitochondrial disorders. In this review, we provide information on diagnosis approach to patients suspected for mitochondrial disorders as well as management on chronic and acute settings. Follow-up should provide comprehensive information on patient's status, since intervention on these diseases is mostly supportive and prognosis is variable and sometimes unpredictable.

What is Known:
- *Mitochondrial disorders are heterogenous and may present at any age, with any symptoms and any type of inheritance.*
- *Mitochondrial disorders may be due to pathogenic variants in mitochondrial DNA (mtDNA) or nuclear genes (nDNA).*

What is New:
- *Since no single score system is satisfactorily accurate, a definite diagnosis is only possible with genetic studies with gene panels proving to be a cost-effective approach.*
- *Clinical and biochemical features of patients without a confirmed diagnosis must be reviewed and other diagnosis must be considered. A wider genetic approach may be applied (WES or WGS).*

Keywords Mitochondria · Mitochondrial disease · mtDNA · Respiratory chain deficiency · Genetic diagnosis

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Abbreviations

ATP	Adenosine triphosphate
DNA	Deoxyribonucleic acid
LHON	Leber hereditary optic neuropathy
MELAS	Mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes
MERRF	Myoclonic epilepsy with red ragged fibers
MRI	Magnetic resonance imaging
mtDNA	Mitochondrial DNA
NARP	Neuropathy with ataxia and retinitis pigmentosa
nDNA	Nuclear DNA
OXPHOS	Oxidative phosphorylation
PEO	Progressive external ophthalmoplegia
RRF	Red ragged fiber
WES	Whole exome sequence
WGS	Whole genome sequence

Introduction

Mitochondria are dynamic and complex organelles located in the cytoplasm with autonomous replication from mitosis. Mitochondria have independent genetic material (mtDNA): a double-stranded circular DNA molecule with 16,569 DNA base pairs containing 37 genes [37], representing 0.4% of total cell DNA. Mitochondrial function and replication are not autonomous from the nucleus, following multiple nuclear signals for mitochondrial protein synthesis and replication and incorporating proteins synthetized in the cytoplasm [1, 37, 38]. An efficient protein synthesis and assembly depends on nuclear (nDNA) and mitochondrial DNA—mitochondrial dual genetic control [37]. Pathways involved in the nucleus–mitochondria communication are complex and over 1500 proteins are listed in the mitochondrial proteome [22, 34].

Mitochondrial metabolism is complex and comprises energy production through oxidative phosphorylation, pyruvate oxidation through Krebs cycle, fatty acid beta-oxidation, amino acid catabolism, apoptosis, autophagy, and signal transduction [14, 23]. Hence, mitochondrial dysfunction may supervene in innumerous situations.

Oxidative phosphorylation (OXPHOS) is obtained through the respiratory chain, formed by five complexes. Its main purpose is to create an electrochemical gradient to allow ATP (adenosine triphosphate) synthesis, allowing the complete oxidation of 1 mol of glucose to have a net gain of 31 ATP molecules (previously considered a net gain of 38 molecules) [35]. A simplified vision of the mitochondrial metabolism and details on the respiratory chain are shown in Fig. 1.

Strictly speaking, mitochondrial disorders primarily affect OXPHOS and have an estimated incidence of 1:5000 to 1:8500 live births [3, 9, 23, 37]. Genetic defects (point mutations or deletions) that can result in a mitochondrial disorder can affect either mitochondrial or nuclear genome or the interplay between the two genomes leading to defects of assembly, mtDNA transcription, translation and maintenance, or mitochondrial fusion and fission [14, 25, 37].

Large-scale mtDNA deletions and rearrangements (breakpoints occurring within directly repeated sequences which allow the mtDNA molecule to maintain stability) are usually sporadic and have a broad phenotypic spectrum [21].

Mitochondrial disorders can also be due to a quantitative defect, i.e., reduced number of copies of mtDNA and are known as mitochondrial DNA (mtDNA) depletion syndromes [30].

Nuclear genome defects are responsible for the majority (80–90%) of mitochondrial disorders manifested in childhood [31, 34].

Mitochondrial disorders may show any kind of inheritance: mitochondrial, recessive, dominant, or X-linked [14]. Mitochondrial DNA transmission has higher variability since mitochondrial genome may be similar in all copies (homoplasmy) or may differ between copies (heteroplasmy). This may have an effect on clinical phenotype (threshold effect) [14] and accounts for the variability observed between offspring of an affected mother (mitochondrial genetic bottleneck), represented in Fig. 2 [37]. Heteroplasmy has unexpected effects in offspring and the heteroplasmy rate cannot always correlate with clinical phenotype.

Objective

This systematic review aims to summarize clinical features and workup and presents a diagnostic approach of mitochondrial disorders. It also reviews management on chronic and acute settings for patients with suspected or confirmed mitochondrial disorders and provides comprehensive follow-up recommendations based on a personalized medicine approach.

Clinical presentation

Mitochondrial disorders are highly heterogeneous with acute, static, or progressive symptoms at any age of onset [1, 3, 9, 15, 45]. As expected, tissues with higher energy demand, such as brain, heart, skeletal muscle, liver, kidney, and eye, will be more affected [34]. Nonetheless, signs and symptoms may associate in an unpredictable manner and patients may be oligosymptomatic.

Clinical suspicion of a respiratory chain disorder must be considered in the presence of the following: (1) consanguineous parents (consider a founder effect on parents from the same birthland); (2) evidence of maternal inheritance; (3) more than one affected system (especially if not embryologically related); (4) progressive disease; (5) worsening with

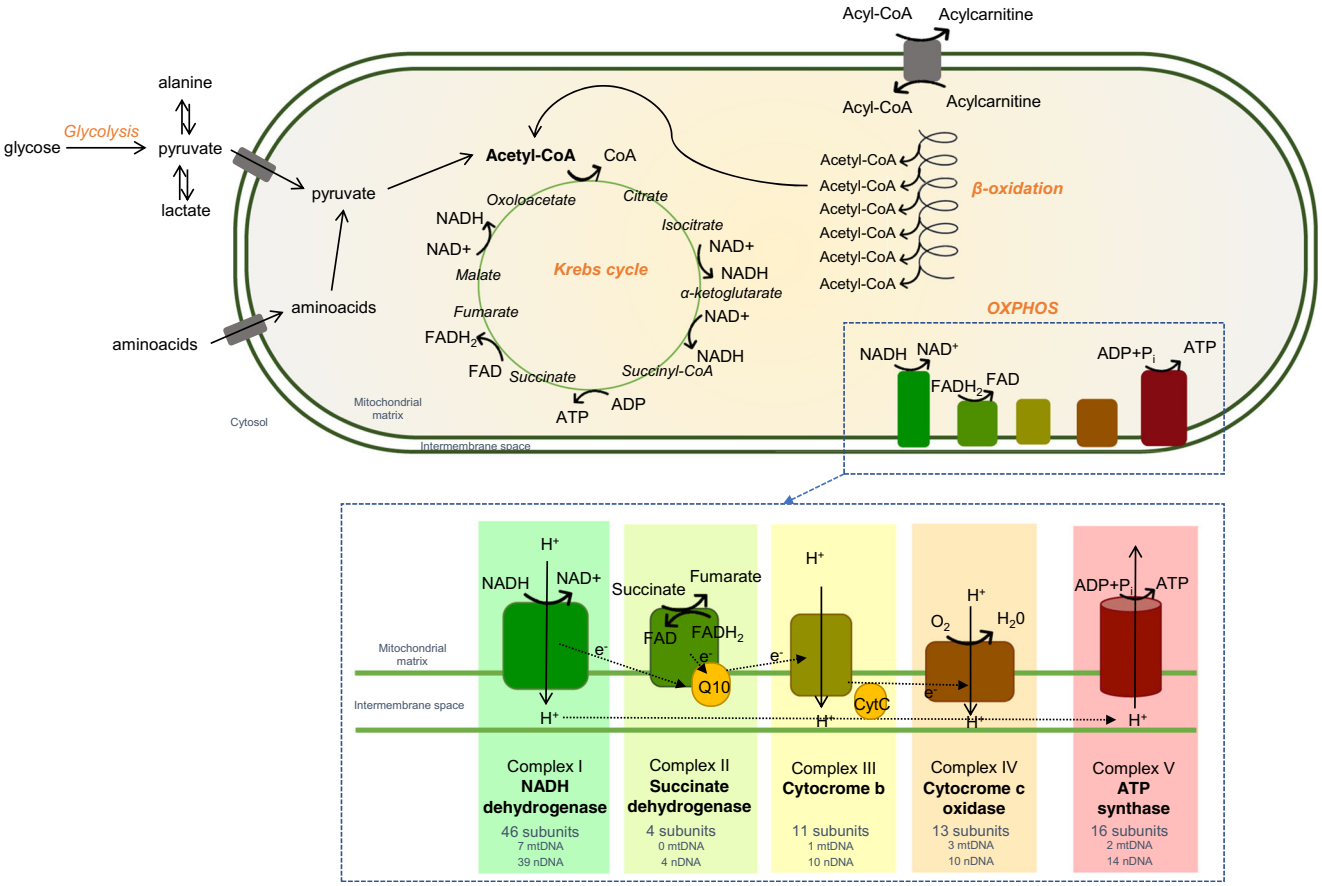

Fig. 1 Mitochondrial metabolism, production of energy through the respiratory chain, and dual regulation of synthesis of respiratory chain complexes (OXPHOS, oxidative phosphorylation; ATP, adenosine triphosphate)

energetic imbalance (in catabolic states such as vomiting, diarrhea, dehydration, fever, prolonged fasting, surgeries); or with the use of drugs metabolized at the mitochondria [14].

Highly suggestive symptoms for mitochondrial disorder in childhood are the following: epilepsy (intractable or dependent on energetic status), encephalopathy, stroke, basal ganglia, thalamus, and/or cerebellum hyperintensities in T2 and FLAIR, neurosensorial deafness, hypertrophic cardiomyopathy, hypotonia, myopathy, ophthalmoplegia, and tubulopathy. A detailed description of possible clinical, biochemical, and histological manifestations of mitochondrial disorders is listed in Table 1, including chronic or acute signs and symptoms [1, 4, 9, 11, 15, 17, 34].

Some patients present with a cluster of clinical features [14, 34] which allowed to describe classical syndromes with a certain genotype–phenotype correlation. Few patients will fit in classical syndromes being oligosymptomatic or having a poorly defined phenotype with overlapping features. The more commonly observed in childhood are Leigh syndrome (subacute necrotizing encephalomyelopathy with typical lesions on brain MRI) and mitochondrial depletion syndromes.

Other syndromes can present in childhood or adolescence and progress to complete clinical phenotypes [3, 14] such as mitochondrial encephalomyophalopathy, lactic acidosis, and stroke-like episodes (MELAS) [40]; myoclonic epilepsy with red ragged fibers (MERRF); neuropathy with ataxia and retinitis pigmentosa (NARP); Leber hereditary optic neuropathy (LHON) with loss of central vision usually starting in adolescence; progressive external ophthalmoplegia (PEO) and Kearns-Sayre syndrome (pigmentary retinopathy and progressive external ophthalmoplegia with onset earlier than 20 years of age combined with cardiac conduction defects and ataxia or elevated cerebrospinal fluid protein) [39]; Pearson syndrome (sideroblastic anemia and exocrine pancreatic dysfunction) and Alpers syndrome (neurological presentation later complicated by liver disease). Coenzyme Q10 deficiency may present heterogeneously with neurological symptoms, renal (steroid-resistant nephrotic syndrome), and rhabdomyolysis [3, 10, 21].

Biochemical features

There is still no single diagnostic biomarker of mitochondrial disorder despite multiple biochemical features may be evidence of mitochondrial dysfunction [34]. A probable

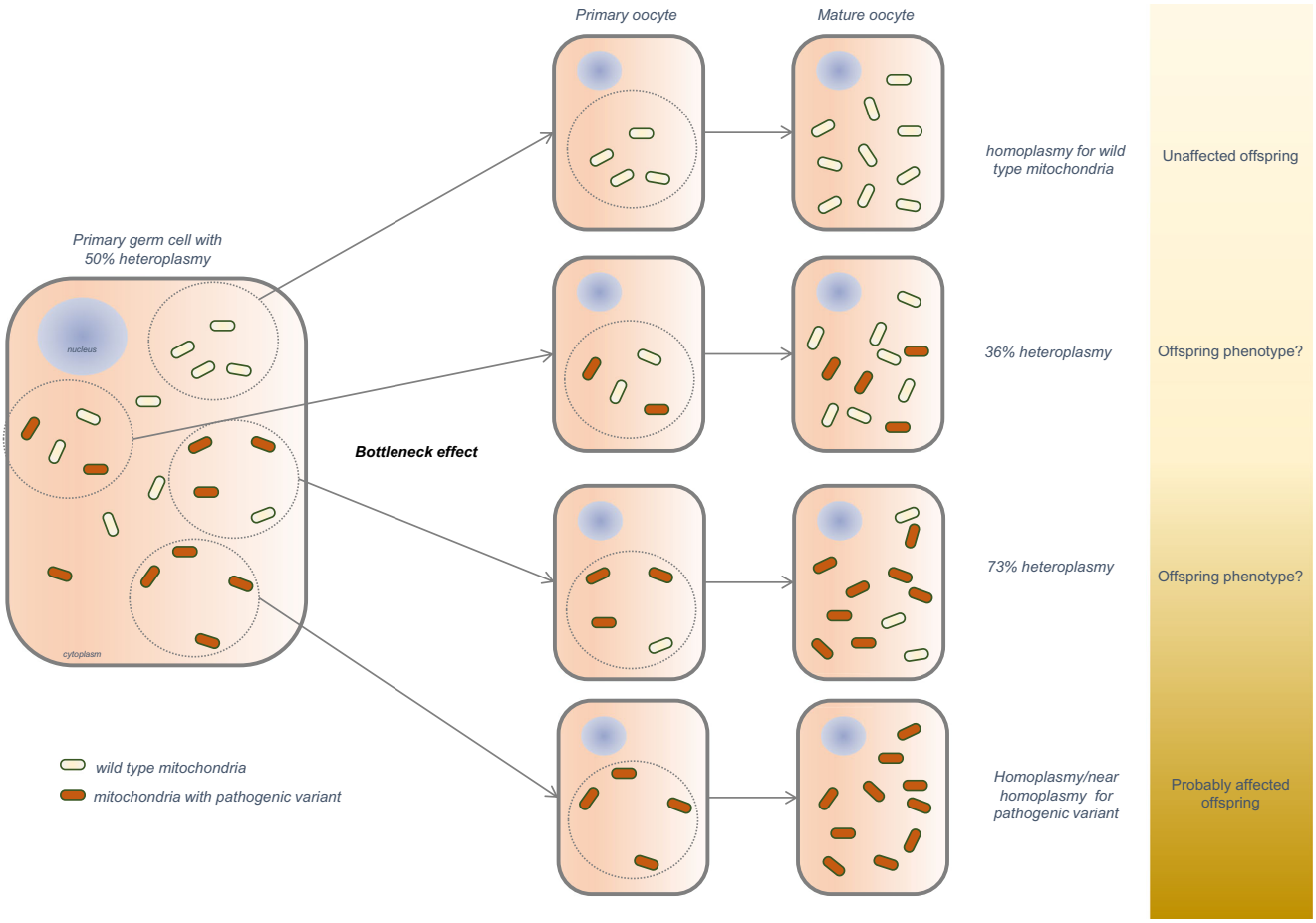

Fig. 2 The threshold effect and the unpredictable effect on offspring

diagnosis is considered with the combination of possible symptoms with biochemical mitochondrial dysfunction.

Metabolic workup can show persistent or recurrent metabolic acidosis and/or hyperlactacidemia (above 2.0 μmol/L) with normo- or hypoglycemia. Lactate may rise in the postprandial period or after a glucose loading test (2 g/kg, maximum 50 g). Pyruvate may be elevated (> 130 μmol/L) but is not specific. Proportion of lactate/pyruvate (L/P ratio) reflects the cytoplasmic redox state and may be considered when lactate level is elevated. For calculating L/P ratio, lactate and pyruvate must be measured at the sample collected without tourniquet. A L/P above 20 (some authors support a cutoff ranging from 17 [3] to 30 [23]) is suggestive of a respiratory chain disorder while a L/P ratio lower than 10 is suggestive of pyruvate dehydrogenase deficiency.

Ketonemia is also a feature of mitochondrial disorders with a β-hydroxybutyrate (βOHBA) higher than acetoacetate (AcAc). βOHBA/AcAc ratio reflects mitochondrial redox state with a ratio above 3 or a paradoxal postprandial increase found to be very suggestive of mitochondrial disease.

Plasma amino acids may show elevation of alanine [34] (above 450 μmol/L or higher than 20% of upper normal limit). Other clues may be present, such as low citrulline in Leigh syndrome [12], mainly in those with the variants m.8993T>G or m.8993T>C. Urinary amino acids may reveal a generalized hyperaminoaciduria.

Urine organic acid cromatography may show elevation of lactic, 3-methylglutaconic, ethylmalonic and/or 2-ethylhydracrilic acids, Krebs cycle metabolites (as fumaric, succinic, and malic acids), and ketonuria (βOHBA and AcAc).

Cerebrospinal fluid (CSF) may be useful in the absence of plasma biomarkers, since elevation of lactate, pyruvate, or alanine can be evident even without plasma elevation.

Novel biomarkers for mitochondrial disorders have promising results. Fibroblast growth factor 21 (FGF-21) and growth and differentiation factor 15 (GDF-15) appear to be useful in the differentiation between primary and secondary mitochondrial dysfunctions [28].

Cerebral magnetic resonance imaging (MRI) can be useful in cases of neurological involvement showing white matter alteration, evidence of stroke (that may or not follow a vascular distribution), basal ganglia, thalamus, and/or cerebellum hyperintensities in T2 and FLAIR. Cerebral magnetic resonance with spectroscopy can demonstrate a characteristic inverted lactate peak. Cerebral MRIs should be thoroughly

Table 1 Possible manifestations of mitochondrial disorders

Central nervous system	Encephalopathy and/or coma*
	Epilepsy*
	Myoclonus
	Ataxia*
	Dystonia
	Migraine*
	Development delay or regression
	Behavioral disorders
	White matter alteration
	Stroke* (following vascular distribution or not)
	Stroke-like*
	Basal ganglia, thalamus, and/or cerebellum hyperintensities in T2 and FLAIR
	Cerebral magnetic resonance with (1.5 or 3 T) spectroscopy showing inverted lactate peak
Audiology	Progressive or congenital neurosensorial deafness (regardless of history of aminoglycoside treatment as precipitating factor)
Ophthalmologic	Ptosis
	Ophthalmoplegia
	Retinitis pigmentosa
	Optic atrophy
	Cataracts
Endocrine	Diabetes mellitus type 1 or 2
	Hypothyroidism
	Hypoparathyroidism
	Adrenal insufficiency
	Short stature
	Growth hormone deficiency
	Primary or secondary hypogonadism
	Premature ovarian failure/premature menopause
Cardiac	Cardiomyopathy (mostly hypertrophic)
	Heart arrhythmia (specially atrioventricular block)*
	Cardiac failure*
Hepatology	Elevated liver enzymes
	Liver failure*
	Reye syndrome*
	Cholestasis
Gastrointestinal	Failure to thrive
	Recurrent vomiting*
	Pancreatic dysfunction
	Gastric dysmotility
	Pseudo-intestinal obstruction*
Cutaneous and subcutaneous	Hypertrichosis
	Lipomas
Kidney	Proximal tubulopathy
	Steroid-resistant nephrotic syndrome
	Tubulointerstitial nephritis
	Chronic kidney disease of unknown etiology
Hematological	Sideroblastic anemia
	Aplasic anemia
	Neutropenia
	Thrombocytopenia
Neuromuscular	Peripheral neuropathy (distal, axonal, or demyelinating; subclinical)
	Hypotonia
	Muscular weakness* (proximal and symmetrical)
	Myalgias*
	Exercise intolerance
	Rhabdomyolysis/myoglobinuria*
	Elevation of creatine kinase (may be asymptomatic)
	Muscle MRI spectroscopy with > Pi/Pcr
Metabolic	Hypoglycemia*
	Metabolic acidosis*
	Persistent or recurrent hyperlactacidemia* (at least 3 different measures with lactate above > 2.0 mmol/L)—worsened by fasting and after a glucose load test

*Possibly features in acute presentation or decompensation

examined by an experienced radiologist or neuroradiologist. Follow-up MRIs may provide further information about relative patterns of disease progression although the risk of an eventual anesthesia in a non-compliant patient should be considered.

Histologic studies and tissue immunohistochemical assays are crucial to the diagnosis [8, 44]. Biopsy sample may also be used to functional studies of the respiratory chain. Virtually all tissues can be used in these studies but tissues highly dependent on mitochondrial metabolism are more useful. Liver, heart, kidney, and skeletal muscle are the best examples, with muscle as the most convenient and easily obtained. Histology must include a modified Gomori trichrome and may show mitochondrial proliferation and enlargement, with accumulation in the subsarcolemical space, abnormal fiber size variation, and red-ragged fibers (fibers with massive accumulation of mitochondria with disrupted structure and loss of cytoplasmic membrane integrity) [4, 44]. Red-ragged fibers higher than 3% in children are considered to be very specific for mitochondrial disorders (cutoff point for adults is 5%, considering age-related muscle modifications).

Cytochrome oxidase (COX) stain may show COX-negative fibers. Succinate dehydrogenase (SDH) stain reflects complex II activity (encoded only by nuclear genes), and strongly positive SDH fibers may be more evident around vessels in MELAS patients [4].

Even though these findings may be highly specific for mitochondrial disorders, it is important to keep in mind that disorders with secondary mitochondrial dysfunction may induce the same histological appearance and some cases of primary mitochondrial disorders may have normal muscle histology (consider muscle-sparing phenotype or age-related alterations).

Electron microscopy can provide more information about mitochondrial structure showing abnormal shape, number, hypertrophy, abnormal architecture of the cristae, or inclusions. None of these features is specific of primary mitochondrial disease.

Functional biochemical testing of complexes of the respiratory chain (global and individually) must be performed in muscle tissue, preferably in fresh tissue (if not possible, the sample can be immediately frozen at − 80 °C until analysis) [34]. Functional assays may show partial or complete deficiencies in a single complex or as a combined deficiency (more than one complex involved). A coenzyme Q10 (ubiquinone) measure in muscle lower than 50% of control reflects Q10 deficiency.

Functional analysis of the OXPHOS chain is a complex and expensive procedure that must be performed in experienced centers with good quality control. Therefore, muscle biopsy must be carefully considered in selected cases and always include histological and functional tests.

Diagnosis

Diagnosis of mitochondrial disorders is challenging, especially in pediatric patients. It is estimated that 30–40% of affected patients, mainly children, do not have a known genetic diagnosis and there is limited knowledge of the biological factors causing their wide clinical heterogeneity [7, 43]. Some authors (such as Wolf [42] and Bernier [2]) attempted to create a diagnostic methodology for mitochondrial disorders, both including clinical, biochemical, and functional criteria [2, 42]. However, patients with confirmed mitochondrial disorders may exhibit normal routine biochemistry, muscle histology, or enzymatic analysis of the respiratory chain [1, 14].

Although sensitive, these criteria are not specific as mitochondrial dysfunction is present in several diseases and is not entirely related to primary mitochondrial respiratory chain disorders [6, 13, 26]. In the event of abnormal or equivocal biochemistry, it can be difficult to differentiate between a primary disorder of the respiratory chain from secondary mitochondrial dysfunction (for instance, in multisystemic disorders like congenital defects of glycosylation, organic acidurias, or neuronal ceroid lipofuscinosis). No single biomarker or functional study accurately distinguishes a primary defect in respiratory chain of secondary mitochondrial dysfunction [5, 7, 9, 11, 36].

A combination of classically described symptoms or biochemical signals can lead an experienced clinician to suspect certain candidate genes, eventually leading to a molecular diagnosis.

Recent development of high-throughput, next-generation sequence (NGS) technology has revolutionized the research and molecular diagnosis of human genetic disease. The ability to generate enormous amount of sequence data in a short time at an affordable cost makes this approach ideal for a wide range of applications from sequencing a group of candidate genes, all coding regions (whole exome sequencing, WES) to the entire human genome (whole genome sequencing, WGS) [7, 27, 43]. NGS may be used in gene panels which may be designed with the more common genes related to a specific disease or clinical presentation and may include a few to hundreds of genes, making it a very attractive cost-affordable option, as opposed to a gene-by-gene Sanger sequencing of individual DNA fragment strategy that is time-consuming and laborious. There are several commercial kits for the diagnosis of mitochondrial disorders. Since multiple genes can be involved, a comprehensive panel should be performed. An example is listed in Table 2, including 209 nuclear genes known to be associated with mitochondrial diseases. It is a customized panel targeting nuclear genes using Suredesign software (Agilent Technologies), initially applied in research.

Table 2 Nuclear genes involved in mitochondrial disorders

OXPHOS subunits	
Complex I	*NDUFS1*; *NDUFS2*; *NDUFS3*; *NDUFS4*; *NDUFS6*; *NDUFS7*; *NDUFS8*; *NDUFV1*; *NDUFV2*; *NDUFA1*; *NDUFA10*; *NDUFA11*; *NDUFA12*; *NDUFA2*; *NDUFA9*; *NDUFB3*; *NDUFB9*
Complex II	*SDHA*; *SDHB*; *SDHC*; *SDHD*
Complex III	*CYC1*; *LYRM7*; *UQCRQ*; *UQCRC2*; *UQCRB*
Complex IV	*COX5A*; *COX5B*; *COX4I2*; *COX6A1*; *COX6B1*; *COX7B*; *NDUFA4*
Complex V	*ATP5A1*; *ATP5E*
OXPHOS assembly factors	
Complex I	*ACAD9*; *FOXRED1*; *NDUFAF1*; *NDUFAF2*; *NDUFAF3*; *NDUFAF4*; *NDUFAF5*; *NDUFAF6*; *NUBPL*
Complex II	*SDHAF1*; *SDHAF2*
Complex III	*BCS1L*; *HCCS*; *TTC19*
Complex IV	*COA3*; *COA5*; *COX10*; *COX15*; *COX14*; *COX20*; *FASTKD2*; *SCO1*; *SCO2*; *SURF1*
Complex V	*ATPAF2*; *TMEM70*
mtDNA maintenance	*C10ORF2*; *DNA2*; *DGUOK*; *FBXL4*; *POLG*; *MGME1*; *MPV17*; *POLG2*; *SLC25A4*; *SUCLA2*; *SUCLG1*; *TYMP*; *TK2*; *RRM2B*
Mitochondrial translation	*AARS2*; *C12ORF65*; *CARS2*; *DARS2*; *EARS2*; *ELAC2*; *FARS2*; *GFM1*; *GTPBP3*; *HARS2*; *IARS2*; *KARS*; *LARS*; *LARS2*; *LRPPRC*; *MARS2*; *MRPL23*; *MRPL3*; *MRPL44*; *MRPL50*; *MRPL57*; *MRPS16*; *MRPS22*; *MTFMT*; *MTO1*; *MTPAP*; *NARS2*; *PARS2*; *PNPT1*; *PUS1*; *RARS2*; *RMND1*; *RNASEH1*; *SARS2*; *TACO1*; *TARS2*; *TRMU*; *TRNT1*; *TSFM*;*TUFM*; *VARS2*; *WARS2*; *YARS2*
Membrane function and import	*AGK*; *C19ORF12*; *CISD2*; *DNAJC19*; *DNM1L*; *GDAP1*; *GFER*; *MFF*; *MFN1*; *MFN2*; *MICU1*; *MPC1*; *OPA1*; *SERAC1*; *SLC25A22*; *SLC25A3*; *SLC25A42*; *SLC4A3*; *SLC52A1*; *SLC52A2*; *SLC52A3*; *TAZ*; *TIMM8A*; *TMEM126A*; *TMEM126B*
Cofactor biosynthesis (CoQ10, lipoic acid, FeS clusters, others)	*ABCB7*; *ADCK3*; *BOLA3*; *COQ2*; *COQ4*; *COQ6*; *COQ9*; *FDX1L*; *GLRX5*; *IBA57*;*ISCU*; *LIAS*; *LIPT1*; *LYRM4*; *NFU1*; *PDSS1*; *PDSS2*; *TPK1*
Others	*ACO2*; *AIFM1*; *ALAS2*; *ALG13*; *APTX*; *AUH*; *C9ORF116*; *CHKB*; *CLN3*; *CLN5*; *CLN6*; *CLN8*; *CLPB*; *CTSD*; *CWF19L1*; *DLAT*; *DLD*; *DNAJC5*; *DOLK*; *ECHS1*; *ETFA*; *ETFB*; *ETFDH*; *ETHE1*; *FLAD1*; *HACE1*; *HIBCH*; *KCTD7*; *KIF5A*; *MEGF10*; *MFSD8*; *MPI*; *NOTCH3*; *OPA3*; *PC*; *PCK2*; *PDHA1*; *PDHB*; *PDHX*; *PDP1*; *PLD1*; *PMM2*; *PPT1*; *SLC19A3*; *SPATA5*; *TPP1*; *WDR45*; *WFS1*

NGS has been successfully applied to the analysis of whole mtDNA which greatly facilitates the characterization of patients with probable mitochondrial disease in a timely and cost-effective approach [11, 25].

Considering that a *definite diagnosis* is only possible with genetic confirmation (in mitochondrial or nuclear genes) [5, 7, 24, 25, 45] or in the presence of a primary mitochondrial DNA depletion syndrome (> 70% depletion), establishing the likelihood of mitochondrial disease is helpful in deciding which patients to undergo further studies [34]. If genetic studies are not conclusive, one should reconsider clinical presentation and biochemical features, and eventually consider other diagnosis.

A diagnosis is *possible* if presentation is suggestive (particularly if neurological and/or muscular) and may be accompanied by biochemical mitochondrial dysfunction (as previously mentioned).

A *probable diagnosis* is considered with the combination of clinical presentation, biochemical workup and/or imagiological findings and/or immunohistochemical findings or functional studies show decreased function of one or more complexes of the respiratory chain.

A diagnostic approach is proposed in Fig. 3.

If after genetic testing (including mitochondrial and nuclear genes through NGS) a definite diagnosis is not possible, a mitochondrial disorder must not be excluded but other

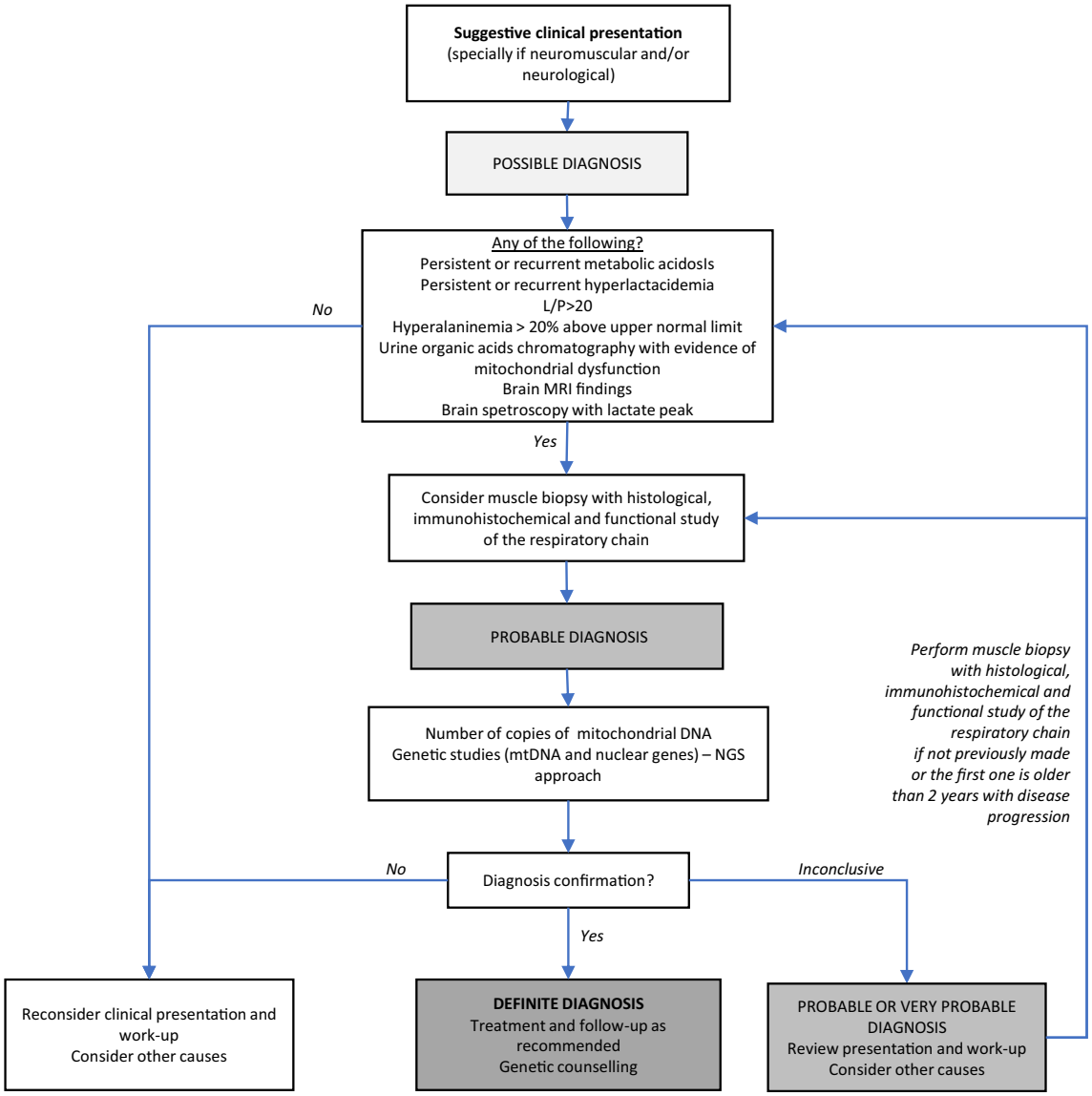

Fig. 3 Algorithm for the diagnosis of a mitochondrial disorder

diagnoses must be considered after reviewing clinical and workup findings.

Treatment

Specific treatment of mitochondrial disorders (disease-modifying treatment) is based on different strategies such as increasing mitochondrial biogenesis, boost of respiratory chain function, energy buffering, scavenging toxic compounds, vascular effects, or alteration of mitochondrial dynamics [18, 41].

Coenzyme Q10 is a component of the respiratory chain and can be used for augmenting its function. It can be effective in cases of Q10 deficiency administered 5 mg/kg/day (60 a 250 mg/day) orally, with an expected neuromuscular improvement but without a clear neurological benefit (poor crossing of the blood–brain barrier). For those without confirmed Q10 deficiency, it may prescribed with limited clinical evidence. Idebenone is a coenzyme Q10 analog and evidence of its value is limited [18].

L-Arginine 150–300 mg/kg/day orally is recommended in the cases of MELAS or previous history of stroke-like episodes. It acts as a vasoactive through the nitric oxide pathway [18].

Riboflavin (B2) is the flavin adenine dinucleotide precursor and doses of 10–100 mg/day have some evidence in the treatment of complex I deficiency.

L-Carnitine is important for fatty acid transport to the mitochondria and may be used to enhance fatty acid oxidation or

Table 3 Clinical, biochemical, and imaging follow-up of mitochondrial disorders in childhood

Interval	Workup
Annual	Blood count
	Plasma: glycemia, liver enzymes, urea, creatinine, creatine-kinase, sodium, potassium, chloride, total calcium, magnesium, phosphorus, vitamin D
	Acid–base balance and lactate
	Urine: microalbuminuria, alpha-1-microglobulin, beta-2-microglobulin, creatinine, proteinuria, amino acids, phosphorus, sodium, calcium, and glycose
	HbA1c, ACTH, cortisol, IGF1, PTH, FT4, and TSH
	ECG and echocardiogram
	Clinical neurological evaluation
	EEG if epilepsy
Every 2 years	Scoliosis, contractures, luxation, limb deformities
	Bone densitometry (earlier if bone fractures)
	Chromatography of urine organic acids and plasma amino acids
	Visual acuity, visual fields, eye movements, ptosis, and fundoscopy
	Hearing evaluation
Every 5 years	Brain MRI (with spectroscopy if not previously made)—earlier if acute neurological symptoms or development regression
	Formal developmental or cognitive evaluation
	Neuropsychological evaluation

in secondary carnitine deficiency; usual dose is 50 mg/kg/day (maximum 3 g/day) [18].

Other supplements, such as thiamine and creatine, may be used per os as chronic treatment with controversial or limited clinical benefit. Thiamine (B1) is a cofactor of the pyruvate dehydrogenase complex and may have an effect on decreasing lactate while increasing acetyl-CoA; it may be used at maximum 900 mg/day. Creatine phosphate (maximum 10 g/day) may be administered in the presence of myopathy or cardiomyopathy and aims to improve ATP storage via creatine phosphatase system.

Other therapeutics include cysteine to increase the availability of antioxidant glutathione peroxidase, nuclear respiratory factors (NRF1 and 2), peroxisomal proliferator activator receptors (PPAR α, β, and γ), and their agonists to positively influence the transcription of OXPHOS genes. EPI-743 (the parabenzoquinone analog) is a novel promising therapeutic approach with some evidence of reversion of disease [16, 18]. Undergoing clinical trials also include deoxycytidine monophosphate (dCMP) and deoxythymidine monophosphate (dTMP) in thymidine kinase 2 (PK2) deficiency, a disorder leading to mtDNA instability and depletion [41].

Enzyme replacement, shift of heteroplasmy rate, and stem cell and gene therapy are still experimental.

Despite several clinical trials, there is no specific treatment for mitochondrial disorders and their management is mainly supportive [16, 18, 33]. Symptomatic treatment of mitochondrial disorders includes specific-organ management such as a pacemaker for rhythm disease, dialysis for renal insufficiency, ventilatory support, or invasive procedures such as surgical ptosis correction. Organ transplantation may be considered depending on global patient status and comorbidities.

General recommendations

Avoidance of the following drugs are strongly recommended: (1) those metabolized at the mitochondria (sodium valproate and barbiturates as phenobarbital); (2) mitochondrial protein synthesis inhibitors (gentamycin, tetracyclines, and chloramphenicol); and (3) inhibitors of the respiratory chain (statins, metformin, phenytoin, propofol) [19, 33]. A high glucose infusion rate is not recommended (above 5% dextrose), except for immediate hypoglycemia treatment (see below).

Surgeries and anesthesia pose a particular problem within these patients, with respiratory failure, neurological lesions, cardiac depression, conduction defects, and hypoglycemia as the main problems [29]. Volatile anesthetics and propofol are more likely to depress mitochondrial function. Patients should own a written plan in the event of any surgery or need of anesthesia and start intravenous fluids with normal saline and 5% dextrose during fasting and avoid temperature variations [29].

Physical exercise is recommended, adjusted to the patient's capacity and tolerance [3, 32].

Management in the acute setting

In the presence of acute presentation or decompensation of mitochondrial disorders, acid–base balance, lactate, ammonia,

liver enzymes, creatine-kinase, myoglobin, electrolytes, and ketonemia should be assessed [3]. Urinalysis may also be informative. If diagnosis is not definite, plasma amino acids, acylcarnitine profile, and urine organic acids chromatography should also be considered.

Intravenous fluids should be started while avoiding glycose overload. Vomiting should be treated and ondansetron (0.1 mg/kg, maximum 8 mg) is a safe option. If shock or severe dehydration, isotonic crystalloid or colloid bolus 10–20 mL/kg must be the first approach.

Hypoglycemia (lower than 45 mg/dL) can be treated with intravenous glucose 2 mL/kg and severe hyperlactacidemia (lactate > 7 mmol/L or bicarbonate < 10 mmol/L) can be treated with intravenous sodium bicarbonate at a low infusion rate (1–2 mEq/kg in 12 h) [3, 23].

In case of seizures, valproic acid and phenobarbital are contraindicated; benzodiazepines and levetiracetam are safe options [20]. In stroke or stroke-like episode, start intravenous L-arginine (150–500 mg/kg/day) straight away and reassess after 3 days [32]. Brain MRI should be performed in every de novo neurological deficits or seizures or change in the pattern of a previously known epilepsy.

If antibiotics are prescribed, aminoglycosides (especially in neonates), tetracyclines, and chloramphenicol should be avoided [32].

Rhabdomyolysis must be actively treated with 120–150% of daily needs avoiding glucose overload (preference for normal saline with 5% dextrose) while maintaining normoglycemia.

In the event of an eminent death or immediately postmortem, muscle and skin biopsies must be performed as well as blood collection for posterior genetic studies.

Follow-up

Every patient with a very likely or definite diagnosis should be handed a medical report and acute setting and surgery guidelines [32]. Genetic counseling should be offered to the individual and their family, particularly in face of a new pregnancy (or desire to have more children) or reaching adulthood [11]. Pneumococcus and seasonal flu vaccination should be proposed.

Phenotypical variants and poor correlation of genotype–phenotype justify similar follow-up for all mitochondrial disorders. At each consultation, assessment must include auxological data (weight, height, BMI, and head circumference), blood pressure, heart frequency, complete physical exam, and development milestones. Regular evaluation must include annual workup and cardiac and neurological evaluations [33].

Ophthalmologic and hearing evaluations should be as regular as every 2 years. Some comorbidities will require specific care, namely, nutritional support (with supplements such as medium chain triglycerides), enteric nutrition through nasogastric tube or gastrostomy if feeding difficulties, respiratory support if respiratory insufficiency or sleep apnea, and immunological evaluation if recurrent or severe infections.

A recommendation on complete evaluation is presented in Table 3 and implies the collaboration in multidisciplinary teams (nephrology, endocrine, cardiology, neurology, orthopedics, ophthalmology, otorhinolaryngology, etc.). Most recommendations can be applied to adult patients.

Conclusions

Mitochondrial disorders are highly variable in clinical presentation, biochemistry, and molecular etiology. Biochemical screening tests may be inconclusive or misleading, since patients with confirmed mitochondrial disorders may exhibit normal routine biochemistry, muscle histology, or enzymatic analysis of the respiratory chain, especially within pediatric patients.

Awareness between physicians is of major importance regarding clinical heterogenicity and biochemical features of mitochondrial disorders. Prompt diagnosis and intervention are crucial for optimizing care, considering the possibility of a rapidly progressive and devastating disease course in infancy or early childhood, where therapy options are still rather ineffective.

Molecular diagnosis of mitochondrial disorders is a great challenge with gene panels proving to be a cost-effective approach. Clinical and biochemical features of patients without a confirmed diagnosis must be reviewed and other diagnoses must be considered. A wider genetic approach may be applied (WES or WGS).

Follow-up should provide comprehensive information on patient's status, since intervention on these diseases is mostly supportive and prognosis is variable and sometimes unpredictable.

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Esmeralda Martins: critical manuscript review
Laura Vilarinho: critical manuscript review

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Compliance with ethical standards

This article does not contain any studies with human participants or animals performed by any of the authors.

Conflict of interests The authors declare that they have no conflict of interest.

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