REVIEW

Diagnosis of 'possible' mitochondrial disease: an existential crisis

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ABSTRACT

Primary genetic mitochondrial diseases are often difficult to diagnose, and the term 'possible' mitochondrial disease is used frequently by clinicians when such a diagnosis is suspected. There are now many known phenocopies of mitochondrial disease. Advances in genomic testing have shown that some patients with a clinical phenotype and biochemical abnormalities suggesting mitochondrial disease may have other genetic disorders. In instances when a genetic diagnosis cannot be confirmed, a diagnosis of 'possible' mitochondrial disease may result in harm to patients and their families, creating anxiety, delaying appropriate diagnosis and leading to inappropriate management or care. A categorisation of *'diagnosis uncertain'*, together with a specific description of the metabolic or genetic abnormalities identified, is preferred when a mitochondrial disease cannot be genetically confirmed.

INTRODUCTION

Primary mitochondrial disorders (PMD) are genetic metabolic disorders that directly impair normal mitochondrial structure or function including electron-transport chain (ETC) activity.[1] They are due to mutations in either maternally inherited mitochondrial DNA (mtDNA) or one of hundreds of nuclear DNA (nDNA) genes that encode components involved in mitochondrial structure and function. PMDs can present at any age and be multisystemic or selectively involve only a single organ. They can present as a well-defined canonical syndrome or a constellation of phenotypes, although typically at least one 'red-flag' symptom is usually present at disease onset.[2]

With advances in next-generation sequencing (NGS) and the discovery of a multitude of new disease genes, the ability to diagnose PMDs has improved enormously compared with just a few years ago. The diagnosis still remains challenging due to heterogeneous manifestations combined with limitations of currently available biochemical and genetic testing methods. Despite recent advances, many individuals with suspected mitochondrial disease may remain without a confirmed genetic diagnosis, presenting a challenge to the clinician in establishing a mitochondrial disease diagnosis and in knowing how to categorise, counsel and manage the patient with a suspected PMD where a genetic diagnosis is not yet possible.[3–7]

These limitations contribute to continued variation in diagnostic categorisation of patients depending on the opinion of the treating provider.[8] Diagnostic terms such as 'unlikely', 'possible' or 'probable' mitochondrial disease, originally proposed as part of research diagnostic criteria[9–11] were developed prior to genetic advances and may end up being inaccurate and misleading to patients and care providers, impacting or limiting proper counselling and the pursuit of further diagnostic testing. The complex and variable clinical presentation of mitochondrial diseases means that many unexplained disorders could conceivably have a mitochondrial aetiology, so if a concrete alternative diagnosis cannot be made using conventional investigations, there is a tendency to use the label 'possible' mitochondrial as a working diagnosis until an alternative emerges. Patients and families may inadvertently be burdened by the fear of the progressive nature of PMDs, the potential complications and early demise, as PMDs have no known cure. Thus, a diagnosis of 'possible' mitochondrial disease may do more harm than good and consequently a new categorisation for these patients is necessary.

Mitochondrial disease can no longer be diagnosed on the basis of phenotypic features alone

A high index of suspicion for the possibility of a mitochondrial disease is appropriate when there is multisystem involvement or the presence of so-called 'red-flags' such as stroke-like episodes in a non-vascular distribution (seen in Mitochondrial Encephalomyopathy, Lactic Acidosis and Stroke (MELAS) syndrome), bilateral symmetrical T2-weighted hyperintense MRI lesions in the basal ganglia and/or brainstem (Leigh syndrome) or chronic progressive external ophthalmoplegia (CPEO) and myopathy. Some of these 'red-flag'

Table 1 Differential diagnosis of selected phenotypes commonly associated with mitochondrial disease

Phenotype	Mitochondrial cause	Limited differential diagnosis
Dystonia	Leigh syndrome, deafness-dystonia syndrome, other mitochondrial encephalomyopathies	Biotinidase deficiency, thiamine transporter deficiency 2, *ADAR* mutations (Aicardi- Goutières syndrome 6), organic acidaemias (especially glutaric aciduria type I), NBIA, acute (viral) necrotising encephalopathy, mutations in *NUP62*, *RANBP2* and *PDE8B*, primary genetic dystonias
Epileptic encephalopathy	Alpers-Huttenlocher syndrome, many other mitochondrial disorders	Many genetic epileptic encephalopathies, including Dravet syndrome and *KCNQ2* mutations, Pyridoxine dependent epilepsies (Antiquitin deficiency, PNPO deficiency), viral encephalitis
Progressive myoclonic epilepsy	MERRF	Ramsay Hunt syndrome, Unverricht-Lundborg disease, Lafora body disease, sialidosis, *PRICKLE1* mutations
Leukoencephalpathy	Complex I deficiency, Complex II deficiency, SURF1 deficiency (rarely), disorders of mitochondrial translation and Fe-S cluster assembly	Vanishing white matter disease, lysosomal storage disorders, Canavan disease, Alexander disease, Pelizaeus-Merzbacher(-like), hypo/dysmyelination
Ataxia	*ADCK3* mutations, ataxia-neuropathy syndromes, for example, SCAE, MIRAS, MERRF, NARP, disorders of coenzyme Q_{10} biosynthesis	Spinocerebellar ataxias, CAPOS syndrome
Demyelination	MNGIE	ADEM, multiple sclerosis
Peripheral neuropathy	Mutations in *POLG, MPV17, KARS* and *SURF1*; part of multisystem disease in many mitochondrial disorders, for example, MNGIE	Other non-mitochondrial genetic causes of Charcot-Marie-Tooth syndromes, riboflavin transporter deficiency, toxic neuropathies, critical illness
Ptosis and ophthalmoplegia	PEO, KSS, MNGIE, MELAS	Some congenital myopathies, pseudo upgaze impairment in *OPMD*, horizontal gaze palsy and scoliosis (*ROBO3* mutation)
Optic neuropathy	LHON, ADOA, Leigh syndrome	Toxic optic neuropathy (eg, methanol, cyanide, tobacco)
Hypertrophic cardiomyopathy with lactic acidosis	Complex I deficiency, *TMEM70* mutations, Sengers syndrome (AGK deficiency), disorders of mitochondrial translation	Viral infection
Dilated cardiomyopathy with lactic acidosis	Barth syndrome, disorders of mitochondrial phospholipid remodelling, other mitochondrial cardiomyopathies	Viral infection
Exocrine pancreatic insufficiency	Pearson syndrome	Cystic fibrosis
Diabetes and deafness	MIDD, other mtDNA mutations	Type II diabetes mellitus with incidental non-syndromic deafness
Sideroblastic anaemia	Pearson syndrome, MLASA, TRNT1 deficiency, *PUS1* or *YARS2* mutations	Blackfan-Diamond syndrome, Schwachman-Diamond syndrome, X linked sideroblastic anaemia
B cell immune deficiency	TRNT1 deficiency	Primary immunodeficiency disorder
Liver failure	Mitochondrial DNA (mtDNA) depletion syndromes,	NBAS, LARS and IARS deficiencies, viral infection, lysosomal storage disorders, other syndromic genetic conditions
Renal tubulopathy/failure	Pearson and Kearns-Sayre syndromes, *RMND1*-related disease	Gitelman syndrome, Fanconi Bickel (*SLC2A2* mutations) syndrome, other syndromic genetic conditions
Myopathy	Part of multisystem disease in many mitochondrial disorders, especially mtDNA depletion syndromes	Congenital muscular dystrophies, myositis, many other disorders
Rhabdomyolysis	Mitochondrial myopathies (eg, *MTCO1*, *MTCO2*, *MTCO3* and *MTCYB* mutations)	*LPIN1* mutations, fatty acid oxidation defects (VLCAD, LCHAD), TANGO deficiency, glycolytic defects, toxic, postexercise
Low copper	Cytochrome oxidase deficiency	Menkes, *SLC33A1* mutations
Complex multisystem disorders	Many mitochondrial disorders, particularly in childhood	Congenital disorders of glycosylation, peroxisomal disorders, lysosomal storage disorders, other syndromic genetic conditions

ADEM, acute disseminated encephalomyelitis; ADOA, autosomal dominant optic atrophy; CAPOS, cerebellar ataxia, areflexia, pes cavus, optic atrophy and sensorineural hearing loss; Fe-S, iron-sulphur; KSS, Kearns-Sayre syndrome; LHON, Leber hereditary optic neuropathy; MERRF, myoclonic epilepsy with ragged red fibres; MIDD, maternally inherited diabetes and deafness; MIRAS, mitochondrial recessive ataxia syndrome; MLASA, myopathy, lactic acidosis, sideroblastic anaemia; MNGIE, mitochondrial neurogastrointestinal encephalomyopathy; NBIA, neurodegeneration with brain iron accumulation; PEO, progressive external ophthalmoplegia; SCAE, spinocerebellar ataxia with epilepsy.

symptoms were the subject of a previous review.[2] However, the growing list of genetically confirmed mitochondrial diseases has also led to an expanding list of variable phenotypes that should be suspected in the differential diagnosis of PMD, some of which are outlined in table 1.[12–14]

In contrast, genetic testing has revealed that non-mitochondrial disorders may present with symptoms suggestive of mitochondrial disease. Without confirmatory genetic evidence, an erroneous diagnosis of a PMD may be made. For example, myopathy with ophthalmoplegia may also be seen in some cases of congenital myopathies with mutations in *MYH2*,[15] *MTM1* in male patients and female carriers,[16] *DNM2*[17] and recessive *RYR1* mutations[18–22] as well as in late-onset nemaline myopathy[23] and in congenital myasthenia caused by mutations in *CHAT* encoding the choline acetyltransferase.[24] Similarly, patients with branched-chain organic acidurias can manifest with non-haemodynamic strokes,[25] as can patients with congenital disorders of glycosylation.[26] Bilateral striatal necrosis (with MRI lesions resembling those observed in Leigh syndrome) has been reported with genetic mutations in the nuclear pore proteins *NUP62*[27] and *RANBP2*[28] or in the cyclic nucleotide phosphodiesterase *PDE8B*.[29] Many of these disorders may also be associated with secondary mitochondrial dysfunction on biochemical testing as discussed later and illustrated in table 2. Clinicians clearly need to exercise caution when considering mitochondrial disease, in order not to narrow the differential too quickly simply based on aspects of the clinical phenotype and astutely ensure that even 'red flag' features of a PMD are placed in the correct context of the patient's comorbid symptoms, family history and course of disease.

Table 2 Mitochondrial dysfunction identified in select other genetic disorders

Disorder	Mitochondrial defect	Reference by PubMed ID number
AOA1 (*APTX* mutations)	Coenzyme Q_{10}	15699391
Desminopathy	CS, mtDNA depletion (35%)	26097489
Dravet syndrome (*SCN1A* mutations)	Variable OXPHOS deficiencies	20392657; 21906962
EXOSC3 and *EXOSC8* related diseases	Low Complex I and pyruvate dehydrogenase activities, low mtDNA copy number, increased expression of mitochondrial genes	28687512; 24989451
GLUT1 deficiency	Complex I	22156785
GM3 synthase deficiency	Respiratory chain dysfunction in fibroblasts and liver	22990144
LCHADD	Complex III, COX	16417669
Limb immobilisation	COX and CS	19654872
Lysosomal diseases: GM1-gangliosidosis, mucopolysaccharidosis IIIC, multiple sulfatase deficiency, Krabbe disease, Gaucher disease, Niemann Pick disease type C	Multiple OXPHOS deficiencies attributed to excessive production of mitochondrial reactive oxygen species and dysregulated calcium homeostasis with mitochondria-induced apoptosis and neurodegeneration	28132808
MADD (*ETFDH, ETFA* or *ETFB* mutations)	Complex I and II deficiencies; Riboflavin and Coenzyme Q_{10} responsive	17412732
Molybdenum cofactor deficiency	COX	16417669
MTHFR mutations	Complex I deficiency	21131308
Multiple carboxylase deficiency	Complex III	16417669
NBIA (PKAN)	Complex III	16417669
Neonatal haemochromatosis	Complex III (liver)	16417669
Neuroferritinopathy (*FTL1)*	Complex I or multiple Complex deficiency	17142829
NPHS3 (PLCE1 deficiency)	COX	21365190
Neuronal Ceroid Lipofuscinosis (*CLN2* and *CLN3*-related)	Partial deficiency in fatty acid oxidation enzymes and the storage of subunit c of mitochondrial ATP synthase in fibroblasts	8971698
ORAI1 related disease	Impaired lipid metabolism and fatty acid oxidation in skeletal muscle, heart and liver due to abnormal store-operated Ca^{2+} entry	28132808
Organic acidemias	Coenzyme Q_{10}, multiple OXPHOS deficiencies and free radical induced oxidative damage	21329767; 28753922; 28753922
Ras/MAPK pathway mutations	Variable OXPHOS deficiencies	26097489
Rett syndrome (*MECP2* mutations)	Variable OXPHOS deficiencies	26741492
SCAR10	Coenzyme Q_{10}	25182700
Spinal muscular atrophy	Complexes I-IV, mtDNA depletion	12557011; 25844556
STXBP1 mutation (de novo)	Complex I	25418441
Zellweger syndrome	Complexes II+III, COX	25287621; 28753922

Biochemical diagnostic tests remain imperfect

Consensus criteria to help standardise the evaluation of patients with potential PMD, outlining a streamlined approach and reviewing the strengths and limitations of many of the current testing modalities were suggested in 2015 by the Mitochondrial Medicine Society (MMS), an international group of clinicians specialising in mitochondrial disease.[30] This exercise aimed to decrease the variability that exists in approaches used by clinicians to diagnose PMDs.[8]

When a mitochondrial disorder is suspected, biochemical screening in blood, urine and cerebrospinal fluid remain the initial tests of choice quickly followed by NGS of mtDNA and nDNA from white blood cells, with additional genetic studies in muscle when needed, particularly in adult-onset cases. Whole exome sequencing (WES) is useful, and along with whole genome sequencing is quickly becoming the first or second line genetic test in patients with suspected mitochondrial disease.[1 5]

Histopathological, biochemical and genetic analysis of tissue including muscle remain important tools to further delineate the phenotype and ascertain the relevance of any genetic variants identified in blood, but should no longer be considered first or second line tests when suspicion of a PMD is high and appropriate genetic testing is available.[30] Select disorders, such as CPEO, may warrant the need for further diagnostic testing in muscle. Additional considerations regarding these tests have been reviewed previously[30] and are summarised below and in box 1 and discussed in detail in the supplementary material (online supplementary testing).

Box 1 Limitations of testing

Current Limitations of biochemical testing
- Imperfect sensitivity and specificity.
- Secondary mitochondrial dysfunction leading to abnormal results.
- Interlab variability of methods and reference ranges.
- Challenges with tissue processing.

Current limitations of genetic testing
- Incomplete understanding of the role of the entire genome in mitochondrial function.
- Novel genes still being identified.
- Interpretation of nuclear and mtDNA variants of uncertain significance.
- Lack of understanding of tissue-specificity of mtDNA mutations.
- Unclear relevance of low heteroplasmy levels of pathogenic mtDNA mutations mtDNA deletions and depletion may be observed in non-mitochondrial disease.

Challenges with biochemical testing

Biochemical studies in blood and urine such as lactate, amino acids, organic acids and including the recently identified biomarkers growth differentiation factor 15 and fibroblast growth factor 21 (FGF21), along with functional assays in various tissues such as ETC enzyme analysis, all have less than optimal sensitivity and specificity, especially when interpreted in isolation from the clinical context.[30–35]

Abnormalities on ETC enzyme analysis may occur for a multitude of reasons outside of PMD including secondary mitochondrial dysfunction from other causes such as other genetic diseases, limb immobilisation[36] and in liver failure from non-mitochondrial causes.[37 38] The list of other genetic disorders where some degree of secondary mitochondrial dysfunction in various tissues is seen seems ever-growing (table 2) and includes spinal muscular atrophy ,[39] X linked adrenoleukodystrophy,[40] Phelan-McDermid syndrome, Down syndrome, Zellweger syndrome, the 'rasopathies' (disorders caused by mutations in the Ras-MAPK pathway) and a variety of other conditions.[41–46] Causes of this secondary dysfunction have been discerned for very few of these disorders and the extent of mitochondrial dysfunction is variable and may not meet the diagnostic criteria threshold for 'definite' mitochondrial disease.[47] Therefore, evidence of biochemical dysfunction on functional testing alone, especially when mild or moderate, should not lead to a conclusive diagnosis of PMD.[42 45 47–49] When used with rigour, mitochondrial disease criteria may help the clinician selectively better stratify truly high-risk patients.[50] However, mitochondrial disease diagnostic criteria were all developed at a time prior to the advent of NGS, when only limited genetic testing was available, and strongly emphasised the importance of abnormal biochemical findings in tissue.[10 50 51] This inevitably led to many patients being diagnosed with 'possible' mitochondrial disease.

Challenges with genetic testing

The advent of rapid, relatively low cost, NGS technologies has allowed for a genetic diagnosis to be made in many more patients with PMD. A growing number of nuclear genes has been associated with mitochondrial function (1500 to-date)[52 53] although only around 350 or so have firmly been linked to causing human mitochondrial disease.[1 54 55] With more routine use of WES, new nuclear genes impacting mitochondrial function continue to be discovered. In some patients with a prior suspected but unconfirmed mitochondrial disease diagnosis, WES has also identified non-mitochondrial diseases.[56] In other cases, variant and milder phenotypes of PMD have been identified.[57] The ability to detect clearly pathogenic mutations in suspected PMD via genetic studies remains imperfect, with a reported diagnostic yield ranging from 25% to 75%.[3–7] The lack of understanding of the entire genome beyond the exome and increasing findings of variants of unknown significance (VUS) add to the diagnostic complexity.

MtDNA can now be accurately sequenced in its entirety for a relatively low cost and it is possible to detect levels of heteroplasmy of less than 5% in tissue, including blood. Genetic testing of mtDNA continues to be impacted by aspects of tissue specificity of mutations in mtDNA and varying degrees of heteroplasmy in easily attainable tissue. With newer testing methods able to detect low levels of heteroplasmy, common pathogenic mtDNA mutations (such as m.3243A>G) at low mutation load may mistakenly be attributed to cause a patient's phenotype.[58] These issues and others are discussed in further detail in the supplementary material (online supplementary testing) but lead to the clear concern that simply testing the mitochondrial genome in leucocytes is not always adequate and that mtDNA testing including quantification and deletion analysis in other tissues (skeletal muscle, liver, buccal, urine sediment) may be needed. Furthermore, even though many defects in mtDNA maintenance may be diagnosed by WES, there remains a significant number in which the causative genes remain unknown. Muscle or liver biopsy (depending on the phenotype), along with reliable assessment of mtDNA copy number compared with age specific control ranges and/or long PCR for multiple deletions, are needed to diagnose these patients.

Despite the current limitations of genetic testing, the need for genetic confirmation of a PMD diagnosis is becoming a necessity. The number of phenocopies identified together with the less than perfect specificity of biochemical studies raises the concern of a mistaken diagnosis and the potential of missing a separate treatable disease. Accurate genetic diagnosis of a PMD allows care providers and affected families to better understand the condition, for the provision of appropriate genetic counselling, and for the development of targeted therapies. For some PMDs where the natural history is better known, clinicians and families can more accurately predict the disease course and provide appropriate clinical management and preventative care.[59] The need for a genetic diagnosis in PMD is now essential for eligibility in clinical trials. Preimplantation genetic diagnosis for nuclear and mtDNA disorders and mitochondrial donation techniques also requires a prior confirmed genetic diagnosis.

Ending a 'possible' diagnosis of mitochondrial disease

Previously established diagnostic criteria,[9–11] developed prior to advances in genetic testing, relied heavily on biochemical functional tests. They were intended to serve as research categorisation tools in the era of only a basic understanding of mtDNA as it relates to mitochondrial illness and prior to our knowledge of any but a handful of the hundreds of nuclear genes that are now known to cause mitochondrial disease. In addition, they were often not adhered to in the strictest fashion by clinicians. These diagnostic categorisations subsequently infiltrated the clinic and many more patients began to be labelled as having 'possible' mitochondrial disease. Others have received the diagnosis of 'mitochondrial myopathy' because of abnormalities seen in muscle histology or microscopy alone, even though this finding may exist due to other genetic, metabolic or neurodegenerative diseases.

While genetic testing has improved, it is not currently possible to confirm the diagnosis at a genomic level in every case. Some patients may have a coincidentally identified pathogenic mtDNA mutation with low levels of heteroplasmy or a VUS in a nuclear gene bioinformatically predicted to impact mitochondrial function that may make a clinician consider a 'possible' mitochondrial disease diagnosis.

Given that patients with symptoms suggestive of mitochondrial disease may or may not ultimately have a PMD, it is increasingly important to establish better diagnostic criteria or at least a unified approach to categorising these patients, to avoid significant variability in diagnostic labelling, genetic counselling and management. With the growing number of clinical, biochemical and genetic phenocopies of PMD being identified, it has become prudent that a definitive diagnosis of mitochondrial disease should only be provided when a confirmed pathogenic genetic defect has been identified. Utmost caution must be used when providing a diagnosis based on biochemical abnormalities in tissue alone and the strictest application of biochemical

diagnostic criteria is needed. Patients with strong biochemical and clinical evidence for a PMD should be periodically re-evaluated as diagnostic testing advances.

There is a clear concern that a diagnosis of 'possible' mitochondrial disease may result in harm. First and foremost, some patients who receive a diagnosis of a 'possible' or 'suspected' mitochondrial disease may not recognise the impermanence of such a diagnosis and remain carrying this label for many years without having their symptoms periodically reassessed and a more specific diagnosis investigated as knowledge and diagnostic tools improve. Over time, the categorisation of 'possible' is often dropped by some providers and non-mitochondrial specialists providing routine care for the patient. Some families may cling to the diagnosis even after having had a different genetic disease confirmed, as it is the diagnosis they have become most familiar with over time. Testing for another disorder may be delayed from the clinician's side if they are not aware of this diagnostic uncertainty. Other treatable disorders may not be diagnosed or diagnosis may be delayed.

A diagnosis of 'possible' mitochondrial disease may also create an unfounded fear of worsening morbidity and mortality. Certain families of patients given a diagnosis of 'possible' mitochondrial disease often overlook the uncertainty of the diagnosis and become overly concerned that they or a family member may manifest all of the symptoms a patient with a PMD may develop, including neurodegeneration or early death, even in instances where their symptoms are relatively mild.

Last, patients with a diagnosis of 'possible' mitochondrial disease may receive inappropriate care or be overmedicalised. Counselling of disease expectations and management may vary based on how patients are categorised.[60] Unnecessary medical interventions may be offered to some during times of catabolic stress. Some medications may not be used due to a concern of potential mitochondrial toxicity. New symptoms that a patient may manifest may inappropriately be explained away by the underlying diagnostic label rather than looking for other potentially treatable causes. These and other concerns are summarised in box 2.

Some of these very issues and challenges are outlined in example cases provided in the supplementary material (online supplementary cases). In addition to the disorders outlined in table 2, the online supplementary cases illustrate instances where a patient may have symptoms suggesting the possibility of mitochondrial disease, often with biochemical abnormalities suggesting mitochondrial dysfunction, but the final diagnosis is not a PMD. Diagnosis is often delayed due to the mistaken diagnosis. Examples include a manganese transporter disorder with bilateral basal ganglia hyperintensities and elevated FGF21 levels (Case 1), oculopharyngeal muscular dystrophy with ragged red and cytochrome *c* oxidase (COX)-negative fibres (Case 2), Lesch-Nyhan syndrome with putaminal and thalamic abnormalities, lactic acidosis and reduced Complex I enzymatic activity in muscle (Case 3) and Niemann-Pick Type C with Complex I deficiency leading to a delay in being prescribed Miglustat (Case 4). In some of these instances, mitochondrial functional testing was notably abnormal, meeting biochemical diagnostic criteria for a mitochondrial disease. In contrast, select other cases (Cases 5–8) illustrate a delayed PMD diagnosis due to limitations of genetic testing in blood, findings of low levels of heteroplasmy or findings of a VUS. Case 5 illustrates an instance of a female with MELAS-like symptoms. Other cases (Cases 6–8) show the challenges in interpreting nuclear and mtDNA VUS.

Box 2 Potential harms arising from a diagnosis of 'possible' mitochondrial disease

- Ending diagnostic odyssey prematurely.
- Missing potentially treatable disorders.
- Psychological burden of mitochondrial disease diagnosis: parent/patient fear of progressive or degenerative disorder.
- Inaccurate recurrence risk counselling.
- Inappropriate preventative care.
- Unnecessary medical interventions at times of catabolic stress.
- Avoidance of needed medications owing to fear of mitochondrial toxicity.
- Inappropriate reproductive decisions taken.

Box 3 Terminology to avoid when a mitochondrial diagnosis is uncertain

- 'Possible,' 'probable' or 'suspected' mitochondrial disease.
- Mitochondrial myopathy.
- Mitochondrial cytopathy.
- Mitochondrial metabolism disorder.
- Defect of mitochondrial metabolism.

Recommendations

In patients without a confirmed genetic diagnosis, there is a need for clinicians and the mitochondrial disease community to use diagnostic labels that clearly state that the diagnosis is uncertain even when mitochondrial dysfunction has been identified. A category of '*genetic diagnosis uncertain; mitochondrial biochemical dysfunction or mitochondrial genetic variant of unknown significance identified*' is preferable to a diagnosis of 'possible' or 'probable' or 'suspected' mitochondrial disease. Other terminology that should be avoided is listed in box 3. Depending on the clinical situation, patients may be further stratified into a '*high risk*' for a PMD to guide management.

Our proposal to use a diagnostic label of '*genetic diagnosis uncertain*' for all such cases would allow clinicians and patients to remain actively engaged in the diagnostic odyssey, review the prior data periodically and take advantage of technological advances in genetic testing and new disease descriptions. Conducting relevant screening of other systems and monitoring for other organ involvement would allow better definition of the phenotype and not overlook disease progression. The clarity of the diagnostic label may prevent inappropriate or unnecessary care and allay fears of a progressive or degenerative disease.

Further categorisation of selected patients as possible 'high risk' for a PMD would allow for closer monitoring for mitochondrial disease-related systemic comorbidities or extra cautions during times they are at risk of metabolic decompensations. If the phenotype is especially suggestive of a PMD, it may be appropriate to manage such a patient as if they have a genetically confirmed PMD for the time being—especially if they have previously experienced metabolic decompensation during times of illness or medical stress. Unexpected, acute changes in clinical status warrant thorough medical evaluation including laboratory testing to investigate potential mitochondrial dysfunction. However, the '*diagnosis uncertain*' designation would prevent any misunderstanding among medical teams. If the phenotype is not as suggestive of a PMD, it may be prudent

to avoid overmedicalisation of the patient and simply continue more routine monitoring.

As diagnostic standards for mitochondrial disease continue to evolve, these patients should remain under the care of a clinician who can assist in providing up-to-date recommendations regarding further testing. The MMS has such recommendations available online at http://www.mitosoc.org.

CONCLUSION

Despite advances in diagnostic techniques and molecular genetics, a subset of patients with suspected mitochondrial disease remains without a confirmed genetic diagnosis. The path these patients take to receiving a diagnosis is arduous and, at times, circuitous. Newer NGS-based genetic studies offer the ability to streamline the approach to diagnosis for some patients. Others remain with a constellation of symptoms, findings of mitochondrial dysfunction on functional testing and no clear pathogenic genetic mutation. Patients diagnosed with a 'possible' mitochondrial disease might be found to have a non-mitochondrial genetic disorder once new testing modalities are used. A mistaken diagnosis of mitochondrial disease may prematurely end their diagnostic journey, overmedicalise their care and potentially limit access to appropriate treatments for the actual underlying condition. To alleviate this dilemma, such patients would be better served by clinicians avoiding the diagnostic term 'possible' mitochondrial disease.

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REFERENCES

1 Rahman J, Rahman S. Mitochondrial medicine in the omics era. *Lancet* 2018;391:2560–74.
2 Haas RH, Parikh S, Falk MJ, Saneto RP, Wolf NI, Darin N, Cohen BH. Mitochondrial disease: a practical approach for primary care physicians. *Pediatrics* 2007;120:1326–33.
3 Calvo SE, Compton AG, Hershman SG, Lim SC, Lieber DS, Tucker EJ, Laskowski A, Garone C, Liu S, Jaffe DB, Christodoulou J, Fletcher JM, Bruno DL, Goldblatt J, Dimauro S, Thorburn DR, Mootha VK. Molecular diagnosis of infantile mitochondrial disease with targeted next-generation sequencing. *Sci Transl Med* 2012;4:118ra10.
4 Wortmann SB, Koolen DA, Smeitink JA, van den Heuvel L, Rodenburg RJ. Whole exome sequencing of suspected mitochondrial patients in clinical practice. *J Inherit Metab Dis* 2015;38:437–43.
5 Taylor RW, Pyle A, Griffin H, Blakely EL, Duff J, He L, Smertenko T, Alston CL, Neeve VC, Best A, Yarham JW, Kirschner J, Schara U, Talim B, Topaloglu H, Baric I, Holinski-Feder E, Abicht A, Czermin B, Kleinle S, Morris AA, Vassallo G, Gorman GS, Ramesh V, Turnbull DM, Santibanez-Koref M, McFarland R, Horvath R, Chinnery PF. Use of whole-exome sequencing to determine the genetic basis of multiple mitochondrial respiratory chain complex deficiencies. *JAMA* 2014;312:68–77.
6 Lieber DS, Calvo SE, Shanahan K, Slate NG, Liu S, Hershman SG, Gold NB, Chapman BA, Thorburn DR, Berry GT, Schmahmann JD, Borowsky ML, Mueller DM, Sims KB, Mootha VK. Targeted exome sequencing of suspected mitochondrial disorders. *Neurology* 2013;80:1762–70.
7 Kohda M, Tokuzawa Y, Kishita Y, Nyuzuki H, Moriyama Y, Mizuno Y, Hirata T, Yatsuka Y, Yamashita-Sugahara Y, Nakachi Y, Kato H, Okuda A, Tamaru S, Borna NN, Banshoya

K, Aigaki T, Sato-Miyata Y, Ohnuma K, Suzuki T, Nagao A, Maehata H, Matsuda F, Higasa K, Nagasaki M, Yasuda J, Yamamoto M, Fushimi T, Shimura M, Kaiho-Ichimoto K, Harashima H, Yamazaki T, Mori M, Murayama K, Ohtake A, Okazaki Y. A comprehensive genomic analysis reveals the genetic landscape of mitochondrial respiratory chain complex deficiencies. *PLoS Genet* 2016;12.
8 Parikh S, Goldstein A, Koenig MK, Scaglia F, Enns GM, Saneto R, Anselm I, Collins A, Cohen BH, DeBrosse SD, Dimmock D, Falk MJ, Ganesh J, Greene C, Gropman AL, Haas R, Kahler SG, Kamholz J, Kendall F, Korson MS, Mattman A, Milone M, Niyazov D, Pearl PL, Reimschisel T, Salvarinova-Zivkovic R, Sims K, Tarnopolsky M, Tsao CY, van Hove J, Walsh L, Wolfe LA. Practice patterns of mitochondrial disease physicians in North America. Part 1: diagnostic and clinical challenges. *Mitochondrion* 2014;14:26–33.
9 Morava E, van den Heuvel L, Hol F, de Vries MC, Hogeveen M, Rodenburg RJ, Smeitink JA. Mitochondrial disease criteria: diagnostic applications in children. *Neurology* 2006;67:1823–6.
10 Bernier FP, Boneh A, Dennett X, Chow CW, Cleary MA, Thorburn DR. Diagnostic criteria for respiratory chain disorders in adults and children. *Neurology* 2002;59:1406–11.
11 Thorburn DR, Sugiana C, Salemi R, Kirby DM, Worgan L, Ohtake A, Ryan MT. Biochemical and molecular diagnosis of mitochondrial respiratory chain disorders. *Biochim Biophys Acta* 2004;1659:121–8.
12 Martikainen MH, Chinnery PF. Mitochondrial disease: mimics and chameleons. *Pract Neurol* 2015;15:424–35.
13 Needham M, Duley J, Hammond S, Herkes GK, Hirano M, Sue CM. Mitochondrial disease mimicking charcot-marie tooth disease. *BMJ Case Rep* 2009;2009.
14 Gardeitchik T, Wyckmans J, Morava E. Complex phenotypes in inborn errors of metabolism: overlapping presentations in congenital disorders of glycosylation and mitochondrial disorders. *Pediatr Clin North Am* 2018;65:375–88.
15 D'Amico A, Fattori F, Bellacchio E, Catteruccia M, Servidei S, Bertini E. A new de novo missense mutation in MYH2 expands clinical and genetic findings in hereditary myosin myopathies. *Neuromuscul Disord* 2013;23:437–40.
16 Biancalana V, Scheidecker S, Miguet M, Laquerrière A, Romero NB, Stojkovic T, Abath Neto O, Mercier S, Voermans N, Tanner L, Rogers C, Ollagnon-Roman E, Roper H, Boutte C, Ben-Shachar S, Lornage X, Vasli N, Schaefer E, Laforet P, Pouget J, Moerman A, Pasquier L, Marcorelle P, Magot A, Küsters B, Streichenberger N, Tranchant C, Dondaine N, Schneider R, Gasnier C, Calmels N, Kremer V, Nguyen K, Perrier J, Kamsteeg EJ, Carlier P, Carlier RY, Thompson J, Boland A, Deleuze JF, Fardeau M, Zanoteli E, Eymard B, Laporte J. Affected female carriers of MTM1 mutations display a wide spectrum of clinical and pathological involvement: delineating diagnostic clues. *Acta Neuropathol* 2017;134:889–904.
17 Catteruccia M, Fattori F, Codemo V, Ruggiero L, Maggi L, Tasca G, Fiorillo C, Pane M, Berardinelli A, Verardo M, Bragato C, Mora M, Morandi L, Bruno C, Santoro L, Pegoraro E, Mercuri E, Bertini E, D'Amico A, D'Amico A. Centronuclear myopathy related to dynamin 2 mutations: clinical, morphological, muscle imaging and genetic features of an Italian cohort. *Neuromuscul Disord* 2013;23:229–38.
18 Bevilacqua JA, Monnier N, Bitoun M, Eymard B, Ferreiro A, Monges S, Lubieniecki F, Taratuto AL, Laquerrière A, Claeys KG, Marty I, Fardeau M, Guicheney P, Lunardi J, Romero NB. Recessive RYR1 mutations cause unusual congenital myopathy with prominent nuclear internalization and large areas of myofibrillar disorganization. *Neuropathol Appl Neurobiol* 2011;37:271–84.
19 Wilmshurst JM, Lillis S, Zhou H, Pillay K, Henderson H, Kress W, Müller CR, Ndondo A, Cloke V, Cullup T, Bertini E, Boennemann C, Straub V, Quinlivan R, Dowling JJ, Al-Sarraj S, Treves S, Abbs S, Manzur AY, Sewry CA, Muntoni F, Jungbluth H. RYR1 mutations are a common cause of congenital myopathies with central nuclei. *Ann Neurol* 2010;68:717–26.
20 Clarke NF, Waddell LB, Cooper ST, Perry M, Smith RL, Kornberg AJ, Muntoni F, Lillis S, Straub V, Bushby K, Guglieri M, King MD, Farrell MA, Marty I, Lunardi J, Monnier N, North KN. Recessive mutations in RYR1 are a common cause of congenital fiber type disproportion. *Hum Mutat* 2010;31:E1544-50.
21 Kondo E, Nishimura T, Kosho T, Inaba Y, Mitsuhashi S, Ishida T, Baba A, Koike K, Nishino I, Nonaka I, Furukawa T, Saito K. Recessive RYR1 mutations in a patient with severe congenital nemaline myopathy with ophthalomoplegia identified through massively parallel sequencing. *Am J Med Genet A* 2012;158A:772–8.
22 Das S, Dowling J, Pierson CR. X-linked myotubular myopathy. 1993 http://www.ncbi.nlm.nih.gov/pubmed/20301605 (accessed 27 Apr 2018).
23 Wengert O, Meisel A, Kress W, Dekomien G, Angstwurm K, Heppner FL, Goebel HH, Stenzel W. Progressive external ophthalmoplegia as initial manifestation of sporadic late-onset nemaline myopathy. *J Neurol* 2011;258:915–7.
24 Mullaney P, Vajsar J, Smith R, Buncic JR. The natural history and ophthalmic involvement in childhood myasthenia gravis at the hospital for sick children. *Ophthalmology* 2000;107:504–10.
25 Testai FD, Gorelick PB. Inherited metabolic disorders and stroke part 2: homocystinuria, organic acidurias, and urea cycle disorders. *Arch Neurol* 2010;67:148–53.
26 Briones P, Vilaseca MA, García-Silva MT, Pineda M, Colomer J, Ferrer I, Artigas J, Jaeken J, Chabás A. Congenital disorders of glycosylation (CDG) may be underdiagnosed when mimicking mitochondrial disease. *Eur J Paediatr Neurol* 2001;5:127–31.
27 Basel-Vanagaite L, Muncher L, Straussberg R, Pasmanik-Chor M, Yahav M, Rainshtein L, Walsh CA, Magal N, Taub E, Drasinover V, Shalev H, Attia R, Rechavi G, Simon AJ, Shohat M. Mutated nup62 causes autosomal recessive infantile bilateral striatal necrosis. *Ann Neurol* 2006;60:214–22.
28 Neilson DE, Adams MD, Orr CM, Schelling DK, Eiben RM, Kerr DS, Anderson J, Bassuk AG, Bye AM, Childs AM, Clarke A, Crow YJ, Di Rocco M, Dohna-Schwake C, Dueckers G, Fasano AE, Gika AD, Gionnis D, Gorman MP, Grattan-Smith PJ, Hackenberg A, Kuster A, Lentschig MG, Lopez-Laso E, Marco EJ, Mastroyianni S, Perrier J, Schmitt-Mechelke T, Servidei S, Skardoutsou A, Uldall P, van der Knaap MS, Goglin KC, Tefft DL, Aubin C, de Jager P, Hafler D, Warman ML. Infection-triggered familial or recurrent cases of acute necrotizing encephalopathy caused by mutations in a component of the nuclear pore, RANBP2. *Am J Hum Genet* 2009;84:44–51.
29 Appenzeller S, Schirmacher A, Halfter H, Bäumer S, Pendziwiat M, Timmerman V, De Jonghe P, Fekete K, Stögbauer F, Lüdemann P, Hund M, Quabius ES, Ringelstein EB, Kuhlenbäumer G. Autosomal-dominant striatal degeneration is caused by a mutation in the phosphodiesterase 8B gene. *Am J Hum Genet* 2010;86:83–7.
30 Parikh S, Goldstein A, Koenig MK, Scaglia F, Enns GM, Saneto R, Anselm I, Cohen BH, Falk MJ, Greene C, Gropman AL, Haas R, Hirano M, Morgan P, Sims K, Tarnopolsky M, Van Hove JL, Wolfe L, DiMauro S. Diagnosis and management of mitochondrial disease: a consensus statement from the Mitochondrial Medicine Society. *Genet Med* 2015;17:689–701.
31 Lehtonen JM, Forsström S, Bottani E, Viscomi C, Baris OR, Isoniemi H, Höckerstedt K, Österlund P, Hurme M, Jylhävä J, Leppä S, Markkula R, Heliö T, Mombelli G, Uusimaa J, Laaksonen R, Laaksovirta H, Auranen M, Zeviani M, Smeitink J, Wiesner RJ, Nakada K, Isohanni P, Suomalainen A. FGF21 is a biomarker for mitochondrial translation and mtDNA maintenance disorders. *Neurology* 2016;87:2290–9.
32 Montero R, Yubero D, Villarroya J, Henares D, Jou C, Rodríguez MA, Ramos F, Nascimento A, Ortez CI, Campistol J, Perez-Dueñas B, O'Callaghan M, Pineda M, Garcia-Cazorla A, Oferil JC, Montoya J, Ruiz-Pesini E, Emperador S, Meznaric M, Campderros L, Kalko SG, Villarroya F, Artuch R, Jimenez-Mallebrera C. GDF-15 is elevated in children with mitochondrial diseases and is induced by mitochondrial dysfunction. *PLoS One* 2016;11:e0148709–15.
33 Yatsuga S, Fujita Y, Ishii A, Fukumoto Y, Arahata H, Kakuma T, Kojima T, Ito M, Tanaka M, Saiki R, Koga Y. Growth differentiation factor 15 as a useful biomarker for mitochondrial disorders. *Ann Neurol* 2015;78:814–23.
34 Davis RL, Liang C, Edema-Hildebrand F, Riley C, Needham M, Sue CM. Fibroblast growth factor 21 is a sensitive biomarker of mitochondrial disease. *Neurology* 2013;81.
35 Davis RL, Liang C, Sue CM. A comparison of current serum biomarkers as diagnostic indicators of mitochondrial diseases. *Neurology* 2016;86:2010–5.
36 Abadi A, Glover EI, Isfort RJ, Raha S, Safdar A, Yasuda N, Kaczor JJ, Melov S, Hubbard A, Qu X, Phillips SM, Tarnopolsky M. Limb immobilization induces a coordinate down-regulation of mitochondrial and other metabolic pathways in men and women. *PLoS One* 2009;4:e6518.
37 McKiernan P, Ball S, Santra S, Foster K, Fratter C, Poulton J, Craig K, McFarland R, Rahman S, Hargreaves I, Gupte G, Sharif K, Taylor RW. Incidence of primary mitochondrial disease in children younger than 2 years presenting with acute liver failure. *J Pediatr Gastroenterol Nutr* 2016;63:592–7.
38 Lane M, Boczonadi V, Bachtari S, Gomez-Duran A, Langer T, Griffiths A, Kleinle S, Dineiger C, Abicht A, Holinski-Feder E, Schara U, Gerner P, Horvath R. Mitochondrial dysfunction in liver failure requiring transplantation. *J Inherit Metab Dis* 2016;39:427–36.
39 Berger A, Mayr JA, Meierhofer D, Fötschl U, Bittner R, Budka H, Grethen C, Huemer M, Kofler B, Sperl W. Severe depletion of mitochondrial DNA in spinal muscular atrophy. *Acta Neuropathol* 2003;105:245–51.
40 Fourcade S, Ferrer I, Pujol A, stress O. Oxidative stress, mitochondrial and proteostasis malfunction in adrenoleukodystrophy: A paradigm for axonal degeneration. *Free Radic Biol Med* 2015;88:18–29.
41 Frye RE, Cox D, Slattery J, Tippett M, Kahler S, Granpeesheh D, Damle S, Legido A, Goldenthal MJ. Mitochondrial dysfunction may explain symptom variation in phelan-mcdermid syndrome. *Sci Rep* 2016;6:19544.
42 Hui J, Kirby DM, Thorburn DR, Boneh A. Decreased activities of mitochondrial respiratory chain complexes in non-mitochondrial respiratory chain diseases. *Dev Med Child Neurol* 2006;48:132–6.
43 Niyazov DM, Kahler SG, Frye RE. Primary mitochondrial disease and secondary mitochondrial dysfunction: importance of distinction for diagnosis and treatment. *Mol Syndromol* 2016;7:122–37.
44 García-Cazorla A, Serrano M, Pérez-Dueñas B, González V, Ormazábal A, Pineda M, Fernández-Alvarez E, Campistol JM, Artuch RM. Secondary abnormalities of neurotransmitters in infants with neurological disorders. *Dev Med Child Neurol* 2007;49:740–4.
45 Prince J, Jia S, Båve U, Annerén G, Oreland L. Mitochondrial enzyme deficiencies in Down's syndrome. *J Neural Transm Park Dis Dement Sect* 1994;8:171–81.
46 Kleefstra T, Wortmann SB, Rodenburg RJ, Bongers EM, Hadzsiev K, Noordam C, van den Heuvel LP, Nillesen WM, Hollody K, Gillessen-Kaesbach G, Lammens M, Smeitink JA, van der Burgt I, Morava E. Mitochondrial dysfunction and organic aciduria in five patients carrying mutations in the Ras-MAPK pathway. *Eur J Hum Genet* 2011;19:138–44.

47 Panneman DM, Smeitink JA, Rodenburg RJ. Mining for mitochondrial mechanisms: Linking known syndromes to mitochondrial function. *Clin Genet* 2018;93.
48 Frye RE. 15q11.2-13 duplication, mitochondrial dysfunction, and developmental disorders. *J Child Neurol* 2009;24:1316–20.
49 Maynard TM, Meechan DW, Dudevoir ML, Gopalakrishna D, Peters AZ, Heindel CC, Sugimoto TJ, Wu Y, Lieberman JA, Lamantia AS. Mitochondrial localization and function of a subset of 22q11 deletion syndrome candidate genes. *Mol Cell Neurosci* 2008;39:439–51.
50 Witters P, Saada A, Honzik T, Tesarova M, Kleinle S, Horvath R, Goldstein A, Morava E. Revisiting mitochondrial diagnostic criteria in the new era of genomics. *Genet Med* 2018;20:1–8.
51 Wolf NI, Smeitink JA. Mitochondrial disorders: a proposal for consensus diagnostic criteria in infants and children. *Neurology* 2002;59:1402–5.
52 Calvo SE, Clauser KR, Mootha VK. MitoCarta2.0: an updated inventory of mammalian mitochondrial proteins. *Nucleic Acids Res* 2016;44:D1251–7.
53 Smith AC, Robinson AJ. MitoMiner v3.1, an update on the mitochondrial proteomics database. *Nucleic Acids Res* 2016;44:D1258–61.
54 Frazier AE, Thorburn DR, Compton AG. Mitochondrial energy generation disorders: genes, mechanisms and clues to pathology. *J Biol Chem* 2017:jbc.R117.809194.
55 Ormondroyd E, Mackley MP, Blair E, Craft J, Knight JC, Taylor JC, Taylor J, Watkins H. "Not pathogenic until proven otherwise": perspectives of uk clinical genomics professionals toward secondary findings in context of a genomic medicine multidisciplinary team and the 100,000 genomes project. *Genet Med* 2018;20:320–8.
56 McCormick EM, Kenyon L, Falk MJ. Desmin common mutation is associated with multi-systemic disease manifestations and depletion of mitochondria and mitochondrial DNA. *Front Genet* 2015;6:1–5.
57 Cámara Y, Carreño-Gago L, Martín MA, Melià MJ, Blázquez A, Delmiro A, Garrabou G, Morén C, Díaz-Manera J, Gallardo E, Bornstein B, López-Gallardo E, Hernández-Lain A, San Millán B, Cancho E, Rodríguez-Vico JS, Martí R, García-Arumí E. Severe TK2 enzyme activity deficiency in patients with mild forms of myopathy. *Neurology* 2015;84:2286–8.
58 Elliott HR, Samuels DC, Eden JA, Relton CL, Chinnery PF. Pathogenic mitochondrial DNA mutations are common in the general population. *Am J Hum Genet* 2008;83:254–60.
59 Parikh S, Goldstein A, Karaa A, Koenig MK, Anselm I, Brunel-Guitton C, Christodoulou J, Cohen BH, Dimmock D, Enns GM, Falk MJ, Feigenbaum A, Frye RE, Ganesh J, Griesemer D, Haas R, Horvath R, Korson M, Kruer MC, Mancuso M, McCormack S, Raboisson MJ, Reimschisel T, Salvarinova R, Saneto RP, Scaglia F, Shoffner J, Stacpoole PW, Sue CM, Tarnopolsky M, Van Karnebeek C, Wolfe LA, Cunningham ZZ, Rahman S, Chinnery PF. Patient care standards for primary mitochondrial disease: a consensus statement from the Mitochondrial Medicine Society. *Genet Med* 2017;19.
60 Parikh S, Goldstein A, Koenig MK, Scaglia F, Enns GM, Saneto R. Practice patterns of mitochondrial disease physicians in North America. Part 2: treatment, care and management. *Mitochondrion* 2013;13:681–7.