

## REVIEW

### Mitochondrial disorders and drugs: what every physician should know

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#### Abstract

Mitochondrial disorders are a group of metabolic conditions caused by impairment of the oxidative phosphorylation system. There is currently no clear evidence supporting any pharmacological interventions for most mitochondrial disorders, except for coenzyme Q10 deficiencies, Leber hereditary optic neuropathy, and mitochondrial neurogastrointestinal encephalomyopathy. Furthermore, some drugs may potentially have detrimental effects on mitochondrial dysfunction. Drugs known to be toxic for mitochondrial functions should be avoided whenever possible. Mitochondrial patients needing one of these treatments should be carefully monitored, clinically and by laboratory exams, including creatine kinase and lactate. In the era of molecular and 'personalized' medicine, many different physicians (not only neurologists) should be aware

of the basic principles of mitochondrial medicine and its therapeutic implications. Multicenter collaboration is essential for the advancement of therapy for mitochondrial disorders. Whenever possible, randomized clinical trials are necessary to establish efficacy and safety of drugs. In this review we discuss in an accessible way the therapeutic approaches and perspectives in mitochondrial disorders. We will also provide an overview of the drugs that should be used with caution in these patients.

**Keywords:** coenzyme Q10, drugs, mitochondria, mtDNA, mitochondrial diseases, toxicity

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## Introduction

Mitochondrial disorders (MD) are an extraordinarily complex group of diseases caused by impairment of mitochondrial functions, especially of the mitochondrial respiratory chain.<sup>1</sup> Mitochondria are dynamic organelles whose maintenance requires about 1500 proteins. Mutations in either the mitochondrial (mtDNA) or nuclear (nDNA) genome can disrupt myriads of metabolic and homeostatic functions of the cell.<sup>2</sup>

For this reason, from a genetic perspective, there are two major groups of MD: the diseases due to defects in mtDNA and those due to mutations in nDNA. Only the last ones follow the Mendelian laws of inheritance. MtDNA large-scale single deletions are sporadic and not inheritable. Inherited MtDNA point mutations follow the rules of mitochondrial inheritance (maternal transmission, mitotic segregation, heteroplasmy, and the threshold effect).<sup>1</sup>

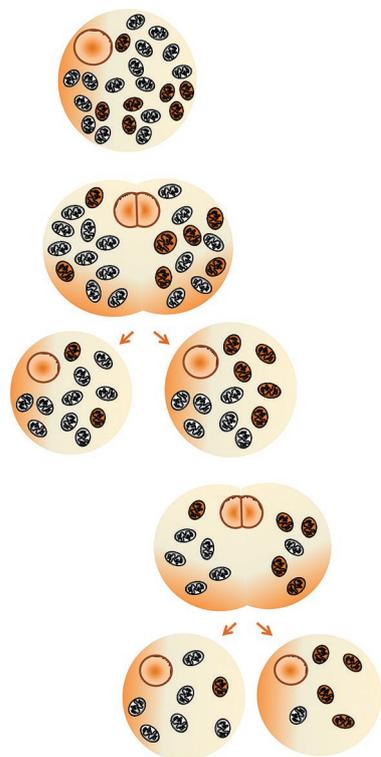
Cells contain multiple copies of mtDNA (polyplasmidy), which, in usual conditions, are identical to one another (homoplasmy). Heteroplasmy refers to the coexistence of two populations of mitochondria: normal and mutated. Mutated mtDNA has to reach a certain critical 'threshold' in a tissue to cause

dysfunction (threshold effect). Because of the mitotic segregation (which is the random assignment of mitochondria between the daughter cells), the mutation load can change from one cell generation to the next, and therefore it can either surpass or fall below the pathogenic threshold (Figure 1).<sup>1</sup>

It has been observed that cells attempt to maintain wild-type mtDNA density through cell volume reduction, and thus power demand reduction, until a minimum volume. These studies provide evidence for the physiological significance of mtDNA density and emphasize the need for performing single-cell volume measurements jointly with mtDNA quantification.<sup>3</sup> This new concept of 'mtDNA density' may have implications for the development of new therapies for MD due to mtDNA mutations.<sup>3</sup>

Recently, the paradigm of maternal inheritance of mtDNA has been questioned.<sup>4</sup> Biparental mtDNA transmission was reported in three unrelated multigeneration families, with an autosomal dominant inheritance mode.<sup>4</sup> In our opinion, these exceptional families where paternal mtDNA could be passed to the offspring do not invalidate the central dogma of maternal inheritance of mtDNA.<sup>4</sup> However, we agree that

**Figure 1. Heteroplasmy and mitotic segregation: schematic representation.** Because of the mitotic segregation (random share-out of mutated and nonmutated mitochondria between the daughter cells), the mutation load can change from one cell generation to the next, and with time, it can either surpass or fall below the pathogenic threshold.



**Table 1. Some of the most frequent clinical features of mitochondrial diseases.**

<b>Central nervous system</b>	Migraine, myoclonus, cognitive impairment, stroke-like episodes, seizures, ataxia, dystonia, parkinsonism, tremor, psychiatric involvement
<b>Peripheral nervous system</b>	Axonal multifocal neuropathy
<b>Skeletal muscle</b>	Weakness, ophthalmoparesis, eyelid ptosis, exercise intolerance, myoglobinuria, respiratory impairment, hypotonia
<b>Visual system</b>	Pigmentary retinopathy, cataract, optic neuropathy
<b>Acoustic system</b>	Sensorineural hearing loss
<b>Digestive system</b>	Malabsorption, intestinal pseudo-obstruction
<b>Kidney</b>	Tubulopathy, Fanconi syndrome
<b>Metabolic/endocrine apparatus</b>	Lactic acidosis, multiple lipomatosis, short stature, diabetes, hypothyroidism, hypoparathyroidism
<b>Heart</b>	Cardiomyopathy, conduction system defects, Wolff–Parkinson–White syndrome
<b>Hematopoietic system</b>	Sideroblastic anemia

there is a strong need for further study aimed at elucidating the molecular mechanisms and the biomedical implications of this unusual mode of inheritance.<sup>4</sup>

The effects of mutations (in both nuclear and mitochondrial genomes) affecting the respiratory chain may be variable, heterogeneous, and multisystemic, with possible involvement of nearly all tissues and organs, including visual and auditory pathways, heart, central nervous system (CNS), and skeletal muscle (Tables 1 and 2). Some of the ‘red flags’ for MD are exercise intolerance and myopathy, ophthalmoparesis and/or eyelid ptosis, sensorineural hearing loss, axonal neuropathy, hypertrophic cardiomyopathy, optic neuropathy, pigmentary retinopathy, diabetes, short stature, migraine, and lactic acidosis.<sup>1</sup>

The heterogeneity is further increased by other not-modifiable factors, including gender<sup>5</sup> and nuclear and mtDNA genetic background,<sup>6</sup> and by potentially modifiable gene–environment interactions (i.e. smoke<sup>5</sup> and drugs).<sup>7</sup>

Because of the multisystem involvement, a wide range of medical specialists (general practitioners, internists,

pediatricians, cardiologists, endocrinologist, audiologists, ophthalmologists, etc.) may first encounter these patients, and the clinical approach may become a ‘diagnostic odyssey’.<sup>8</sup> The prevalence of MD is 1–2/10,000, and in fact they represent one of the most frequently encountered metabolic conditions.<sup>9</sup> Therefore, we believe that in the era of molecular and ‘personalized’ medicine, physicians (not only neurologists) should know the basic principles of mitochondrial medicine and its therapeutic implications.

This review will focus on the ‘properly called’ MD (diseases of the mitochondrial respiratory chain). Other potentially treatable metabolic and neurodegenerative conditions ‘indirectly’ linked to mitochondrial dysfunction will not be discussed here. These diseases, which are not directly caused by dysfunction of the electron transport chain, include Friedreich ataxia,<sup>10</sup> ethylmalonic encephalopathy (treated with metronidazole plus N-acetylcysteine),<sup>11</sup> acyl-CoA dehydrogenation deficiency (treated with riboflavin),<sup>12</sup> primary carnitine deficiency, vitamin E deficiency, and secondary coenzyme Q10 deficiencies (treated with administration of the defective metabolite).<sup>7</sup>

**Table 2. Mitochondrial syndromes.** Selected, well-established mitochondrial syndromes are shown. However, many patients do not show these specific clinical pictures and are affected by a variety of complex syndromes (myopathies, neuropathies, cardiomyopathies, encephalomyopathies, multisystemic diseases, etc.) or partial pictures.

	Main feature(s)	Associated feature(s)	Inheritance	Common genetic findings	Treatment of choice
Alpers syndrome	Childhood myocerebrohepatopathy		Autosomal recessive	<i>POLG</i> mutations	Symptomatic (avoid valproate)
Autosomal dominant optic atrophy (ADOA)	Optic neuropathy (blindness)		Autosomal dominant	<i>OPA1</i> mutations	Symptomatic
Coenzyme Q10 deficiency	Ataxia or myopathy or multisystem disease		Autosomal recessive	Different genes	Coenzyme Q10
Kearns–Sayre Syndrome (KSS)	Ocular myopathy (ptosis, ophthalmoparesis)	Ataxia, cardiac conduction defects	Sporadic	Single large-scale deletion of mtDNA	Symptomatic
Leber hereditary optic neuropathy (LHON)	Optic neuropathy (blindness)		Maternal (low penetrance, higher in male smokers)	Different mtDNA mutations	Idebenone
Leigh syndrome	Severe pediatric encephalopathy		Autosomal recessive or maternal	Different nuclear genes or mtDNA (e.g. m.8993T>G)	Symptomatic
Mitochondrial encephalopathy with lactic acidosis and stroke-like episodes (MELAS)	Stroke-like episodes	Hearing loss, diabetes	Maternal	m.3243A>G	Symptomatic
Myoclonic epilepsy with ragged red fiber (MERRF)	Myoclonus	Ataxia, myopathy	Maternal	m.8344A>G	Symptomatic (e.g. Levetiracetam)
Mitochondrial Neurogastrointestinal Encephalomyopathy (MNGIE)	Gastrointestinal dysmotility	Leukodystrophy, ocular myopathy, peripheral neuropathy	Autosomal recessive	<i>TYMP</i> mutations	Allogeneic hematopoietic stem cell transplantation
Neuropathy, Ataxia, Retinitis Pigmentosa (NARP)	Ataxia	Neuropathy, Retinitis Pigmentosa	Maternal	m.8993T>G	Symptomatic
Non syndromic hearing loss (NSHL)	Hearing loss		Maternal	m.1555A>G	Symptomatic (avoid aminoglycosides )
Progressive external ophthalmoplegia (PEO)	Ocular myopathy	Myopathy	Autosomal dominant, recessive, maternal, or sporadic	Different nuclear genes, different mtDNA mutations, mtDNA single deletion	Symptomatic

mtDNA, mitochondrial DNA.

Here, we will discuss the therapeutic approaches and perspectives in MD. We will also provide an overview of the drugs that should be used with caution in these patients. We searched PubMed in May 2019 for all articles about 'mitochondrial disorder\* OR mitochondrial disease\* OR mitochondrial \*myopath\*', and we reviewed the abstracts to identify relevant publications.

## Etiopathogenetic approaches

### Can we treat mitochondrial dysfunction?

Despite great progress in the molecular pathogenesis of MD, the treatment possibilities are still very limited.<sup>7</sup> Apart from symptomatic treatments, therapies that have been attempted include antioxidants, respiratory chain cofactors, and other metabolites. However, there is no clear evidence supporting the use of any of these interventions in most MD,<sup>13</sup> and further research is needed. There have been very few randomized controlled clinical trials, the majority of which were short and involved few participants with heterogeneous phenotypes.<sup>13</sup>

Coenzyme Q10, or ubiquinone, is widely used for MD, because of the generally positive anecdotal data together with the lack of major toxicity.<sup>7</sup> This cofactor is located on the inner mitochondrial membrane and shuttles electrons from complexes I and II and from the oxidation of fatty acids and branched chain aminoacids to complex III of the mitochondrial respiratory chain. Reduced coenzyme Q10 has also antioxidant properties.<sup>7</sup> A randomized, double-blind crossover trial with coenzyme Q10 was performed on eight patients with MD.<sup>14</sup> Several measures showed a trend toward improvement on treatment.<sup>14</sup> In another more recent crossover trial, 30 patients with MD received coenzyme Q10 (1200 mg/day) for 60 days.<sup>15</sup> The treatment had some effects on aerobic capacity and postexercise lactate, without affecting other relevant variables.<sup>15</sup>

Unfortunately, crystalline CoQ10 is lipophilic, water insoluble, and poorly absorbed in the gut.<sup>16</sup> Therefore, there is a strong need for using solubilized formulations with certified bioavailability. Coenzyme Q10 analogs that target mitochondria, such as 'MitoQ', have been also developed and widely used as antioxidants,<sup>17</sup> but they have not been evaluated yet in patients with MD.

Regarding other metabolites and cofactors, carnitine (up to 3 g/day) resulted in some improvement in isolated cases, but objective evidence is unavailable.<sup>7</sup> However, it has been observed that chronic oral L-carnitine supplementation to MD patients increases a plasma metabolite (trimethylamine oxide) that has been linked to cardiovascular disease.<sup>18</sup> Therefore, further studies to evaluate both the efficacy and long-term safety are warranted before routine carnitine supplementation.<sup>18</sup> A randomized, controlled trial with creatine on six MD patients showed some improvement of high-intensity activities.<sup>19</sup> Another controlled crossover trial studied

the effects of creatine in 16 participants with mitochondrial myopathy.<sup>20</sup> No significant effects of treatment were noted.<sup>20</sup> More recently, a randomized, double-blind, placebo-controlled, crossover study with a combination therapy (creatine, coenzyme Q10, and lipoic acid) was performed on 17 patients with heterogeneous MD.<sup>21</sup> The combination therapy could lower lactate and oxidative stress biomarkers.<sup>21</sup> Riboflavin is a cofactor for the respiratory chain that was reported to elicit some positive effects in children with complex II deficiency<sup>22</sup> and other rare patients with specific biochemical defects, such as the myopathic form of coenzyme Q10 deficiency due to mutations in the electron-transferring flavoprotein dehydrogenase (*ETFDH*) gene.<sup>23</sup>

A high fat (or ketogenic)<sup>24</sup> diet can be beneficial for some MD patients with complex I deficiency, but further clinical studies are needed.<sup>25</sup> However, to date, no specific dietary manipulation has shown consistent benefit.

Looking forward to an effective treatment, one of the choice therapies, well tolerated and safe, is coenzyme Q10, possibly in its reduced form (ubiquinol, at least 300 mg a day), alone or combined with other metabolites (i.e. riboflavin,  $\alpha$ -lipoic acid, or creatine).<sup>7</sup>

### Can we treat oxidative stress?

A plausible pathogenic mechanism in MD is oxidative stress.<sup>7</sup> Protein oxidative damage is increased in patients with mitochondrial myopathy, especially in the more severely affected ones, supporting the tight links between mitochondrial dysfunction and oxidative stress.<sup>26</sup>

There has been interest in cysteine donors. Glutathione deficiency has been reported in MD, and the biosynthesis of glutathione depends on cysteine availability. Our group performed a crossover double-blind trial to evaluate if 30-day supplementation with a whey-based cysteine donor could modify oxidative stress markers.<sup>26</sup> Clinical scores and lactate at rest and after exercise were not modified by the 30-day supplementation, but the cysteine donor markedly improved oxidative stress markers as well as total glutathione levels.<sup>26</sup>

Cysteine is also required for the 2-thiomodification of mitochondrial tRNAs.<sup>27</sup> Interestingly, supplementation with L-cysteine could partially rescue the mitochondrial translation defect in fibroblasts carrying the m.8344A>G or the m.3243A>G mutation.<sup>27</sup> N-acetyl-cysteine had some beneficial effects on mitochondrial translation in fibroblasts from patients with autosomal recessive mutations in genes affecting mitochondrial translation.<sup>27</sup> Further studies are needed to explore the full potential of cysteine supplementation as a treatment for patients with MD.

A recent preclinical study evaluated the therapeutic potential of cysteamine bitartrate in three MD models spanning three evolutionarily distinct species (*Caenorhabditis elegans*, zebrafish, human cells).<sup>28</sup> Cysteamine bitartrate is an approved therapy for nephropathic cystinosis, potentially able to enhance

glutathione biosynthesis.<sup>28</sup> It could improve mitochondrial function in these models, even if not through modulation of total glutathione levels. Careful consideration is required to evaluate safe and effective dosages of cysteamine bitartrate for clinical trials.<sup>28</sup>

Furthermore, treatment of complex I deficient cells with JP4-039, a novel mitochondrion-targeted reactive oxygen species (ROS) scavenger, could decrease levels of superoxide and increase basal and maximal respiratory rate.<sup>29</sup>

Another study showed that the mitochondrial ROS-redox modulator KH176 could have some beneficial effects in a mammalian model of Leigh disease.<sup>30</sup> Leigh disease is a devastating pediatric neurometabolic disorder, for which an effective treatment is not available. It has also been suggested that the antioxidant EPI-743 may have some beneficial effects in MD, especially in Leigh disease. This molecule is supposed to enhance the biosynthesis of glutathione.<sup>31</sup> Definitive results of well-conducted clinical trials are not available yet.<sup>32</sup>

Interestingly, exercise may also protect mitochondria against oxidative damage, at least in animal models.<sup>33</sup> Chronic normobaric hypoxic conditions were also able to prevent the development of the disease in a mouse model of MD (*Ndufs4* KO).<sup>34</sup> Median life span was remarkably increased from 58 to 270 days, whereas hyperoxia had the opposite effect. The detailed mechanisms are still unclear, but oxidative stress is likely involved.<sup>34</sup>

It must be noted that mitochondrial ROS production at physiological concentrations appears to be integral to cell signaling and the modulation of gene expression.<sup>2</sup> Further studies are strongly needed to clarify if oxidative stress may represent a therapeutic target. In the meanwhile, antioxidants should be used with caution in MD as well as in other diseases linked to mitochondrial dysfunction.

## The example of primary coenzyme Q10 deficiency

Primary coenzyme Q10 deficiency is a very rare autosomal recessive MD.<sup>35</sup> It is due to mutations in coenzyme Q10 biosynthetic genes, and it has been mainly associated with the infantile multisystemic and cerebellar ataxic phenotypes.<sup>7</sup>

Coenzyme Q10 deficiency is a treatable condition; therefore it is important to consider this diagnostic possibility.<sup>7</sup> An early supplementation with high-dose coenzyme Q10 could radically change the natural history. Patients with all phenotypes of coenzyme Q10 deficiency have shown improvement with oral coenzyme Q10 administration. CNS symptoms are only partially ameliorated, likely because of irreversible neural damage before treatment and because of poor penetration of coenzyme Q10 across the blood–brain barrier.<sup>7</sup>

Primary coenzyme Q10 deficiency should be treated with coenzyme Q10 supplementation and not with short-tail Q10

analogs, such as idebenone, because these analogs cannot replace coenzyme Q10 in the respiratory chain.<sup>7</sup> Patients were given various doses of coenzyme Q10 ranging from 90 to 2000 mg daily. The beneficial effects of exogenous coenzyme Q10 require high doses and long-term administration.<sup>7</sup>

A therapeutic attempt with coenzyme Q10 should be considered in every MD patient with myopathy and/or ataxia in whom coenzyme Q10 deficiency has not been excluded.<sup>7</sup>

Although some human coenzyme Q10 deficiencies respond to dietary supplementation with coenzyme Q10, in general the uptake and assimilation of this very hydrophobic lipid is inefficient.<sup>36</sup> Simple coenzyme Q10 derivatives may act to enhance biosynthesis of coenzyme Q10 or may bypass certain deficient steps in the coenzyme Q10 biosynthetic pathway.<sup>36</sup> For instance,  $\beta$ -resorcylic acid was reported to be more effective than coenzyme Q10 in a mouse model of coenzyme Q10 deficiency with an encephalopathic phenotype.<sup>37</sup>  $\beta$ -resorcylic acid is an analog of the coenzyme Q10 precursor 4-hydroxybenzoic acid.<sup>37</sup> Further studies are needed to evaluate if its use could be considered in human coenzyme Q10 deficiencies.

## Idebenone in Leber disease

Idebenone is a coenzyme Q10 short-tail analog with a more favorable pharmacokinetic profile.<sup>7</sup> In recent years, it has been largely used in Leber hereditary optic neuropathy. Leber disease is a mtDNA-related MD mainly involving the optic nerves. It may lead to blindness, especially in male smokers.<sup>5</sup>

Idebenone could increase ATP production and reduce ROS levels in Leber fibroblasts, but the effect was cell specific and partial. Other Q10 analogs had more variable effects.<sup>38</sup>

A 24-week multicenter, double-blind, randomized, placebo-controlled trial with idebenone was performed on 85 patients with Leber hereditary optic neuropathy.<sup>39</sup> No difference in visual recovery was reported, but *post hoc* analysis suggested that patients with discordant visual acuities were the most likely to benefit from this treatment.<sup>39</sup>

A large retrospective study has also suggested that idebenone treatment could significantly improve the frequency of visual recovery in Leber patients, possibly changing the natural history of this disease.<sup>40</sup>

Further studies are still needed to definitively confirm these preliminary observations.

Other medications, including elamipretide (a small molecule that binds to cardiolipin and stabilizes the inner mitochondrial membrane) and curcumin, are currently under study for Leber disease.<sup>31</sup> Furthermore, a number of clinical trials are investigating the efficacy of viral-based gene therapy for Leber patients harboring the m.11778G>A mtDNA mutation,<sup>41</sup> but definitive results are not available to date.

## The particular case of mitochondrial neurogastrointestinal encephalomyopathy

Mitochondrial neurogastrointestinal encephalomyopathy (MNGIE) is an autosomal recessive disease that usually leads to death in early adulthood.<sup>42</sup> It is caused by mutations of thymidine phosphorylase (*TYMP*) gene that lead to deoxynucleoside accumulation in plasma and tissues and subsequently to mitochondrial dysfunction. MNGIE is characterized by gastrointestinal dysmotility with cachexia, leukodystrophy, ocular myopathy, and peripheral neuropathy.<sup>42</sup>

Therapeutic options aimed at counterbalance the nucleoside accumulation are available, and in fact MNGIE is one of the few MD susceptible to effective treatment.<sup>42</sup> Attempted strategies include platelets infusion to restore *TYMP* activity, continuous ambulatory peritoneal dialysis, hemodialysis, enzyme replacement therapy using recombinant *Escherichia coli* thymidine phosphorylase entrapped in encapsulated autologous erythrocytes (EE-TP), and liver transplantation.<sup>43–49</sup>

To date, the treatment of choice is allogeneic hematopoietic stem cell transplantation (HSCT). HSCT is available only for very selected patients<sup>50</sup> and may restore the thymidine phosphorylase enzyme function and improve the clinical picture, even if the complication rate is very high in these patients. Hematopoietic gene therapy may represent a promising permanent approach for MNGIE in the future.<sup>42</sup>

Unfortunately, these approaches are not useful for most MDs that are not caused by the accumulation of noxious metabolites.

## Innovative metabolic strategies

Targeting mitochondrial biogenesis is a possibility for treating MD.<sup>51</sup> Medications able to enhance mitochondrial biogenesis, and therefore oxidative metabolism, have been defined exercise-mimetic drugs,<sup>51</sup> because exercise has been shown to promote mitochondrial biogenesis. In fact, exercise may provide a safe therapeutic option to patients with mitochondrial myopathy, benefiting the biochemical and clinical endpoints.<sup>51</sup>

Fibrates, which are peroxisomal proliferator-activated receptors (PPAR) pan-agonists, have been suggested as a potential therapeutic agents to correct oxidative phosphorylation in MD.<sup>52</sup> Bezafibrate was able to delay the accumulation of cytochrome c (COX)-negative fibers and multiple mtDNA deletions in Twinkle-mutated mice.<sup>53</sup>

More recently, these positive preliminary findings on fibrates were not confirmed in three recombinant mouse models characterized by defective COX activity.<sup>54</sup> Furthermore, bezafibrate showed adverse effects in these models (myopathy, marked hepatomegaly, and body weight loss).<sup>54</sup> Another

strategy appeared more promising. Two principal systems, PPARs and PGC-1 $\alpha$  (PPAR- $\gamma$  coactivator-1 $\alpha$ ), are supposed to increase biogenesis of mitochondria and thus may enhance aerobic metabolism acting on partially overlapping sets of genes. AMP-dependent kinase (AMPK) is a key regulator of PGC-1 $\alpha$ . Double-recombinant animal models overexpressing PGC-1 $\alpha$  in skeletal muscle on a *Surf1* knockout background showed marked induction of mitochondrial biogenesis and increased mitochondrial respiratory activities, including COX.<sup>54</sup> Interestingly, treatment with the AMPK agonist AICAR (5-aminoimidazole-4-carboxamide ribonucleoside) led to partial correction of COX deficiency in COX-deficient models, with significant motor improvement in the knockout/knockin *Sco2* mouse. Therefore, pharmacological activation of mitochondrial biogenesis may be a potentially useful approach.<sup>54</sup>

Another recent study screened 10 different compounds using fibroblasts derived from seven patients with complex I deficiency, harboring different mutations. AICAR was found to be the most effective molecule, improving growth and ATP content and decreasing oxidative stress; AICAR also activated AMPK, thus increasing mitochondrial biogenesis.<sup>55</sup> Therefore, further studies on this drug are warranted. As AICAR may have effects on different homeostatic and regulatory pathways,<sup>56</sup> further studies are needed to establish its safety and translatability to humans.<sup>55</sup>

A study recently appeared in *Nature*<sup>57</sup> showed that *de novo* nicotinamide adenine dinucleotide (NAD<sup>+</sup>) synthesis enhanced mitochondrial function. NAD<sup>+</sup> production could be pharmacologically increased, ultimately improving mitochondrial function.<sup>57</sup>

Nicotinamide riboside, a form of vitamin B<sub>3</sub> and a natural precursor of NAD<sup>+</sup>, may be a promising treatment strategy for MD.<sup>58</sup> It could prevent both the development and progression of mitochondrial myopathy in a mouse model.<sup>58</sup> The treatment also resulted in a significant induction of mitochondrial biogenesis and oxidative metabolism.<sup>58</sup> A NAD<sup>+</sup> precursor compound named acipimox may also be tested, but data on MD are not available yet.<sup>59</sup>

Very recently, the administration of febuxostat and inosine was suggested as another potential strategy to enhance cellular ATP levels in MD patients, but further studies are needed.<sup>60</sup>

Nucleoside supplementation may be beneficial in mtDNA depletion syndromes by substrate enhancement of the purine nucleoside salvage pathway, thus improving liver pathology in these young patients. Studies are also needed to confirm this hypothesis.<sup>61</sup>

Deoxynucleoside substrate enhancement is a novel therapy, which may ameliorate thymidine kinase 2 (TK2) deficiency in patients.<sup>62</sup> TK2 is required for mtDNA maintenance, and autosomal recessive TK2 gene mutations cause depletion and multiple deletions of mtDNA that manifest predominantly

as a progressive myopathy, which usually begins in childhood.<sup>63</sup> A recent open-label clinical study investigated deoxynucleoside administration to 16 TK2-deficient patients. This study indicated favorable side-effect profiles and clinical efficacy of deoxynucleoside therapies for TK2 deficiency.<sup>63</sup>

Another recent suggestion is the potential possibility of bypassing blockade of the complex III–IV segment of the respiratory chain by alternative oxidase (AOX). AOX is a nonmammalian enzyme that shunts electrons from quinols directly to oxygen, thus restoring electron flow upstream of complex III.<sup>64</sup> AOX transgene could prevent lethal mitochondrial cardiomyopathy in a mouse model of complex III deficiency,<sup>64</sup> and it may hypothetically represent a potential therapeutic strategy for MD patients with this specific biochemical defect. A similar study in another MD model (*COX15* knockout mouse)<sup>65</sup> led to decreased life span and a substantial worsening of the myopathy. Decreased ROS production led to impaired AMPK/PGC-1 $\alpha$  signaling and compromised the compensatory responses.<sup>65</sup> Noticeably, the antioxidant N-acetylcysteine had a similar effect, decreasing the life span in these mice.<sup>65</sup> This study highlighted the benefits of ROS signaling and the potential hazards of indiscriminate antioxidant treatment. However, the idea of bypassing blockade of the respiratory chain is intriguing and further studies may be warranted.

Furthermore, the bromodomain inhibitor I-BET525762A could remodel the mitochondrial proteome, thus increasing levels and activity of the respiratory chain and leading to rescue of the bioenergetic defects and cell death caused by blockade of Complex I.<sup>66</sup>

A recent pilot study reported that the bacterial protein CNF1 could boost the mitochondrial ATP production in cells derived from a patient with the m.8344A>G mitochondrial mutation, likely acting on the actin cytoskeleton.<sup>67</sup> Further studies are needed to clarify if the cytoskeleton may represent a pharmacological target against MD.

Targeting the regulation of lipid dynamics may represent another possibility. For instance the drug, elamipretide, targets the lipid cardiolipin and may stabilize the mitochondrial lipid structure.<sup>68</sup> This drug has been assessed in mitochondrial myopathy patients and could improve exercise intolerance and walking distance when administered at the higher dose.<sup>68</sup> Further studies are ongoing.

Mitochondria are dynamic organelles undergoing frequent fission and fusion cycles that result in major morphological changes. OPA1 protein is involved in these modifications, and therefore autosomal dominant optic atrophy (ADOA), which is due to *OPA1* mutations, has been defined as a disorder of mitochondrial fusion. Active fusion is important to maintain mitochondrial integrity, to reduce ROS production and to activate mtDNA replication to elevate mtDNA copy number. Pharmacological modulation of mitochondrial fusion (e.g. by

the hydroxylamine derivative BGP-15) may represent a potential therapeutic strategy for ADOA and possibly other MD, but further studies are needed.<sup>69</sup>

Mitochonic acid (MA-5) could increase ATP and improve mitochondrial function in MD fibroblasts, and it prolonged the life span of the disease model 'Mitomouse'. MA-5 was reported to facilitate mitochondrial ATP production and to modulate oxidative stress.<sup>70</sup> Furthermore, it may reduce mitochondrial fragmentation and restore crista shape and dynamics.<sup>70</sup> Further studies are needed to clarify if MA-5 may be useful in MD patients.

Rapamycin is a mTORC1 inhibitor that had beneficial effects in all the models of MD tested, independently of the genetic lesion.<sup>71</sup> Given the broad effects of mTOR inhibition, including immunosuppressive action, side effects are a major concern for the use of these compounds. Rapamycin analogs with a better safety profile are being developed. Similarly, rapamycin administration is not a valid therapeutic strategy for every case of MD, and appropriate investigations on patients are warranted.<sup>71</sup>

Mitochondrial RNA translation may also represent a potential treatment target, but further studies are needed.<sup>73</sup>

## Gene therapy and germline therapy

Future approaches to MD may also include gene therapy and germline therapy. For MD due to mutations in nuclear genes, the problems are not different from those valid for other Mendelian disorders, including choice of appropriate vectors, delivery to the affected tissues, and potential immunological reactions.<sup>7</sup> The problems are even more complex for mtDNA-related diseases because of heteroplasmy and polyplasmmy.

To achieve mitochondrial gene therapy, developing a mitochondrial transgene expression system that produces therapeutic proteins in mitochondria is essential. For instance, mitochondrial transfection may be achieved using a liposome-based carrier for delivering a cargo to mitochondria via membrane fusion.<sup>74</sup>

Mitochondria-targeted endonucleases may provide an alternative strategy for treating mtDNA-related MD via targeted destruction of the mutant mtDNA and induction of heteroplasmic shifting.<sup>75</sup> This was successfully achieved in cell lines harboring a high proportion of the m.3243A>G mtDNA mutation, suggesting a potential new approach for the treatment of MD as well as the prevention of germline transmission of mutant mtDNA.<sup>75</sup> Two recent studies showed the feasibility of this approach *in vivo* as well.<sup>76,77</sup>

Interestingly, it has been observed that mtDNA could be efficiently replaced in mature primate oocytes by spindle–chromosomal complex transfer from one egg to an enucleated, mitochondrial-replete egg.<sup>78</sup> The reconstructed

oocytes with the replaced mtDNA were capable of supporting normal fertilization, embryo development and could produce healthy offspring. Genetic analysis confirmed that nuclear DNA in the infants born so far originated from the spindle donors whereas mtDNA came from the cytoplasm donors.<sup>78</sup>

In future, similar approaches may offer a reproductive option to prevent mutated mtDNA transmission in MD families. A comprehensive discussion of these options goes beyond the aims of this paper. Recent approaches and advances in the field of mitochondrial replacement therapies are excellently discussed elsewhere.<sup>79–81</sup>

## Treatment of specific clinical features

### Stroke-like episodes

'Mitochondrial encephalopathy with lactic acidosis and stroke-like episodes' (MELAS) is a mitochondrial phenotype mainly associated with the m.3243A>G mutation of the mtDNA.<sup>82</sup> A mitochondrial stroke-like episode is a subacute, evolving brain syndrome linked to seizure activity in genetically determined MD. Unfortunately, experience with the treatment of stroke-like episodes derives only from case report or small series. 'MELAS' denote patients with histological, biochemical, and/or molecular evidence of MD who experience stroke-like episodes.<sup>82</sup>

A double-blind, placebo-controlled, randomized, crossover trial of the lactate-lowering dichloroacetate in MELAS patients harboring the m.3243A>G mutation had to be terminated because of peripheral nerve toxicity, which overshadowed any potential beneficial effect.<sup>83</sup>

Several evidences point toward a benefit of arginine, a nitric oxide precursor, to both prevent and reduce the severity of stroke-like episodes in MELAS patients.<sup>84</sup>

Administration of L-arginine, a nitric oxide precursor, to MELAS patients has been suggested to reduce neurological symptoms due to stroke-like episodes and to prevent recurrences.<sup>85</sup> Two years of supplementation with oral L-arginine could significantly improve endothelial function.<sup>86</sup> L-arginine administration within 30 minutes of a stroke-like episode could decrease frequency and severity of stroke-like episodes.<sup>87</sup> L-arginine is usually applied intravenously in a dosage of 0.4–0.5 g/kg.<sup>7</sup>

Recently, the efficacy and safety of oral and intravenous L-arginine was studied in an open-label trial on pediatric and adult patients with MELAS.<sup>88</sup> Oral L-arginine (0.3–0.5 g/kg/day) could extend the interictal phase and decrease the incidence and severity of stroke-like episodes. L-arginine was well tolerated, and no treatment-related adverse events were reported.<sup>88</sup> Arginine is safe in pediatric MD patients as well as in adults.<sup>89</sup>

However, we do not advocate the use of intravenous L-arginine as part of the routine treatment of stroke-like episodes. There is no robust scientific evidence supporting its use. Placebo-controlled studies of oral and intravenous L-arginine in patients with MELAS are strongly needed.

Until specific drugs will be available, the treatment of choice of a stroke-like episode is based on antiepileptic drugs (excluding valproic acid) and other supportive treatments. During stroke-like episodes, steroids may be safely used and could play some benefit.<sup>90</sup>

### Seizures

Treatment of CNS manifestations, including epilepsy and status epilepticus, in patients with MD does not differ from treatment of the same conditions in the general population.<sup>91</sup> However, mitochondrion-toxic drugs, such as the antiepileptic drug valproate, mainly in patients with *POLG* disease, should be avoided.<sup>7</sup>

Because of its antimitochondrial effects,<sup>7</sup> valproate may also have detrimental effects in patients with MELAS<sup>92</sup> and in other MD. For instance, children with Alpers syndrome (a severe hepatoencephalopathy due to nuclear mutations in the *POLG* gene) are at high risk of death from status epilepticus or liver failure, if exposed to valproate.<sup>93</sup>

Finally, carbonic anhydrase inhibitors topiramate and zonisamide have been reported to cause severe metabolic acidosis in some patients,<sup>94–96</sup> and if used in subjects with MD, acidosis may require monitoring.<sup>7</sup> Lacosamide can be safe and potentially effective.<sup>97</sup>

Ketogenic diet may be attempted for treating intractable epilepsy, especially in the treatment of MD due to complex I deficiency.<sup>98</sup> Carefully monitoring these patients is critical.

Regarding status epilepticus, attempted treatments include antiepileptic drugs, anesthetic agents, high-dose steroids, and others, with variable success.<sup>99</sup> The outcome of mitochondrial status epilepticus is poor.<sup>99</sup> Perampanel has been recently suggested as a potential therapeutic alternative in refractory status epilepticus associated with MELAS.<sup>100</sup> Intravenous magnesium as anticonvulsant therapy, adapted from practice in eclampsia, was attempted in two unrelated, previously healthy teenage girls who developed refractory status epilepticus.<sup>101</sup> In both cases, *POLG* mutations were found. Magnesium therapy resulted in clinical and neurophysiological improvement and extubation of both patients.<sup>101</sup>

### Myoclonus

Myoclonus is one of the typical features of MERRF syndrome, defined as 'myoclonic epilepsy with ragged red fibers', or maybe more correctly 'myoclonic encephalopathy with ragged red fibers'.<sup>102</sup> MERRF is more frequently, but not invariably, associated to the m.8344A>G mtDNA mutation.<sup>103</sup>

Although valproic acid may be beneficial, it should be used with caution.<sup>7</sup> Levetiracetam seems to be a safe and effective alternative<sup>104</sup> and may possibly have some mitochondrioprotective effect.<sup>105</sup>

## Other CNS manifestations

As a general rule, treatment of CNS signs and symptoms (e.g. parkinsonism, mood disorders, migraine) in patients with MD does not differ from treatment of the same conditions in general population.<sup>91</sup> This is also true for most neuromuscular and internist features such as endocrine abnormalities, cardiomyopathy with heart failure, renal insufficiency, and respiratory insufficiency. Hardly accessible to treatment are cerebellar manifestations ('mitochondrial ataxias').<sup>106</sup>

For instance, mitochondrial parkinsonism does not have distinctive features from idiopathic Parkinson disease.<sup>107</sup> Reduced striatal dopamine uptake and good response to levodopa or dopamine agonists have been well documented. Levodopa-induced dyskinesias and motor fluctuations may also occur.<sup>107</sup>

Regarding antidepressants, an adult MELAS patient with major depressive disorder was reported to show an excellent response to the selective serotonin-norepinephrine reuptake inhibitor medication duloxetine.<sup>108</sup>

## Be careful with these drugs

The potential mitochondrial toxicity of some antiepileptics, with particular reference to valproic acid, has been discussed earlier. Valproate should be avoided, because of the risk of liver failure and status epilepticus, especially in *POLG* mutation carriers.<sup>7</sup> However, for most of the antiepileptic drugs, there is not *in vivo* evidence of severe mitotoxicity and, therefore, they may be used safely.

Furthermore, because of their prokaryotic origins, mitochondria are susceptible to the antibiotics targeting the bacterial ribosome. This vulnerability is increased by specific mtDNA mutations. Among them, aminoglycosides should be avoided, whenever possible, in patients with known or suspected MD.<sup>7</sup> In fact, they may even lead to deafness if administered to patients with some mtDNA mutations, especially the m.1555A>G.<sup>109</sup> If aminoglycosides need to be used, it is strongly recommended to screen for mitochondrial DNA mutations before treatment. One MELAS patient developed severe lactic acidosis shortly after the beginning of linezolid treatment.<sup>110</sup> Linezolid induces lactic acidosis by inhibiting mitochondrial ribosomal protein synthesis and thus disrupting crucial mitochondrial functions.<sup>111</sup> The risk of side effects of use of chloramphenicol is low but serious, namely acute hepatitis or blood dyscrasia. Therefore, preferably alternatives should be prescribed, especially in patients with mitochondrial genetic defects affecting liver and/or bone marrow, such as mtDNA depletion syndromes or Pearson

**Table 3. Potentially mitochondrion-toxic agents. Mitochondrial patients needing one of these treatments should be carefully monitored, clinically and by laboratory exams, including creatine kinase and lactate (for further details, see text).**

Aminoglycosides
Antiretrovirals
Clevudine
Chloramphenicol
Dichloroacetate
Isoflurane
Linezolid
Metformin
Propofol
Statins
Topiramate
Valproic acid
Zonisamide

syndrome. MD patients needing one of these antibiotics should be carefully monitored.<sup>7</sup>

Metformin is an effective agent for reducing hyperglycemia in type 2 diabetes mellitus. Lactic acidosis can develop during metformin intoxication, possibly because of mitochondrial dysfunction.<sup>112</sup> Therefore, this drug should be given to patients with MD with monitoring lactate levels.

Statins are currently the most effective medications for reducing low-density lipoprotein (LDL) cholesterol concentrations. The most frequent side effects of statins are myopathic complaints ranging from myalgia to fatal rhabdomyolysis. Statins block production of farnesyl pyrophosphate, an intermediate of coenzyme Q10 biosynthesis.<sup>113</sup> Muscle coenzyme Q10 levels and respiratory chain activities were significantly reduced by simvastatin, but not by atorvastatin.<sup>114</sup> Simvastatin, but not atorvastatin, reduced muscle mtDNA levels.<sup>115</sup> Patients with MD may be prone to develop statin myopathy.<sup>116</sup> Statin-induced myopathy was linked to mitochondrial complex III inhibition.<sup>117</sup> Therefore, statins should be used with caution and strictly monitoring creatine kinase levels in MD patients.<sup>7</sup> Atorvastatin seems to be preferable to simvastatin, but convincing studies are pending.

In some instances,  $\beta$ -blockers, including metoprolol, were reported to aggravate an underlying MD.<sup>118</sup> However, we do not have enough clinical data to suggest to avoid  $\beta$ -blockers in MD. Other drugs potentially interfering with mitochondrial function, including some antiviral drugs (antiretrovirals and clevudine), are extensively discussed elsewhere.<sup>7</sup> Long-term oral antiviral therapy with nucleoside analogs for hepatitis B

can induce chronic oxidative damage to mtDNA resulting in qualitative mtDNA abnormalities and toxic myopathy.<sup>119</sup>

Many other medications are associated with muscle toxicity (e.g. corticosteroids, amiodarone, chloroquine/hydroxychloroquine, colchicine, vincristine, interferon- $\alpha$ , some neuroleptics, cyclosporine, and other cytotoxic drugs)<sup>7</sup> and/or can cause metabolic acidosis (e.g. spironolactone, prostaglandin inhibitors, triamterene, amiloride, trimethoprim, pentamidine).<sup>7</sup> Inhibition of the AMPK pathway, and thus of ATP production, is one of the proposed mechanisms possibly mediating mitochondrial toxicity due to chemotherapeutics such as doxorubicin, trastuzumab, and sunitinib.<sup>120</sup> Bortezomib was also reported to cause a symptomatic myopathy with storage of lipid droplets together with mitochondrial abnormalities in a patient with multiple myeloma.<sup>121</sup> Further studies are also needed to establish the safety of the chemotherapeutics used for HSCT in MNGIE patients, such as ciclosporin and melphalan (see earlier discussion).

Patients with MD needing one of these treatments (Table 3) should be carefully monitored, clinically and by laboratory exams (including creatine kinase and lactate).

## Anesthesia and MD

Patients with MD have received a variety of general and regional anesthetic regimens with no adverse consequences.<sup>7</sup> Perioperative complications of lactic acidosis were reported more in inhalation anesthesia than intravenous anesthesia.<sup>122</sup> The use of lactate-free intravenous fluids with dextrose should be considered in all MD patients to prevent lactic acidosis. Potentially mitochondrion-toxic agents include isoflurane and propofol.<sup>7</sup> Furthermore, children with MD have increased sensitivity to volatile anesthetics.<sup>123</sup> MD patients may be at higher risk of developing propofol infusion syndrome.<sup>124</sup> Mitochondrial patients are generally considered at increased risk of adverse surgical outcome compared to the general population, but adequate longitudinal studies are still lacking.<sup>125</sup> Common malignant hyperthermia precautions are strongly recommended.<sup>126</sup> Furthermore, mitochondrial patients should be carefully monitored in the perioperative period.<sup>127</sup> Normothermia and normoglycemia should be maintained and metabolic stresses avoided (including prolonged fasting).

## Conclusion and perspectives

The symptomatic treatment of the various clinical features (e.g. epilepsy, migraine, parkinsonism, diabetes, heart failure, renal insufficiency, respiratory insufficiency) in subjects with MD does not substantially differ from the treatment of the same conditions in nonmitochondrial patients. However, well-known mitochondrion-toxic drugs such as valproate, aminoglycosides, or linezolid should be used with extreme caution.<sup>7</sup>

Patient care standards for MD are described in detail elsewhere.<sup>128</sup> These include the use of digital hearing aids or cochlear implantation for hearing loss; pacemaker and/

or implantable defibrillator for arrhythmias; deep brain stimulation for untreatable movement disorders, etc.<sup>128</sup> When needed, renal or cardiac transplantation should be offered after careful multidisciplinary review.<sup>128</sup> Furthermore, MD patients should be offered age-appropriate vaccination.<sup>128</sup>

Appropriate care standards also include enteral nutrition and/or assisted ventilation in MD patients with advanced disease.

When the administration of a mitochondrion-toxic drug is needed, a careful clinical and laboratory follow-up is warranted to precociously recognize and treat possible side effects, such as rhabdomyolysis, lactic acidosis, hepatic failure, and others.<sup>7</sup> Furthermore, in MD patients, possible causes of metabolic acidosis should be timely recognized and treated (e.g. sepsis, diabetic ketoacidosis, alcohol intoxications, other intoxications, diarrhea, prolonged fasting, etc.). The physician should not be scared of treating mitochondrial patients when needed. For instance, a not-treated bacterial infection may be more dangerous than the use of appropriate drugs, because infections may precipitate metabolic crises in MD patients.<sup>7</sup>

Alongside the traditional biochemical and histochemical assays, the molecular diagnosis of MD has been revolutionized by the introduction of next-generation sequencing (NGS). In recent years, high-throughput 'omics' techniques capable of detecting differences in a multitude of molecular constituents in organisms (including metabolomics, proteomics, transcriptomics, genomics, and epigenomics) accompanied by sophisticated bioinformatics tools have revealed new details about mitochondrial function and dysfunctions.<sup>2</sup>

The application of patient-derived induced pluripotent stem cells may allow the development of innovative, more effective cellular models.<sup>129</sup> Their use may benefit the search for effective treatments against MD.

Future approaches to these pathologies could include germline therapy and gene therapy.<sup>7</sup> Mitochondrial gene therapy seems to be a valuable and promising strategy to treat MD.<sup>130</sup> In future, these approaches may offer a reproductive option to prevent mtDNA disease transmission in affected families.

The use of sensitive and valid endpoints is essential to test the effectiveness of potential treatments.<sup>131</sup> A set of recommended outcome measures to be implemented in clinical studies has been identified.<sup>132</sup> The development of new biomarkers to facilitate clinical development of promising new therapies is a critical issue.<sup>133</sup> Furthermore, innovative neuroimaging techniques may in future serve as surrogate biomarkers in trials investigating therapeutic options in MD.<sup>134</sup>

Multicenter collaboration is needed for rare diseases, and a cooperative effort of the centers with specific expertise in mitochondrial medicine is essential for the advancement of the treatment options. In some countries, including Italy, this collaboration is an established fact and it appears timely for large, multicenter, well-controlled clinical trials.<sup>7</sup>

There is still a strong need for more effective therapies, but mitochondrial medicine has entered the evidence-based era.

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