HHS Public Access

Author manuscript

Mol Genet Metab. Author manuscript; available in PMC 2018 November 01.

Published in final edited form as:

Mol Genet Metab. 2017 November ; 122(3): 1–9. doi:10.1016/j.ymgme.2017.09.009.

Therapies for mitochondrial diseases and current clinical trials

Ayman W. El-Hattab[a], **Ana Maria Zarante**[b], **Mohammed Almannai**[c,d], and **Fernando Scaglia**[c,d,*]

[a]Division of Clinical Genetics and Metabolic Disorders, Pediatrics Department, Tawam Hospital, Al-Ain, United Arab Emirates

[b]Instituto de Ortopedia Infantil Roosevelt, Bogota, Colombia

[c]Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA

[d]Texas Children's Hospital, Houston, TX, USA

Abstract

Mitochondrial diseases are a clinically and genetically heterogeneous group of disorders that result from dysfunction of the mitochondrial oxidative phosphorylation due to molecular defects in genes encoding mitochondrial proteins. Despite the advances in molecular and biochemical methodologies leading to better understanding of the etiology and mechanism of these diseases, there are still no satisfactory therapies available for mitochondrial disorders. Treatment for mitochondrial diseases remains largely symptomatic and does not significantly alter the course of the disease. Based on limited number of clinical trials, several agents aiming at enhancing mitochondrial function or treating the consequences of mitochondrial dysfunction have been used. Several agents are currently being evaluated for mitochondrial diseases. Therapeutic strategies for mitochondrial diseases include the use of agents enhancing electron transfer chain function (coenzyme Q_{10}, idebenone, ribo-flavin, dichloroacetate, and thiamine), agents acting as energy buffer (creatine), antioxidants (vitamin C, vitamin E, lipoic acid, cysteine donors, and EPI-743), amino acids restoring nitric oxide production (arginine and citrulline), cardiolipin protector (elamipretide), agents enhancing mitochondrial biogenesis (bezafibrate, epicatechin, and RTA 408), nucleotide bypass therapy, liver transplantation, and gene therapy. Although, there is a lack of curative therapies for mitochondrial disorders at the current time, the increased number of clinical research evaluating agents that target different aspects of mitochondrial dysfunction is promising and is expected to generate more therapeutic options for these diseases in the future.

Keywords

Mitochondrial diseases; Arginine; Citrulline; RP103; EPI-743; Elamipretide; Bezafibrate; Epicatechin; RTA 408

*Corresponding author at: Department of Molecular and Human Genetics, Baylor College of Medicine, One Baylor Plaza, MS BCM225, Houston, TX 77030, USA. fscaglia@bcm.edu (F. Scaglia).

1. Introduction

Mitochondrial diseases are a clinically and genetically heterogeneous group of disorders that result from dysfunction of the mitochondrial electron transport chain (ETC) and oxidative phosphorylation due to pathogenic variants in mitochondrial DNA (mtDNA) or nuclear DNA (nDNA) encoding mitochondrial proteins [1,2]. In addition to a wide range of cellular perturbations such as aberrant calcium homeostasis, excessive reactive oxygen species (ROS) production, and dysregulated apoptosis, dysfunctional mitochondria are unable to generate sufficient energy to meet the needs of various organs, particularly these with high energy demand, including the nervous system, skeletal and cardiac muscles, kidneys, liver, and endocrine system. Energy deficiency in various organs results in multiorgan dysfunction leading to the variable manifestations observed in mitochondrial diseases including cognitive impairment, epilepsy, cardiac and skeletal myopathies, nephropathies, hepatopathies, and endocrinopathies [3,4].

With the advances in molecular and biochemical methodologies, the etiology and mechanism underlying these disorders have been better understood and the number of identified mitochondrial diseases has increased. However, advances in treating these conditions have been lagging behind. Thus, the treatment for the vast majority of mitochondrial diseases remains mainly symptomatic and does not significantly alter the course of the disease. With a limited base of evidence and little data from randomized clinical trials, the treatment of mitochondrial diseases is still largely anecdotal [5]. Over the past two decades, multiple agents have been evaluated through open-label and randomized clinical trials.

In this review, we present the current therapeutic options for mitochondrial diseases and existing clinical trials for treatment of mitochondrial diseases. Symptomatic treatment, exercise, and diet for mitochondrial diseases are presented first. Subsequently, different agents aiming to enhance mitochondrial function and treat the consequences of mitochondrial dysfunction are presented. These treatment include: 1) agents enhancing ETC function (coenzyme Q_{10} (CoQ_{10}), idebenone, riboflavin, dichloroacetate, and thiamine), 2) energy buffer (creatine), 3) antioxidants (vitamin C, vitamin E, lipoic acid, cysteine donors, and EPI-743), 4) amino acids restoring nitric oxide production (arginine and citrulline), 5) cardiolipin protector (elamipretide), 6) agents enhancing mitochondrial biogenesis (bezafibrate, epicatechin, and RTA 408), and 7) nucleotide bypass therapy (Table 1). Finally, gene therapy and liver transplantation for mitochondrial diseases are discussed.

2. Symptomatic treatment, exercise, and diet

Examples of symptomatic treatment in mitochondrial diseases include physical therapy for hypotonia and motor delays, hearing aids or cochlear implants for hearing loss, slow infusion of sodium bicarbonate during acute exacerbation of lactic acidosis, cardiac pacing for rhythm abnormalities, surgical correction of ptosis, administration of pancreatic enzymes for exocrine pancreatic dysfunction, and treating diabetes with diet, sulfonylurea, and insulin [1,3].

Exercise can be helpful for mitochondrial disease. Lack of exercise in healthy individuals leads to an overall reduction in mitochondrial ETC activity, whereas endurance training can improve ETC activity and resistance training can stimulate the incorporation of satellite cells into existing muscle fibers. It has been suggested that resistance training in individuals with mtDNA mutations can lead to an overall reduction in the proportion of mutated mtDNA, as satellite cells contain a low or negligible amount of mutated mtDNA. Endurance training might also improve the mitochondrial function. Furthermore, exercise can result in mitochondrial proliferation through inducing PGC-1α, which is the master transcription regulator that stimulates mitochondrial biogenesis [6,7].

No specific dietary manipulation has shown consistent benefit for individuals with mitochondrial disorders. On the other hand, secondary mitochondrial dysfunction was observed with extreme malnutrition and individuals with mitochondrial diseases may have altered caloric needs and inadequate caloric intake because of feeding difficulties. As optimizing the number and quality of calories was shown to improve oxidative phosphorylation capacity in individuals with mitochondrial diseases, a comprehensive nutritional evaluation and support are needed for these individuals [8–10]. A high-lipid, low-carbohydrate diet has been suggested because glucose oxidation is largely aerobic and a high-carbohydrate diet can be metabolically challenging in individuals with impaired oxidative phosphorylation [2]. Ketogenic diet can be helpful in pyruvate dehydrogenase deficiency as it has been shown that affected individuals who either had the diet initiated earlier in life or who were placed on greater carbohydrate restriction had increased longevity and improved mental development [11]. Hypoglycemia, which occurs occasionally in mitochondrial diseases, can be managed with fasting avoidance by frequent or continuous feedings. In addition, uncooked cornstarch may reduce symptomatic hypoglycemia in a limited number of conditions associated with liver dysfunction [12].

3. Increasing electron transfer chain function

Some mitochondrial treatments aim to enhance ETC function by either augmenting ETC components and hence enhancing electron transfer (CoQ_{10}, idebenone, and riboflavin) or increasing ETC substrate availability (dichloroacetate, and thiamine).

CoQ_{10} (ubiquinone) is an integral component of ETC, shuttling electrons from complexes I and II to complex III. Primary CoQ_{10} deficiency results from molecular defects in enzymes involved in CoQ_{10} biosynthesis [13]. CoQ_{10} supplementation results in restoring the electron flow and an improvement in clinical manifestations associated with CoQ_{10} deficiency [14–16]. It was suggested that CoQ_{10} supplementation to individuals with other mitochondrial diseases would improve the efficacy of electron transfer through ETC. Some case reports and open-label studies suggested that CoQ_{10} treatment may have beneficial effects in individuals with mitochondrial diseases. However, a randomized double-blinded study only showed minor effects of CoQ_{10} supplementation on cycle exercise aerobic capacity and post-exercise lactate and did not show any effect on other clinically relevant variables such as strength or resting lactate [17]. Therefore, apart from CoQ_{10} deficiency, this supplementation has limited benefits on other mitochondrial diseases. CoQ_{10} (ubiquinone) dose is 5 to 30 mg/kg/day divided in two doses. Reduced CoQ_{10} (ubiquinol) is three to five

times better absorbed when compared with the oxidized form of CoQ_{10} (ubiquinone) and its dosage is 2 to 8 mg/kg/day divided in two doses. There could be a potential effect for ubiquinol, however, there have been no proper studies assessing the effect of ubiquinol in individuals with mitochondrial diseases [10].

Idebenone is an analog of CoQ_{10} but has a higher efficacy and more favorable pharmacokinetic profile. Idebenone has been used in treating Leber hereditary optic neuropathy (LHON). A randomized, placebo-controlled, double-blind clinical trial evaluating the effect of idebenone in LHON did not show statistically significant difference in the primary outcome, which was the best recovery in visual acuity. However, post hoc analysis suggested that idebenone can protect from further vision loss particularly in individuals with discordant visual acuities [18]. Interestingly, a follow up report demonstrated that the beneficial effect from idebenone treatment during the previous study persisted despite discontinuation of therapy [19]. A separate randomized, placebo-controlled, double-blind clinical trial demonstrated that idebenone treatment in individuals with LHON protected from loss of color vision and the effect of idebenone was most prominent in individuals with discordant visual acuity [20]. Doses range from 30 to 300 mg/dose three times daily [5].

Riboflavin (vitamin B2) serves as a flavoprotein precursor. Therefore, it is a key building block in complexes I and II, and a cofactor in several other key enzymatic reactions involving fatty acid β-oxidation and the Krebs cycle. Multiple acyl-CoA dehydrogenase deficiency, typically caused by electron-transport flavoprotein dehydrogenase (*ETFDH*) gene mutations, is an inborn error of metabolism involving several of these enzymatic reactions, and riboflavin supplementation can ameliorate its symptoms and slow its progression [21]. Some non-randomized studies have shown that treatment with riboflavin is also helpful in treating mitochondrial diseases with complexes I and II deficiencies [22,23]. Riboflavin supplementation is of particular usefulness for individuals with complex I deficiency due to acyl-CoA dehydrogenase-9 (ACAD9) deficiency. ACAD9 is a FAD-containing flavoprotein. Riboflavin supplementation improves symptoms in individuals with ACAD9 deficiency and results in increased complex I activity in their fibroblasts. Therefore, ACAD9 deficiency has been considered as a potentially treatable mitochondrial disorder [24,25]. The dose of riboflavin is usually 50–200 mg/day divided in 2–3 doses [5,26,27].

Dichloroacetate activates the pyruvate dehydrogenase enzyme by inhibiting the activity of pyruvate dehydrogenase kinase, that normally phosphorylates and inhibits the enzyme [28]. By preventing the in-activation of the pyruvate dehydrogenase enzyme, dichloroacetate increases the catabolism of pyruvate to acetyl-CoA which enters the Krebs cycle generating reduced cofactors (NADH and $FADH_2$) used to generate ATP through the oxidative phosphorylation. Increased pyruvate catabolism will prevent its accumulation and alleviate lactic acidosis observed in many mitochondrial diseases. Dichloroacetate has been used to treat lactic acidosis in mitochondrial diseases based on some individual case reports [10]. A controlled study demonstrated that dichloroacetate treatment was well tolerated and blunted the postprandial lactate increase in children with congenital lactic acidosis. However, it did not improve neurologic or other measures of clinical outcome [29]. Another open-label study showed reduction of the baseline lactate level with dichloroacetate treatment. In

addition, a standardized neurologic inventory showed stabilization or improvement concluding that dichloroacetate appears to provide at least temporary benefits for some mitochondrial diseases [30]. Although dichloroacetate is generally well-tolerated in individuals with congenital lactic acidosis and it can be effective in alleviating lactic acidosis in mitochondrial diseases [31], it can cause peripheral neuropathy in individuals with MELAS syndrome [32]. Dichloroacetate doses range from 10 to 25 mg/kg/day divided in two doses [10,26].

Thiamine (vitamin B1) also enhances pyruvate dehydrogenase activity, thereby increasing pyruvate oxidation and reduced cofactors (NADH and $FADH_2$) generation. Thiamine has been used in mitochondrial disorders individually or in combination with other agents. Thiamine supplementation in a family with MELAS syndrome and thiamine deficiency was reported to improve lactic acidosis and myopathy [33]. The use of thiamine, along with CoQ_{10}, carnitine, and vitamins C and E, resulted in a marked clinical recovery in an individual with adult-onset Leigh disease presenting as severe brainstem encephalopathy of subacute onset [34]. The dose of thiamine is 10 mg/kg/day for children and 100–1000 mg/day for adults [26]. Thiamine transporter-2 deficiency is caused by mutations in the *SLC19A3* gene. This disease manifests with encephalopathy, features of Leigh syndrome on neuroimaging, and lactic acidosis. Higher doses of thiamine (20 mg/kg/day) are needed to treat the neurological and biochemical abnormalities in this disease [35].

4. Energy buffer

Creatine combines with phosphate in the mitochondria to form phosphocreatine that serves as a source of high energy phosphate released during anaerobic metabolism. Therefore, creatine acts as an intracellular buffer for ATP and as an energy shuttle for the movement of high energy phosphates from mitochondrial sites of production to cytoplasmic sites of utilization. The highest concentrations of creatine are found in tissues with high energy demands, including muscle and brain [10]. A reduction in phosphocreatine measured in muscle tissue was shown in individuals with mitochondrial myopathies, and lower brain creatine in MR spectroscopy was demonstrated in individuals with mitochondrial encephalopathies [36,37]. Creatine monohydrate supplementation can improve exercise capacity in some individuals with mitochondrial myopathies [38]. A randomized cross-over study showed that creatine monohydrate increased the strength of high-intensity anaerobic and aerobic type activities in individuals with mitochondrial diseases but had no apparent effects upon lower intensity aerobic activities [39]. The doses are 100–300 mg/kg/day in children and 2–10 g/day in adults divided in three doses [10,26,27]

5. Antioxidants

During oxidative phosphorylation, a small part of oxygen is partially reduced and converted to ROS (superoxide and hydrogen peroxide). Although ROS play a role in diverse signaling pathways, they can be toxic to the cell. Under normal conditions, ROS can be scavenged by various enzymes, including the mitochondrial superoxide dismutase and glutathione peroxidase [40]. In addition to insufficient energy production resulting from impaired oxidative phosphorylation, ROS generation is enhanced with ETC blockade [41]. ROS can

irreversibly modify many cellular macromolecules. Increased ROS production in mitochondrial diseases can result in protein, lipid, and DNA damage which can potentially lead to further cellular damage and dysfunction [40].

Some agents used to treat mitochondrial diseases are antioxidants that alleviate the toxic effect of excessive ROS produced in these diseases. Vitamin C and vitamin E are occasionally used in individuals with mitochondrial diseases in combination with other agents. A limited number of case reports and small studies has noticed modest benefits for these supplements in some individuals with mitochondrial diseases [42].

Lipoic acid is an essential factor for pyruvate dehydrogenase and ketoglutarate dehydrogenase. It also acts as a potent antioxidant that can decrease oxidative stress markers [43]. Lipoic acid is often administered with other antioxidants to individuals with mitochondrial diseases [10]. A randomized, double-blind, placebo-controlled, crossover study showed that a combination therapy of creatine mono-hydrate, CoQ_{10}, and lipoic acid was beneficial in individuals with mitochondrial diseases in lowering lactate in plasma and oxidative stress markers measured in urine, as well as attenuation of the decline in muscle strength [44]. The usual doses are 25 mg/kg/day or 300–600 mg/day [26,27].

Glutathione is a major intracellular antioxidant and its synthesis depends on the availability of cysteine. As glutathione deficiency can occur in mitochondrial diseases, supplementation with cysteine donors can potentially restore glutathione levels and therefore enhance elimination of excessive ROS in mitochondrial diseases [5]. A whey-based oral supplement with abundance of glutamylcysteine is a cysteine donor that was tried in double-blind cross-over study. This supplement did not modify lactate concentration, clinical scale, or quality of life, but significantly reduced oxidative stress markers in the studied individuals with mitochondrial diseases [45].

N-acetylcysteine also increases cysteine availability and glutathione synthesis, and has been tried in ethylmalonic encephalopathy [46]. Ethylmalonic encephalopathy is caused by mutations in the *ETHE1* gene which encodes a mitochondrial sulfur dioxygenase necessary for the detoxification of sulfide. Hydrogen sulfide, which is synthesized endogenously and formed by intestinal bacteria, accumulates in ethylmalonic encephalopathy and inhibits cytochrome *c* oxidase and short-chain fatty acid oxidation leading to mitochondrial dysfunction and ethylmalonic aciduria, respectively. A combination therapy of oral metronidazole to reduce intestinal bacterial sulfide production and *N*-acetylcysteine to replenish reduced glutathione which can buffer the sulfide has been shown to result in clinical improvement in children with ethylmalonic encephalopathy. In addition, ethylmalonic acid in urine and blood dropped and neuroimaging showed improvement of brain atrophy and leukodystrophy with this combined supplementation [47]. Furthermore, it has been suggested that cysteine or *N*-acetylcysteine supplementation may be a potential treatment for selected subgroups of individuals with mitochondrial translation deficiencies. Supplementation with cysteine was shown to partially rescue the mitochondrial translation defect *in vitro* in fibroblasts of individuals carrying the m.3243A > G and m.8344A > G mutations affecting mitochondrial transfer RNAs. Whereas, *N*-acetylcysteine was shown to have a beneficial effect on mitochondrial translation in fibroblast from individuals with

defects in TRMU and MTO1, which are nDNA encoded proteins involved in mitochondrial transfer RNA modification and are thus important for mitochondrial translation [48].

Cysteamine is an amino thiol that is used to treat cystinosis, a lysosomal storage disease which results from defects in the lysosomal cystine transporter leading to the accumulation of cystine in cells. Cysteamine enters the lysosomes where it breaks the disulfide bond of cystine leading to the formation of cysteine and cysteine-cysteamine disulfide. Cysteine then can leave the lysosome through the cysteine transporter. Cysteamine can increase intracellular glutathione levels by increasing the cysteine available for reduced glutathione synthesis [49]. Therefore, cysteamine is a potential therapy to restore reduced glutathione levels in mitochondrial diseases. RP103, cysteamine bitartrate delayed-release capsules, has been studied in an open-label, dose-escalating study to assess its safety, tolerability, efficacy, pharmacokinetics, and pharmacodynamics in children with mitochondrial diseases. The primary outcome measure evaluated focused on changes in Newcastle Pediatric Mitochondrial Disease Scale Score. Other metabolites are measured as secondary outcome measures including glutathione, glutathione disulfide, and lactate. This study has been completed and data analysis is proceeding (https://clinicaltrials.gov/ct2/show/NCT02023866).

EPI-743 is a *para*-benzoquinone analog with improved pharmacologic properties and therapeutic efficacy. It has a potent cellular protective activity against excessive ROS that targets repletion of reduced intracellular glutathione [50,51]. As ROS damage has been implicated in the pathogenesis of mitochondrial disease and glutathione deficiency was reported in mitochondrial diseases [52], EPI-743 has been evaluated in mitochondrial diseases and initial studies have shown promising results. Initially, an open-label study showed that EPI-743 modified disease progression and resulted in clinical improvement in the majority of studied subjects with heterogeneous mitochondrial diseases who were at risk for progressing to end-of-life care within 90 days [51]. Another open-label study in children with Leigh syndrome showed that EPI-743 supplementation resulted in stabilization and reversal of disease progression [53]. Additionally, a marked increase in reduced glutathione was observed following EPI-743 therapy in individuals with Leigh disease [54]. In another open-label study, EPI-743 arrested disease progression and reverse vision loss in most studied subjects with LHON [55]. Several studies are currently evaluating EPI-743 in mitochondrial diseases. One study evaluates the long-term safety and efficacy of EPI-743 in children with Leigh disease (https://clinicaltrials.gov/ct2/show/NCT02352896). Another randomized, double blind, placebo-controlled, cross-over study has finished recruiting children with mitochondrial diseases and it is in its extension phase. The primary outcome measure is the effect of EPI-743 on quality of life. Secondary outcome measures include various biochemical, imaging, and clinical abnormalities (https://clinicaltrials.gov/ct2/show/NCT01642056).

6. Restoration of nitric oxide production

In addition to reduced energy production, there is growing evidence that nitric oxide (NO) deficiency occurs in mitochondrial diseases and can play a major role in the pathogenesis of several complications observed in mitochondrial diseases including stroke-like episodes,

myopathy, diabetes, and lactic acidosis [56–60]. The amino acids arginine and citrulline act as NO precursors and can be used to restore NO production. Therefore, arginine and citrulline can have therapeutic utility in treating NO deficiency-related manifestations of mitochondrial diseases [59,60].

It has been shown that individuals with MELAS (mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes) syndrome have lower concentrations of NO metabolites (nitrite and nitrate) during stroke-like episodes [57,61]. Furthermore, the NO synthesis rate, measured by stable isotope infusion techniques, has been shown to be lower in children and adults with MELAS syndrome who are not experiencing acute stroke-like episodes [59,60]. Another study has demonstrated that sarcoplasmic NOS activity is reduced in cytochrome *c* oxidase deficient fibers of muscle biopsies obtained from individuals with chronic progressive external ophthalmoplegia (CPEO), mitochondrial myopathy, and MELAS syndrome [56]. Flow-mediated vasodilation (FMD), which is a function of NO synthesized by endothelial cells in response to re-perfusion, was found to be impaired in individuals with mitochondrial myopathy, MELAS, MERRF (myoclonic epilepsy with ragged red fibers), MIDD (maternally inherited diabetes and deafness), and CPEO, providing further evidence of NO deficiency in mitochondrial diseases [58,62]. NO deficiency in mitochondrial disorders is believed to be multifactorial in origin due to impaired NO production and postproduction NO sequestration [63].

Arginine supplementation has only been evaluated in MELAS syndrome. As both arginine and citrulline act as NO precursors; it has been initially proposed that their administration can result in increased NO availability and hence have therapeutic benefits in stroke-like episodes in MELAS syndrome [64]. It was then shown in open-label trials that the administration of intravenous arginine to subjects with MELAS syndrome during stroke-like episodes led to improvement in the clinical symptoms associated with these episodes, and oral arginine supplementation at the interictal phase decreased frequency and severity of stroke-like episodes [57,61,62]. The therapeutic effect of arginine in stroke-like episodes in MELAS is proposed to be due to increased NO availability leading to improving intra-cerebral vasodilation and blood flow. This has been supported by the demonstration that arginine supplementation to subjects with MELAS resulted in increased NO production rate [60] and improved FMD [62]. The use of oral arginine as maintenance therapy and intravenous arginine during the stroke-like episodes have become commonly used in treating individuals with MELAS syndrome. During the acute stroke-like episode, it is recommended to give a bolus of intravenous arginine (500 mg/kg for children or 10 g/m^2 body surface area for adults) within 3 h of symptom onset followed by the administration of similar dosage of intravenous arginine as continuous infusion over 24 h for the next 3–5 days. Once an individual with MELAS has the first stroke-like episode, arginine should be administered prophylactically to reduce the risk of recurrent stroke-like episodes. A daily dose of 150 to 300 mg/kg/day oral arginine in 3 divided doses is recommended [65,66].

The clinical effects of citrulline administration in mitochondrial diseases have not been studied, however, stable isotope studies have demonstrated that, similar to arginine supplementation, citrulline supplementation can increase NO production in children and adults with MELAS syndrome [59,60]. Interestingly, citrulline supplementation induced a

greater increase in the NO synthesis rate than that associated with arginine supplementation, indicating that citrulline is a more effective NO precursor than arginine [59,60]. This can be due to the superiority of citrulline in raising plasma and intracellular arginine levels, leading to more arginine availability for NO synthesis [63].

Increasing NO availability with arginine or citrulline supplementation can potentially improve perfusion in all microvasculature compartments. Therefore, the effect of arginine and citrulline supplementation may not be limited to improving stroke-like episodes, but may also lead to improvement in other clinical features of mitochondrial diseases, including muscle weakness, exercise intolerance, diabetes, and lactic acidosis. Interestingly, arginine and citrulline supplementation has been reported to result in a reduction in plasma alanine and lactate concentrations, suggesting that such supplementation may improve lactic acidemia in MELAS syndrome by increasing NO production and improving perfusion and oxygen delivery [60,67]. Based on the finding that citrulline supplementation can result in a higher NO production than arginine supplementation, it has been proposed that citrulline may have a better therapeutic effect than arginine [63]. Clinical research evaluating the effect of arginine and citrulline in mitochondrial diseases is very limited, with the effect of arginine on stroke-like episodes in MELAS syndrome being the only field studied by uncontrolled open label clinical studies. Therefore, additional measures of the clinical effects of arginine and citrulline supplementation on different aspects of mitochondrial diseases are warranted to determine the potential therapeutic effect of such supplementation [63].

Currently, a randomized crossover study is conducted to assess the effect of arginine and citrulline supplementation on endothelial dysfunction in children with mitochondrial diseases. The primary outcome measure is the changes in reactive hyperemic index, which reflects endothelial function, after arginine or citrulline supplementation (https://clinicaltrials.gov/ct2/show/NCT02809170).

7. Cardiolipin protection

Cardiolipin is a unique phospholipid that is only expressed on the mitochondrial inner membrane. It plays important structural roles in modulating the mitochondrial inner membrane curvature leading to cristae formation and organizing the ETC complexes into super-complexes to facilitate optimal electron transfer and energy production. Cardiolipin also plays a role in anchoring cytochrome *c* to the inner membrane facilitating electron transfer from complex III to complex IV [68]. Moreover, cardiolipin plays an important role in cell death when it is oxidized. Cardiolipin is particularly vulnerable to oxidative damage because of its high content of unsaturated fatty acids and its location near the site of ROS production. The oxidation of cardiolipin causes disruption of its microdomains on the inner membrane leading to loss of membrane curvature and cristae. Cardiolipin oxidation also disturbs supercomplexes and causes cytochrome *c* to be detached from the inner membrane resulting in impaired oxidative phosphorylation and energy production [69]. Furthermore, oxidized cardiolipin synergizes with calcium to induce opening of the mitochondrial permeability transition pores leading to the release of cytochrome *c* and other proapoptotic

proteins into the cytosol where they trigger the caspase cascade and cell death by apoptosis [70].

Elamipretide is a tetrapeptide that binds selectively to cardiolipin via electrostatic and hydrophobic interactions, and protects it from oxidation. By inhibiting cardiolipin oxidation, elamipretide protects mitochondrial cristae, promotes oxidative phosphorylation, and inhibit mitochondrial permeability transition pores opening. This agent has been tried in conditions associated with mitochondrial damage including aging, ischemia, and heart failure [70]. A multi-center, randomized, double-blind, multiple ascending dose, placebo-controlled study for individuals with mitochondrial myopathy has been completed. Primary endpoints of this phase 2 trial were safety, tolerability, and improvement in distance walked on 6-min walk test (https://clinicaltrials.gov/ct2/show/NCT02367014). Based on promising results in the 6-min walk test, an observational study with evaluation of natural history of the disease leading to a phase 3 trial is being conducted.

8. Enhancing mitochondrial biogenesis

Through mitochondrial biogenesis cells increase their mitochondrial population in response to increased energy demand. This is driven by the activation of PCG-1α which is a transcriptional coactivator that regulates mitochondrial biogenesis. The activation of PCG-1α can be targeted in a number of ways. AMP-activated protein kinase (AMPK), which is stimulated by elevated levels of AMP, activates PCG-1α by phosphorylation. NAD$^+$-dependent deacetylase sirtuin-1 (SRT1), which is stimulated by elevated NAD$^+$, activates PCG-1α by deacetylation. The PCG-1α expression and activity is also controlled by the peroxi-some proliferative-activated receptors (PPARs). The activation of PCG-1α by factors such as decreased ATP and increased NAH$^+$ results in increased transcription of nDNA-encoded mitochondrial genes and therefore mitochondrial biogenesis, increasing the amount of ATP produced [71,72]. As the activation of PCG-1α can potentially alleviate the ATP deficiency in individuals with mitochondrial diseases, agents that promote mitochondrial biogenesis are another active area of research related to mitochondrial therapeutics.

Bezafibrate, commonly used to treat dyslipidemia, is a PPAR agonist that can affect the PPAR- PCG-1α pathway and induce mitochondrial biogenesis. The administration of bezafibrate to a murine model with cytochrome *c* oxidase deficiency resulted in improvement of phenotype and activation of mitochondrial biogenesis leading to increasing mitochondrial mass, oxidative phosphorylation capacity, and energy production [73]. On the other hand, studying the effect of bezafibrate on *POLG* mutant mice did not show improvement in muscle function or lifespan [74]. An open-label trial showed that treatment of bezafibrate in individuals with carnitine palmitoyltransferase 2 (CPT2) deficiency resulted in increased long-chain fatty acid oxidation, improved physical activity, and a decline in muscle pain [75]. Currently a study is conducted to assess the effect of bezafibrate in adults with mitochondrial myopathy. The primary outcome measure is the change in ETC activity, with multiple other biochemical and clinical secondary outcome measures (https://clinicaltrials.gov/ct2/show/NCT02398201).

Resveratrol is a natural compound that is present in the skin of red grapes and is capable of activating sirtuins, including SIRT1. Studies on healthy and diseased mice models showed the effect of resveratrol on induction of mitochondrial biogenesis via PGC-1α activation [76]. Similarly, SRT2104 is an activator of SIRT1 that exhibits high efficacy and safety. SRT2104 administration to elderly individuals showed trends for more rapid adenosine diphosphate and phosphocreatine recoveries after exercise, assessed by MR spectrometry, consistent with increased mitochondrial oxidative phosphorylation [77]. These agents have potential therapeutic utility in mitochondrial diseases. However, they have not yet been tried in mitochondrial diseases.

AICAR (aminoimidazole carboxamide ribonucleoside), approved drug for hyperinsulinemia, increases the activity of AMPK which activates PCG-1α by phosphorylation. AICAR was shown to result in increased oxidative phosphorylation and mitochondrial biogenesis in several mouse models with mitochondrial diseases [76]. In addition, this agent resulted in increased mitochondrial biogenesis and ATP production in fibroblasts from individuals with complex I deficiency [78]. This agent has not yet been tried in individuals with mitochondrial diseases.

Epicatechin is found in high concentration in dark chocolate and it has mitochondrial biogenic properties. Mice fed with epicatechin demonstrated improved exercise performance and fatigue resistance, and evidence for enhanced mitochondrial biogenesis including increased ETC proteins, mitofilin, porin, mitochondrial transcription factor A (Tfam), mitochondrial volume, and cristae abundance [79]. Studying cultured bovine coronary artery endothelial cells showed that epicatechin is capable of stimulating mitochondrial function as assessed by citrate synthase activity as well as induction of structural and oxidative phosphorylation protein levels [80]. An open label study evaluating the safety and efficacy of epicatechin in subjects with Friedreich ataxia is currently being conducted. The primary outcome measures are changes in a clinical rating scale composite score and changes from baseline in ventricular hypertrophy as shown on cardiac MRI (https://clinicaltrials.gov/ct2/show/NCT02660112).

RTA 408 is a synthetic isoprenoid that activates the nuclear respiratory factor 2 (Nrf 2). Nrf2 is a downstream effector of PGC-1α and activator of mitochondrial biogenesis [72]. RTA 408 was found to elevate levels of glutathione and increase mitochondrial biogenesis in mouse models of amyotrophic lateral sclerosis [81]. MOTOR study is an interventional randomized study that is currently recruiting adults with mitochondrial myopathy and has two parts. The first part is a randomized, placebo-controlled, double-blind, dose-escalation study to evaluate the safety of RTA 408 at various doses in individuals with mitochondrial myopathies. The second part is a randomized, placebo-controlled, double-blind, parallel study to evaluate the safety, efficacy, and pharmacodynamics of up to 2 dose levels of RTA 408 in individuals with mitochondrial myopathies. The primary outcome measure is the change of peak workload during exercise testing. The secondary outcome measure is the change in distance walked during a 6-min walk test (https://clinicaltrials.gov/ct2/show/NCT02255422).

9. Nucleoside bypass therapy

The maintenance of mtDNA depends on a number of nDNA-encoded proteins that function in mtDNA synthesis or maintaining a balanced supply of nucleotides that is achieved by nucleotide recycling inside the mitochondria and import from the cytosol. Mitochondrial DNA maintenance defects are a group of diseases caused by mutations in the nuclear genes involved in mtDNA maintenance resulting in impaired mtDNA synthesis leading to quantitative (mtDNA depletion) and qualitative (multiple mtDNA deletions) defects in mtDNA [82]. Thymidine kinase 2 (TK2) is a key enzyme in the mitochondrial pyrimidine nucleotide salvage pathway. TK2 deficiency causes a depletion of mitochondrial nucleotides leading to impairment of mtDNA synthesis and a myopathic mtDNA depletion syndrome [83]. In Tk2 deficient mice, deoxythymidine and deoxycytidine were found to delayed disease onset, prolonged life span of Tk2-deficient mice, and restored mtDNA content as well as ETC complexes activities and levels [84]. Several individuals with TK2 deficiency have received nucleoside bypass therapy, mostly in Italy and Spain. However, a proper clinical trial has not been conducted thus far. Foreseeable this same strategy may be used to treat mtDNA depletion caused by deoxyguanosine kinase (DGK) deficiency which is the key enzyme in mitochondrial purine nucleoside salvage pathway.

10. Liver and stem cell transplantation

Liver transplantation has been performed for individuals with hepatocerebral mtDNA depletion syndromes which frequently progress to liver failure. However, liver transplantation in mitochondrial hepatopathy remains controversial, largely because of the multi-organ involvement [85]. Although, liver transplantation is not recommended in children with Alpers-Huttenlocher syndrome because liver transplantation does not change the rapid progression of the neurological manifestations, liver transplantation in adults with other *POLG*-related mtDNA depletion syndromes who have an acceptable quality of life may be beneficial [86]. Survival after liver transplantation for DGK-related mtDNA depletion syndromes is lower than survival after liver transplantation for other diseases, however, a significant fraction of affected individuals survived more than five years despite initial neurological abnormalities. The decision to perform liver transplantation for individuals with this disease remains difficult because neurological manifestations may occur or worsen after liver transplantation despite their absence before transplantation [87]. Liver transplantation outcome for *MPV17*-related mtDNA depletion syndrome has not been satisfactory, because approximately half of the children transplanted died in the post-transplantation period because of multiorgan failure or sepsis [88].

Thymidine phosphorylase deficiency due to mutations in the *TYMP* gene results in mitochondrial neurogastrointestinal encephalopathy (MNGIE) disease. Thymidine phosphorylase catalyzes the conversion of thymidine and deoxyuridine to thymine and uracil, respectively. Thymidine phosphorylase deficiency results in the accumulation of cytosolic thymidine which results in an imbalance of the cytosolic nucleotide pool. Because the mitochondrial nucleotide pool relies, in part, on nucleotides imported from the cytosol, an imbalanced cytosolic nucleotide pool can lead to an imbalanced mitochondrial nucleotide pool that can impair mtDNA synthesis in MNGIE disease. Allogeneic stem cell

transplantation for MNGIE disease was shown to produce nearly complete biochemical correction of the thymidine and deoxyuridine imbalances in blood and some clinical improvements. However, because of the high morbidity and mortality, this procedure is not generally recommended to treat individuals with this disease [89].

11. Gene therapy

Most advancements in gene therapy for mitochondrial diseases have been achieved for Leber hereditary optic neuropathy (LHON). Approximately 70% of individuals with LHON have pathogenic variants in the mtDNA gene encoding subunit 4 of complex 4 (*MT-ND4*). An adeno-associated virus (AAV) can carry the mitochondrial gene and the viral capsid VP2 can be fused with a mitochondrial targeting sequence to target the AAV to the mitochondria and achieve *ND4* expression. Expression of the wild type *ND4* in cells with the *ND4* mutation led to restoration of defective ATP synthesis. Furthermore, with injection into the rodent eye, human *MT-ND4* DNA levels in mitochondria reached 80% of its mouse homolog. The construct was expressed in most inner retinal neurons, and it also suppressed visual loss and optic atrophy induced by a mutant *ND4* homolog [90]. The initial results of unilateral intravitreally injected AAV vector into the eyes of 5 blind individuals with LHON and the m. 11778G > A mutation showed unchanged visual acuity in 3 and increased acuity in 2 individuals after 3 months [91]. Additional results for this study have been recently published and showed an improvement in average acuity for 12 subjects with LHON and bilateral visual loss who received unilateral treatment. This study also demonstrated the safety of allotropic gene therapy for LHON [92].

12. Conclusions

Several agents aiming at enhancing mitochondrial function or treating the consequences of mitochondrial dysfunction have been used. The use of these agents is based on limited number of studies and can be beneficial only in some mitochondrial disease. Therefore, treatment of mitochondrial diseases remains largely symptomatic and does not significantly alter the course of the disease. Although, there is a lack of therapies for mitochondrial disorders at the current time, the increased number of clinical research evaluating agents target different aspects of mitochondrial dysfunction is promising and is expected to generate more therapeutic options for these diseases in the future. Agents currently being evaluated for mitochondrial diseases include antioxidants (RP103 and EPI-743), cardiolipin protector (elamipretide), and mitochondrial biogenesis enhancers (bezafibrate, epicatechin, and RTA 408). Gene therapy has shown promising results in treating LHON.

References

1. Chinnery, PF. Mitochondrial disorders overview. In: Pagon, RA.Adam, MP.Ardinger, HH.Wallace, SE.Amemiya, A.Bean, LJ., et al., editors. GeneReviews(®) [Internet]. University of Washington; Seattle, Seattle (WA): 2014. Available from: http://www.ncbi.nlm.nih.gov/books/NBK1224/ [cited 2017 Jan 30]
2. Munnich, A., Rötig, A., Rio, M. Defects of the respiratory chain, Inborn Metabolic Diseases Diagnosis and Treatment. 5th. Springer; Berlin: 2012. p. 223-238.
3. El-Hattab AW, Scaglia F. Mitochondrial cytopathies. Cell Calcium. Sep; 2016 60(3):199–206. [PubMed: 26996063]

4. Ylikallio E, Suomalainen A. Mechanisms of mitochondrial diseases. Ann Med. 2012 Feb; 44(1):41–59. [PubMed: 21806499]
5. Avula S, Parikh S, Demarest S, Kurz J, Gropman A. Treatment of mitochondrial disorders. Curr Treat Options Neurol. 2014 Jun.16(6):292. [PubMed: 24700433]
6. Taivassalo T, Shoubridge EA, Chen J, Kennaway NG, DiMauro S, Arnold DL, et al. Aerobic conditioning in patients with mitochondrial myopathies: physiological, biochemical, and genetic effects. Ann Neurol. 2001 Aug; 50(2):133–141. [PubMed: 11506394]
7. Kang C, Li Ji L. Role of PGC-1α signaling in skeletal muscle health and disease. Ann N Y Acad Sci. 2012 Oct.1271:110–117. [PubMed: 23050972]
8. Morava E, Rodenburg R, van Essen HZ, De Vries M, Smeitink J. Dietary intervention and oxidative phosphorylation capacity. J Inherit Metab Dis. 2006 Aug.29(4):589.
9. Wortmann SB, Zweers-van Essen H, Rodenburg RJT, van den Heuvel LP, de Vries MC, Rasmussen-Conrad E, et al. Mitochondrial energy production correlates with the age-related BMI. Pediatr Res. 2009 Jan; 65(1):103–108. [PubMed: 19096353]
10. Parikh S, Saneto R, Falk MJ, Anselm I, Cohen BH, Haas R, et al. A modern approach to the treatment of mitochondrial disease. Curr Treat Options Neurol. 2009 Nov; 11(6):414–430. [PubMed: 19891905]
11. Wexler ID, Hemalatha SG, McConnell J, Buist NR, Dahl HH, Berry SA, et al. Outcome of pyruvate dehydrogenase deficiency treated with ketogenic diets. Studies in patients with identical mutations. Neurology. 1997 Dec; 49(6):1655–1661. [PubMed: 9409363]
12. El-Hattab AW, Scaglia F. Mitochondrial DNA depletion syndromes: review and updates of genetic basis, manifestations, and therapeutic options. Neurotherapeutics. 2013 Apr; 10(2):186–198. [PubMed: 23385875]
13. Potgieter M, Pretorius E, Pepper MS. Primary and secondary coenzyme Q10 deficiency: the role of therapeutic supplementation. Nutr Rev. 2013 Mar; 71(3):180–188. [PubMed: 23452285]
14. Di Giovanni S, Mirabella M, Spinazzola A, Crociani P, Silvestri G, Broccolini A, et al. Coenzyme Q10 reverses pathological phenotype and reduces apoptosis in familial CoQ10 deficiency. Neurology. 2001 Aug 14; 57(3):515–518. [PubMed: 11502923]
15. Rötig A, Appelkvist EL, Geromel V, Chretien D, Kadhom N, Edery P, et al. Quinone-responsive multiple respiratory-chain dysfunction due to widespread coenzyme Q10 deficiency. Lancet. 2000 Jul 29; 356(9227):391–395. [PubMed: 10972372]
16. Horvath R. Update on clinical aspects and treatment of selected vitamin-responsive disorders II (riboflavin and CoQ 10). J Inherit Metab Dis. 2012 Jul; 35(4):679–687. [PubMed: 22231380]
17. Glover EI, Martin J, Maher A, Thornhill RE, Moran GR, Tarnopolsky MA. A randomized trial of coenzyme Q10 in mitochondrial disorders. Muscle Nerve. 2010 Nov; 42(5):739–748. [PubMed: 20886510]
18. Klopstock T, Yu-Wai-Man P, Dimitriadis K, Rouleau J, Heck S, Bailie M, et al. A randomized placebo-controlled trial of idebenone in Leber's hereditary optic neuropathy. Brain. 2011 Sep; 134(Pt 9):2677–2686. [PubMed: 21788663]
19. Klopstock T, Metz G, Yu-Wai-Man P, Büchner B, Gallenmüller C, Bailie M, et al. Persistence of the treatment effect of idebenone in Leber's hereditary optic neuropathy. Brain. 2013 Feb.136(Pt 2):e230. [PubMed: 23388409]
20. Rudolph G, Dimitriadis K, Büchner B, Heck S, Al-Tamami J, Seidensticker F, et al. Effects of idebenone on color vision in patients with leber hereditary optic neuropathy. J Neuroophthalmol. 2013 Mar; 33(1):30–36. [PubMed: 23263355]
21. Olsen RKJ, Olpin SE, Andresen BS, Miedzybrodzka ZH, Pourfarzam M, Merinero B, et al. ETFDH mutations as a major cause of riboflavin-responsive multiple acyl-CoA dehydrogenation deficiency. Brain. 2007 Aug; 130(Pt 8):2045–2054. [PubMed: 17584774]
22. Bernsen PL, Gabreëls FJ, Ruitenbeek W, Hamburger HL. Treatment of complex I deficiency with riboflavin. J Neurol Sci. 1993 Sep; 118(2):181–187. [PubMed: 8229067]
23. Bugiani M, Lamantea E, Invernizzi F, Moroni I, Bizzi A, Zeviani M, et al. Effects of riboflavin in children with complex II deficiency. Brain and Development. 2006 Oct; 28(9):576–581. [PubMed: 16737791]

24. Garone C, Donati MA, Sacchini M, Garcia-Diaz B, Bruno C, Calvo S, et al. Mitochondrial encephalomyopathy due to a novel mutation in ACAD9. JAMA Neurol. 2013 Sep 1; 70(9):1177–1179. [PubMed: 23836383]
25. Haack TB, Danhauser K, Haberberger B, Hoser J, Strecker V, Boehm D, et al. Exome sequencing identifies ACAD9 mutations as a cause of complex I deficiency. Nat Genet. 2010 Dec; 42(12): 1131–1134. [PubMed: 21057504]
26. Alfadhel M, Al-Thihli K, Moubayed H, Eyaid W, Al-Jeraisy M. Drug treatment of inborn errors of metabolism: a systematic review. Arch Dis Child. 2013 Jun; 98(6):454–461. [PubMed: 23532493]
27. Scaglia F, Northrop JL. The mitochondrial myopathy encephalopathy, lactic acidosis with stroke-like episodes (MELAS) syndrome: a review of treatment options. CNS Drugs. 2006; 20(6):443–464. [PubMed: 16734497]
28. Kato M, Li J, Chuang JL, Chuang DT. Distinct structural mechanisms for inhibition of pyruvate dehydrogenase kinase isoforms by AZD7545, dichloroacetate, and radicicol. Structure. 2007 Aug; 15(8):992–1004. [PubMed: 17683942]
29. Stacpoole PW, Kerr DS, Barnes C, Bunch ST, Carney PR, Fennell EM, et al. Controlled clinical trial of dichloroacetate for treatment of congenital lactic acidosis in children. Pediatrics. 2006 May; 117(5):1519–1531. [PubMed: 16651305]
30. Barshop BA, Naviaux RK, McGowan KA, Levine F, Nyhan WL, Loupis-Geller A, et al. Chronic treatment of mitochondrial disease patients with dichloroacetate. Mol Genet Metab. 2004 Oct; 83(1–2):138–149. [PubMed: 15464428]
31. Abdelmalak M, Lew A, Ramezani R, Shroads AL, Coats BS, Langaee T, et al. Long-term safety of dichloroacetate in congenital lactic acidosis. Mol Genet Metab. 2013 Jun; 109(2):139–143. [PubMed: 23611579]
32. Kaufmann P, Engelstad K, Wei Y, Jhung S, Sano MC, Shungu DC, et al. Dichloroacetate causes toxic neuropathy in MELAS: a randomized, controlled clinical trial. Neurology. 2006 Feb 14; 66(3):324–330. [PubMed: 16476929]
33. Sato Y, Nakagawa M, Higuchi I, Osame M, Naito E, Oizumi K. Mitochondrial myopathy and familial thiamine deficiency. Muscle Nerve. 2000 Jul; 23(7):1069–1075. [PubMed: 10883001]
34. Mermigkis C, Bouloukaki I, Mastorodemos V, Plaitakis A, Alogdianakis V, Siafakas N, et al. Medical treatment with thiamine, coenzyme Q, vitamins E and C, and carnitine improved obstructive sleep apnea in an adult case of Leigh disease. Sleep Breath. 2013 Dec; 17(4):1129–1135. [PubMed: 23389837]
35. Pérez-Dueñas B, Serrano M, Rebollo M, Muchart J, Gargallo E, Dupuits C, et al. Reversible lactic acidosis in a newborn with thiamine transporter-2 deficiency. Pediatrics. 2013 May; 131(5):e1670–1675. [PubMed: 23589815]
36. Moroni I, Bugiani M, Bizzi A, Castelli G, Lamantea E, Uziel G. Cerebral white matter involvement in children with mitochondrial encephalopathies. Neuropediatrics. 2002 Apr; 33(2): 79–85. [PubMed: 12075488]
37. Tarnopolsky MA, Parise G. Direct measurement of high-energy phosphate compounds in patients with neuromuscular disease. Muscle Nerve. 1999 Sep; 22(9):1228–1233. [PubMed: 10454718]
38. Tarnopolsky MA. Creatine as a therapeutic strategy for myopathies. Amino Acids. 2011 May; 40(5):1397–1407. [PubMed: 21399918]
39. Tarnopolsky MA, Roy BD, MacDonald JR. A randomized, controlled trial of creatine monohydrate in patients with mitochondrial cytopathies. Muscle Nerve. 1997 Dec; 20(12):1502–1509. [PubMed: 9390662]
40. Balaban RS, Nemoto S, Finkel T. Mitochondria, oxidants, and aging. Cell. 2005 Feb 25; 120(4): 483–495. [PubMed: 15734681]
41. Smeitink JA, Zeviani M, Turnbull DM, Jacobs HT. Mitochondrial medicine: a metabolic perspective on the pathology of oxidative phosphorylation disorders. Cell Metab. 2006 Jan; 3(1):9–13. [PubMed: 16399500]
42. Enns GM. Treatment of mitochondrial disorders: antioxidants and beyond. J Child Neurol. 2014 Sep; 29(9):1235–1240. [PubMed: 24985754]

43. Marangon K, Devaraj S, Tirosh O, Packer L, Jialal I. Comparison of the effect of alpha-lipoic acid and alpha-tocopherol supplementation on measures of oxidative stress. Free Radic Biol Med. 1999 Nov; 27(9–10):1114–1121. [PubMed: 10569644]
44. Rodriguez MC, MacDonald JR, Mahoney DJ, Parise G, Beal MF, Tarnopolsky MA. Beneficial effects of creatine, CoQ10, and lipoic acid in mitochondrial disorders. Muscle Nerve. 2007 Feb; 35(2):235–242. [PubMed: 17080429]
45. Mancuso M, Orsucci D, Logerfo A, Rocchi A, Petrozzi L, Nesti C, et al. Oxidative stress biomarkers in mitochondrial myopathies, basally and after cysteine donor supplementation. J Neurol. 2010 May; 257(5):774–781. [PubMed: 19960200]
46. Medved I, Brown MJ, Bjorksten AR, Murphy KT, Petersen AC, Sostaric S, et al. *N*-acetylcysteine enhances muscle cysteine and glutathione availability and attenuates fatigue during prolonged exercise in endurance-trained individuals. J Appl Physiol. 2004 Oct; 97(4):1477–1485. [PubMed: 15194675]
47. Viscomi C, Burlina AB, Dweikat I, Savoiardo M, Lamperti C, Hildebrandt T, et al. Combined treatment with oral metronidazole and N-acetylcysteine is effective in ethylmalonic encephalopathy. Nat Med. 2010 Aug; 16(8):869–871. [PubMed: 20657580]
48. Bartsakoulia M, Müller JS, Gomez-Duran A, Yu-Wai-Man P, Boczonadi V, Horvath R. Cysteine supplementation may be beneficial in a subgroup of mitochondrial translation deficiencies. J Neuromuscul Dis. 2016; 3(3):363–379. [PubMed: 27854233]
49. Besouw M, Masereeuw R, van den Heuvel L, Levtchenko E. Cysteamine: an old drug with new potential. Drug Discov Today. 2013 Aug; 18(15–16):785–792. [PubMed: 23416144]
50. Shrader WD, Amagata A, Barnes A, Enns GM, Hinman A, Jankowski O, et al. α-Tocotrienol quinone modulates oxidative stress response and the biochemistry of aging. Bioorg Med Chem Lett. 2011 Jun 15; 21(12):3693–3698. [PubMed: 21600768]
51. Enns GM, Kinsman SL, Perlman SL, Spicer KM, Abdenur JE, Cohen BH, et al. Initial experience in the treatment of inherited mitochondrial disease with EPI-743. Mol Genet Metab. 2012 Jan; 105(1):91–102. [PubMed: 22115768]
52. Atkuri KR, Cowan TM, Kwan T, Ng A, Herzenberg LA, Herzenberg LA, et al. Inherited disorders affecting mitochondrial function are associated with glutathione deficiency and hypocitrullinemia. Proc Natl Acad Sci U S A. 2009 Mar 10; 106(10):3941–3945. [PubMed: 19223582]
53. Martinelli D, Catteruccia M, Piemonte F, Pastore A, Tozzi G, Dionisi-Vici C, et al. EPI-743 reverses the progression of the pediatric mitochondrial disease—genetically defined Leigh Syndrome. Mol Genet Metab. 2012 Nov; 107(3):383–388. [PubMed: 23010433]
54. Pastore A, Petrillo S, Tozzi G, Carrozzo R, Martinelli D, Dionisi-Vici C, et al. Glutathione: a redox signature in monitoring EPI-743 therapy in children with mitochondrial encephalomyopathies. Mol Genet Metab. 2013 Jun; 109(2):208–214. [PubMed: 23583222]
55. Sadun AA, Chicani CF, Ross-Cisneros FN, Barboni P, Thoolen M, Shrader WD, et al. Effect of EPI-743 on the clinical course of the mitochondrial disease Leber hereditary optic neuropathy. Arch Neurol. 2012 Mar; 69(3):331–338. [PubMed: 22410442]
56. Tengan CH, Kiyomoto BH, Godinho RO, Gamba J, Neves AC, Schmidt B, et al. The role of nitric oxide in muscle fibers with oxidative phosphorylation defects. Biochem Biophys Res Commun. 2007 Aug 3; 359(3):771–777. [PubMed: 17560547]
57. Koga Y, Akita Y, Nishioka J, Yatsuga S, Povalko N, Tanabe Y, et al. L-arginine improves the symptoms of strokelike episodes in MELAS. Neurology. 2005 Feb 22; 64(4):710–712. [PubMed: 15728297]
58. Vattemi G, Mechref Y, Marini M, Tonin P, Minuz P, Grigoli L, et al. Increased protein nitration in mitochondrial diseases: evidence for vessel wall involvement. Mol Cell Proteomics. 2011 Apr. 10(4):M110.002964.
59. El-Hattab AW, Emrick LT, Hsu JW, Chanprasert S, Almannai M, Craigen WJ, et al. Impaired nitric oxide production in children with MELAS syndrome and the effect of arginine and citrulline supplementation. Mol Genet Metab. 2016 Apr; 117(4):407–412. [PubMed: 26851065]
60. El-Hattab AW, Hsu JW, Emrick LT, Wong LJC, Craigen WJ, Jahoor F, et al. Restoration of impaired nitric oxide production in MELAS syndrome with citrulline and arginine supplementation. Mol Genet Metab. 2012 Apr; 105(4):607–614. [PubMed: 22325939]

61. Koga Y, Akita Y, Nishioka J, Yatsuga S, Povalko N, Katayama K, et al. MELAS and L-arginine therapy. Mitochondrion. 2007 Apr; 7(1–2):133–139. [PubMed: 17276739]
62. Koga Y, Akita Y, Junko N, Yatsuga S, Povalko N, Fukiyama R, et al. Endothelial dysfunction in MELAS improved by l-arginine supplementation. Neurology. 2006 Jun 13; 66(11):1766–1769. [PubMed: 16769961]
63. El-Hattab AW, Emrick LT, Craigen WJ, Scaglia F. Citrulline and arginine utility in treating nitric oxide deficiency in mitochondrial disorders. Mol Genet Metab. 2012 Nov; 107(3):247–252. [PubMed: 22819233]
64. Naini A, Kaufmann P, Shanske S, Engelstad K, De Vivo DC, Schon EA. Hypocitrullinemia in patients with MELAS: an insight into the “MELAS paradox”. J Neurol Sci. 2005 Mar 15.229-230:187–193. [PubMed: 15760638]
65. Sproule DM, Kaufmann P. Mitochondrial encephalopathy, lactic acidosis, and strokelike episodes: basic concepts, clinical phenotype, and therapeutic management of MELAS syndrome. Ann N Y Acad Sci. 2008 Oct.1142:133–158. [PubMed: 18990125]
66. Koenig MK, Emrick L, Karaa A, Korson M, Scaglia F, Parikh S, et al. Recommendations for the management of strokelike episodes in patients with mitochondrial encephalomyopathy, lactic acidosis, and strokelike episodes. JAMA Neurol. 2016 May 1; 73(5):591–594. [PubMed: 26954033]
67. El-Hattab AW, Emrick LT, Williamson KC, Craigen WJ, Scaglia F. The effect of citrulline and arginine supplementation on lactic acidemia in MELAS syndrome. Meta Gene. 2013 Dec.1:8–14. [PubMed: 25411654]
68. Schlame M, Ren M. The role of cardiolipin in the structural organization of mitochondrial membranes. Biochim Biophys Acta. 2009 Oct; 1788(10):2080–2083. [PubMed: 19413994]
69. Paradies G, Petrosillo G, Paradies V, Ruggiero FM. Oxidative stress, mitochondrial bioenergetics, and cardiolipin in aging. Free Radic Biol Med. 2010 May 15; 48(10):1286–1295. [PubMed: 20176101]
70. Szeto HH. First-in-class cardiolipin-protective compound as a therapeutic agent to restore mitochondrial bioenergetics. Br J Pharmacol. 2014 Apr; 171(8):2029–2050. [PubMed: 24117165]
71. Liang H, Ward WF. PGC-1alpha: a key regulator of energy metabolism. Adv Physiol Educ. 2006 Dec; 30(4):145–151. [PubMed: 17108241]
72. Rai PK, Russell OM, Lightowlers RN, Turnbull DM. Potential compounds for the treatment of mitochondrial disease. Br Med Bull. 2015; 116:5–18. [PubMed: 26590387]
73. Noe N, Dillon L, Lellek V, Diaz F, Hida A, Moraes CT, et al. Bezafibrate improves mitochondrial function in the CNS of a mouse model of mitochondrial encephalopathy. Mitochondrion. 2013 Sep; 13(5):417–426. [PubMed: 23261681]
74. Dillon LM, Hida A, Garcia S, Prolla TA, Moraes CT. Long-term bezafibrate treatment improves skin and spleen phenotypes of the mtDNA mutator mouse. PLoS One. 2012; 7(9):e44335. [PubMed: 22962610]
75. Bonnefont JP, Bastin J, Laforêt P, Aubey F, Mogenet A, Romano S, et al. Long-term follow-up of bezafibrate treatment in patients with the myopathic form of carnitine palmitoyltransferase 2 deficiency. Clin Pharmacol Ther. 2010 Jul; 88(1):101–108. [PubMed: 20505667]
76. Komen JC, Thorburn DR. Turn up the power - pharmacological activation of mitochondrial biogenesis in mouse models. Br J Pharmacol. 2014 Apr; 171(8):1818–1836. [PubMed: 24102298]
77. Libri V, Brown AP, Gambarota G, Haddad J, Shields GS, Dawes H, et al. A pilot randomized, placebo controlled, double blind phase I trial of the novel SIRT1 activator SRT2104 in elderly volunteers. PLoS One. 2012; 7(12):e51395. [PubMed: 23284689]
78. Golubitzky A, Dan P, Weissman S, Link G, Wikstrom JD, Saada A. Screening for active small molecules in mitochondrial complex I deficient patient's fibroblasts, reveals AICAR as the most beneficial compound. PLoS One. 2011; 6(10):e26883. [PubMed: 22046392]
79. Nogueira L, Ramirez-Sanchez I, Perkins GA, Murphy A, Taub PR, Ceballos G, et al. (—)-Epicatechin enhances fatigue resistance and oxidative capacity in mouse muscle. J Physiol. 2011 Sep 15; 589(Pt 18):4615–4631. [PubMed: 21788351]

80. Moreno-Ulloa A, Cid A, Rubio-Gayosso I, Ceballos G, Villarreal F, Ramirez-Sanchez I. Effects of (—)-epicatechin and derivatives on nitric oxide mediated induction of mitochondrial proteins. Bioorg Med Chem Lett. 2013 Aug 1; 23(15):4441–4446. [PubMed: 23791569]
81. Neymotin A, Calingasan NY, Wille E, Naseri N, Petri S, Damiano M, et al. Neuroprotective effect of Nrf2/ARE activators, CDDO ethylamide and CDDO trifluoroethylamide, in a mouse model of amyotrophic lateral sclerosis. Free Radic Biol Med. 2011 Jul 1; 51(1):88–96. [PubMed: 21457778]
82. El-Hattab AW, Craigen WJ, Scaglia F. Mitochondrial DNA maintenance defects. Biochim Biophys Acta. 2017 Jun; 1863(6):1539–1555. [PubMed: 28215579]
83. Chanprasert, S., Wong, LJC., Wang, J., Scaglia, F. TK2-Related mitochondrial DNA depletion syndrome, myopathic form. In: Pagon, RA.Adam, MP.Ardinger, HH.Wallace, SE.Amemiya, A.Bean, LJ., et al., editors. GeneReviews(®) [Internet]. University of Washington; Seattle, Seattle (WA): 1993. Available from: http://www.ncbi.nlm.nih.gov/books/NBK114628/ [cited 2017 Aug 13]
84. Lopez-Gomez C, Levy RJ, Sanchez-Quintero MJ, Juanola-Falgarona M, Barca E, Garcia-Diaz B, et al. Deoxycytidine and deoxythymidine treatment for thymidine kinase 2 deficiency. Ann Neurol. 2017 May; 81(5):641–652. [PubMed: 28318037]
85. Parikh S, Karaa A, Goldstein A, Ng YS, Gorman G, Feigenbaum A, et al. Solid organ transplantation in primary mitochondrial disease: proceed with caution. Mol Genet Metab. 2016 Jul; 118(3):178–184. [PubMed: 27312126]
86. Cohen, BH., Chinnery, PF., Copeland, WC. POLG-related disorders. In: Pagon, RA.Adam, MP.Ardinger, HH.Wallace, SE.Amemiya, A.Bean, LJ., et al., editors. GeneReviews(®) [Internet]. University of Washington; Seattle, Seattle (WA): 1993. Available from: http://www.ncbi.nlm.nih.gov/books/NBK26471/ [cited 2017 Aug 13]
87. Grabhorn E, Tsiakas K, Herden U, Fischer L, Freisinger P, Marquardt T, et al. Long-term outcomes after liver transplantation for deoxyguanosine kinase deficiency: a single-center experience and a review of the literature. Liver Transpl. 2014 Apr; 20(4):464–472. [PubMed: 24478274]
88. El-Hattab, AW., Scaglia, F., Craigen, WJ., Wong, LJC. MPV17-related hepatocerebral mitochondrial dna depletion syndrome. In: Pagon, RA.Adam, MP.Ardinger, HH.Wallace, SE.Amemiya, A.Bean, LJ., et al., editors. GeneReviews(®) [Internet]. University of Washington; Seattle, Seattle (WA): 1993. Available from: http://www.ncbi.nlm.nih.gov/books/NBK92947/ [cited 2017 Aug 13]
89. Hirano, M. Mitochondrial neurogastrointestinal encephalopathy disease. In: Pagon, RA.Adam, MP.Ardinger, HH.Wallace, SE.Amemiya, A.Bean, LJ., et al., editors. GeneReviews(®) [Internet]. University of Washington; Seattle, Seattle (WA): 1993. Available from: http://www.ncbi.nlm.nih.gov/books/NBK1179/ [cited 2017 Aug 13]
90. Yu H, Koilkonda RD, Chou TH, Porciatti V, Ozdemir SS, Chiodo V, et al. Gene delivery to mitochondria by targeting modified adenoassociated virus suppresses Leber's hereditary optic neuropathy in a mouse model. Proc Natl Acad Sci U S A. 2012 May 15; 109(20):E1238–1247. [PubMed: 22523243]
91. Feuer WJ, Schiffman JC, Davis JL, Porciatti V, Gonzalez P, Koilkonda RD, et al. Gene therapy for Leber hereditary optic neuropathy: initial results. Ophthalmology. 2016 Mar; 123(3):558–570. [PubMed: 26606867]
92. Guy J, Feuer WJ, Davis JL, Porciatti V, Gonzalez PJ, Koilkonda RD, et al. Gene therapy for Leber hereditary optic neuropathy: low- and medium-dose visual results. Ophthalmology. 2017 Jun 21.

Table 1

Agents used or being studied for treating mitochondrial diseases.

	Doses	Effect on mitochondrial function	Diseases for which agents are used
Agents increasing electron transfer chain function			
CoQ_{10}	Ubiquinone: 5–30 mg/kg/day divided in 2 doses Ubiquinol: 2–8 mg/kg/day divided in 2 doses	Improving the efficacy of electron transfer through ETC	Primary CoQ_{10} deficiency
Idebenone	30–300 mg/dose 3 times daily	Being a CoQ_{10} analog with higher efficacy	LHON
Riboflavin	50–200 mg/day divided in 2–3 doses	Being a flavoprotein precursor that is a key building block in complexes I and II	Acyl-CoA dehydrogenase-9 deficiency and multiple acyl-CoA dehydrogenase deficiency
Dichloroacetate	10–25 mg/kg/day divided in 2 doses	Increasing pyruvate dehydrogenase activity, thereby increasing the catabolism of pyruvate to acetyl-CoA	Congenital lactic acidosis
Thiamine	10 mg/kg/day (children) 100–1000 mg/day (adults)	Enhancing pyruvate dehydrogenase activity, thereby increasing the catabolism of pyruvate to acetyl-CoA	Leigh disease and thiamine transporter deficiency
Energy buffer			
Creatine monohydrate	100–300 mg/kg/day divided in three doses (children) 2–10 g/day divided in three doses (adults)	Acting as an intracellular buffer for ATP and an energy shuttle for high energy phosphates movement from mitochondrial to cytoplasm	Mitochondrial myopathies
Antioxidants			
Lipoic acid	25 mg/kg/day (children) 300–600 mg/day (adults)	Providing antioxidant action and being an essential factor for pyruvate and ketoglutarate dehydrogenases	MELAS and other mitochondrial diseases
RP103	–	Increasing intracellular glutathione levels by increasing cysteine availability	Leigh and other mitochondrial diseases, ongoing clinical study (https://clinicaltrials.gov/ct2/show/NCT02023866)
EPI-743	–	Protecting against excessive ROS and restoring reduced intracellular glutathione	Leigh disease, ongoing clinical study (https://clinicaltrials.gov/ct2/show/NCT02352896) Mitochondrial diseases, ongoing clinical study (https://clinicaltrials.gov/ct2/show/NCT01642056)
Restoration of nitric oxide production			
Arginine	150 to 300 mg/kg/day divided in 3 doses	Restoring NO production	MELAS Mitochondrial diseases, ongoing clinical study (https://clinicaltrials.gov/ct2/show/NCT02809170)
Cardiolipin protection			
Elamipretide	–	Binding to cardiolipin and protecting it from oxidation	Mitochondrial myopathy, ongoing clinical study (https://clinicaltrials.gov/ct2/show/NCT02367014)
Agents enhancing mitochondrial biogenesis			
Bezafibrate	–	Activating PPAR which activates PCG-1α pathway and induces mitochondrial biogenesis	Mitochondrial myopathy, ongoing clinical study (https://

	Doses	Effect on mitochondrial function	Diseases for which agents are used
			clinicaltrials.gov/ct2/show/NCT02398201)
Epicatechin	–	Enhancing mitochondrial biogenesis	Friedreich ataxia, ongoing clinical study (https://clinicaltrials.gov/ct2/show/NCT02660112)
RTA 408	–	Activating Nrf 2 which stimulates mitochondrial biogenesis	Mitochondrial myopathy, ongoing clinical study (https://clinicaltrials.gov/ct2/show/NCT02255422)

(ETC: electron transport chain; LHON: Leber hereditary optic neuropathy; ROS: reactive oxygen species; NO: nitric oxide; PPAR: peroxisome proliferative-activated receptors; Nrf 2: nuclear respiratory factor 2).