Chapter 11
Therapies Approaches in Mitochondrial Diseases

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Abstract Therapies for mitochondrial diseases has been largely limited to supportive and symptomatic therapies; however, in the last decade, advances in understanding the causes and pathomechanisms of these diverse disorders have enabled development of novel treatment strategies. Here, we highlight current use of dietary supplements and exercise therapy as well as emerging treatments in preclinical and clinical trial stages of development. Broad-spectrum therapies that may be applied multiple diseases include: activation of mitochondrial biogenesis, regulation of mitophagy and mitochondrial dynamics, bypass of mitochondrial biochemical defects, mitochondrial replacement therapy, and hypoxia. Tailored disease-specific therapies in development include: scavenging of toxic compounds, deoxynucleoside therapy, cell replacement therapies, viral-mediated gene-delivery, shifting heteroplasmy of mitochondrial DNA pathogenic variants, and stabilization of mitochondrial transfer RNAs.

11.1 Introduction

Mitochondrial disorders (MDs) represent a heterogeneous group of inborn errors of the oxidative phosphorylation (OXPHOS) system in mitochondria, where most of the cell's ATP is generated. This metabolic pathway is under the dual genetic control of the mitochondrial and nuclear genomes.

The genetic complexity only partially accounts for the clinical heterogeneity of these disorders. Onset varies widely from childhood to adulthood, even among members of the same family. Virtually every organ system can be affected by mitochondrial dysfunction (in particular brain, skeletal and cardiac muscle) and as a consequence, MDs are often multisystemic (DiMauro et al. 2013).

Taken together, MDs have an estimated prevalence greater than 1:5000 individuals (Schaefer et al. 2008). The 10 most common pathogenic mitochondrial DNA

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(mtDNA) mutations alone have an estimated incidence of 1 in 200 infants (Elliott et al. 2008). Given their prevalence and their progressive nature, often worsening over many decades, these disorders cause substantial morbidity among both pediatric and adult populations.

Currently, only supportive care is available for the vast majority of patients with MDs (Pfeffer et al. 2012; Kerr 2013). However, extraordinary progress has been made in recent years in understanding the pathogenesis of these disorders (Hirano et al. 2018). Based on this knowledge, therapeutic strategies have been proposed and experimental evidence is increasing in *in vitro* and *in vivo* studies.

Numerous challenges albeit hamper the translation of these therapies from bench to bedside. The main challenges are caused by the genetic, biochemical, and phenotypic variability of MDs. This heterogeneity, for instance, makes it difficult to collect a sufficient number of patients to conduct reliable natural history studies, clinical trials, and to identify universally validated outcome measures.

In this chapter, we will discuss separately current therapeutic approaches, including supportive therapy, the results of previous clinical trials, and emerging therapies that have shown promising results at preclinical and clinical level.[1]

11.2   Current Treatments in Clinical Practice

11.2.1   Supportive Treatments

Supportive treatments are often the only available option in the management of mitochondrial patients when a specific therapy is lacking. For the vast majority of patients, therapy is limited to either preventing or treating the complications of MDs. Nevertheless, supportive measures are extremely important and can significantly improve quality of life and survival in this group of patients. Symptomatic approaches often require a multidisciplinary team for early recognition and treatment of complications/manifestations.

Epileptic seizures commonly occur in MDs, such as, for example, Mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS), Myoclonic epilepsy with ragged red fibers (MERRF) and Leigh syndrome. Most available anticonvulsants can be used, except sodium valproate that can cause carnitine deficiency and may precipitate hepatic failure in Alpers syndrome (Rahman 2012). Myoclonic epilepsy in MERRF can be treated effectively with levetiracetam, clonazepam, or zonisamide (DiMauro and Hirano 1993). Dystonia, often present in Leigh syndrome, can benefit of anti-dystonia oral medications (e.g. anticholinergic, neuroleptics) or botulinum toxin injection.

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[1] This chapter is based upon our published review article (Hirano et al. 2018) available via Open Access   (https://portlandpress.com/essaysbiochem/article/62/3/467/78638/Emerging-therapies-for-mitochondrial-diseases) CCBY license ELVJWX.

Heart is often affected in MDs. Conduction defects are commonly present in patients with Kearns-Sayre syndrome (KSS), but also in Leber hereditary optic neuropathy (LHON), and m.3243A>G mutation. Timely placement of a pacemaker can be lifesaving in KSS patients. Cardiomyopathy can also occur and it is estimated to affect 20–40% of children with MDs (Scaglia et al. 2004). Hypertrophic cardiomyopathy is the most common manifestation, being present in 50% of mitochondrial patients with cardiac involvement (Finsterer and Kothari 2014). MDs presenting cardiomyopathy can be due to defects in respiratory chain complexes subunits and assembly factors, mitochondrial tRNAs, rRNAs, ribosomal proteins, translation factors, mtDNA maintenance, CoQ_{10} synthesis, or defect of lipid milieau, like Barth syndrome (El-Hattab and Scaglia 2016). Cardiac involvement should be closely monitored with by a cardiologist and pharmacologically or surgically treated when necessary.

Endocrine dysfunction can be present in MDs, and hormone replacement can be necessary (insulin, thyroxine, growth hormone). In the case of diabetes mellitus, for instance in patients with MELAS and Maternally Inherited Diabetes-Deafness (MIDD) syndrome, diet and low doses of insulin/oral hypoglycemic drugs are usually sufficient to maintain the euglycemic state. Metformin should be avoided because it can cause lactic acidosis (Murphy et al. 2008).

Gastrointestinal (GI) problems are also common in patients with mitochondrial disorders and include dysphagia, weight loss, constipation, pseudo-obstruction, nausea, failure to thrive. Among syndromic MDs, Mitochondrial neurogastrointestinal encephalopathy (MNGIE) is the one with prevalent GI involvement (Garone et al. 2011). Adequate nutrition can be achieved with hypercaloric nutritional supplements but may require PEG (percutaneous or parenteral nutrition endoscopic gastrostomy) feeding in severe cases (Finsterer and Frank 2017).

Other supportive therapies include electrolyte replacement, renal dialysis or transplantation in patients with renal involvement, uncommon but described in patients with mitochondrial DNA or nuclear DNA mutations (O'Toole 2014); blood transfusion in case of anemia in Pearson syndrome or other forms of anemia; respiratory support for restrictive lung disease; and psychological support for patients and their families.

Non-pharmacologic approaches, include the use of hearing aids or cochlear implants for patients with hearing loss (Sinnathuray et al. 2003), and eyelid surgery for ptosis, as a mean to improve not only the vision but also the psychological well-being and social interaction of the patient.

11.2.2 Pharmacological Approaches

Multiple vitamins and cofactors are often used in patients with mitochondrial disorders, although these therapies are not yet standardized or definitively proven to be effective. The dietary supplements are used with different purposes, such as: (1) increase the respiratory chain flux (CoenzymeQ_{10} [CoQ_{10}], riboflavin), (2) serve as

antioxidants (e.g. CoQ_{10}, idebenone, alpha-lipoic acid, vitamins C and E), and/or act as cofactors (e.g. riboflavin, thiamine), or (3) function as mitochondrial substrates (L-carnitine). Accumulation of reactive oxygen species (ROS) as a toxic byproduct of a mitochondrial respiratory chain dysfunction may lead to cellular damage contributing to the pathogenesis of MDs. Moreover, a transgenic murine model overexpressing a catalase targeted to mitochondria extended life span (Schriner et al. 2005). Based on this rational, antioxidants have been frequently used in the treatment of mitochondrial patients. CoQ_{10} is the most commonly utilized and many clinical trials have been investigating its efficacy and that of its analogues, like idebenone and EPI-743, in different MDs (see Sect. 11.3). Other antioxidants like vitamins C and E might also be beneficial in patients with MDs. An example is an analogue of vitamin E, trolox ornithylamide hydrochloride, when applied to fibroblasts from patients with Leigh syndrome reduced ROS levels and an increased activities of mitochondrial complexes I and IV, and citrate synthase (Blanchet et al. 2015). The efficacy of antioxidants in patients with MDs nonetheless remains controversial. A Cochrane review of mitochondrial therapies has found little evidence supporting the use of any vitamin or cofactor (Pfeffer et al. 2012). However, benefits of various agents (riboflavin, alpha-lipoic acid, etc) have been anecdotally reported. Consensus recommendations from the Mitochondrial Medicine Society recently reported (Parikh et al. 2015) aimed to standardize treatment options for mitochondrial patients. According to these recommendations, patients with primary mitochondrial disorders, and not only CoQ_{10} deficiency, should be offered CoQ_{10} in its reduced form (ubiquinol), and plasma or leukocyte levels should be monitored to assess adherence to treatment.

In addition, alpha-lipoic acid (ALA) and riboflavin are frequently offered to mitochondrial patients. L-carnitine should be administered when deficient. Folinic acid should be given to mitochondrial patients when deficient and the central nervous system is involved. Moreover, supplements should be given starting with one supplement at the time, avoiding "cocktails" initially.

Effective treatment of acute stroke-like episodes or their prevention has not been established. Open-label studies suggest that treatment of acute mitochondrial stroke-like episodes with intravenous (IV) arginine hydrochloride, a precursor of nitric oxide, is beneficial for patient with the m.3243 A>G mutation in *MTTL1* (Koenig et al. 2016). IV arginine administration should be considered in acute stroke-like episodes associated with other primary mitochondrial disorders. Open-label studies also suggest that daily oral arginine to prevent strokes should be considered in MELAS patients with the m.3243 A>G mutation (Parikh et al. 2015).

Lastly, some drugs should be avoided in patients with mitochondrial dysfunction, or used with caution: valproic acid, statins, metformin, high-dose acetaminophen, and specific antibiotics (i.e. aminoglycosides, linezolid, tetracycline, azithromycin, erythromycin).

A survey on the use of dietary supplements was recently conducted by the North American Mitochondrial Disease Consortium (NAMDC) (Karaa et al. 2016). The survey pointed out how the majority of patients takes four or more dietary supplements, despite the recent recommendation of the Mitochondrial Medicine Society.

Even though no or minor side effects were reported, and patients noted overall some improvements, the economic burden to the families was considerable; 90% of patients purchased the supplements out-of-pocket. The authors conclude that this burden and the potential side effects are not justifiable, considering the lack of evidence for using these "cocktails". Importantly, this survey reveal an increase interest towards patients' perception of their care and quality of life; this approach is fundamental to determine reliable outcome measures to use in future well-conducted clinical trials.

11.2.3 Exercise

Exercise has been proven beneficial in some patients with MDs (Voet et al. 2013; Tarnopolsky 2014). In particular, aerobic endurance training can increase mitochondrial mass, by stimulating mitochondrial biogenesis, and increase muscle mitochondrial enzyme activities and muscle strength. Endurance training has been proven beneficial and safe in trials of patients with mitochondrial DNA mutations (Taivassalo et al. 2001, 2006). A combination of progressive endurance with or without resistance exercise should be recommended to mitochondrial patients (Parikh et al. 2015).

11.3 Clinical Trials

In 2012 a systematic review by the Cochrane collaboration evaluated 1335 studies comparing pharmacological and non-pharmacological treatments for MDs (Pfeffer et al. 2012). Only 12 studies were selected for inclusion in the review, the most common reason for exclusion being lack of randomization/blinding and presence of methodological biases. The primary outcome measures included any change in muscle strength or neurological features. Secondary outcome measures included quality life evaluation, biochemical biomarkers (e.g. lactic acidosis), and negative outcomes. The 12 studies investigated the effects of CoQ_{10}, dichloroacetate (DCA), creatinine, dimethylglycine, whey-based cysteine and combination therapy of creatine, α-lipoic acid and CoQ_{10}. Dramatic effects were not observed in any of these studies, and one trial assessing the effects of DCA in MELAS patients had to be terminated because of toxicity (NCT00068913). Several clinical trials are currently underway or have been recently completed, but the results were not published for most of them and are still unclear. The majority of the studies focused on patients with MELAS and LHON, which could be studied in relatively large cohorts. Many other trials analyzed less homogeneous cohort of patients, including, for instance, patients with similar phenotype (i.e. mitochondrial myopathy) but different genetic background. A summary of the studies is reported in Table 11.1. We will briefly discuss some examples.

Table 11.1 Recent clinical trials in mitochondrial disorders

Treatment	Disease	Design	Mechanism	Status	Outcome	Trial number
ACTIVE STUDIES						
EPI-743	MDs	Phase 2, Emergency use protocol in acutely ill patients (90 days endo-of-life care)	Antioxidant	Active/not recruiting	na	NCT01370447
EPI-743	Children (2–11 years of age) with MDs or metabolic diseases	Phase 2, randomized, double blind, placebo-controlled, crossover	Antioxidant	Active/not recruiting	na	NCT01642056
MTP-131 (Elamipretide) ophthalmic	LHON	Phase 2, prospective, randomized, double blind, vehicle-controlled	Cardiolipin stabilization	Active/not recruiting	na	NCT0293119
DCA (dichloroacetate)	PDH deficiency	Phase 3, randomized, placebo-controlled, crossover	Lowering lactate levels	Recruiting	na	NCT02616484
Resistance exercise	Barth syndrome	Phase 2, open label	Increase glycolitic type 1 muscle fibers	Recruiting	na	NCT01629459
scAAV2-P1ND4v2	LHON m.11778G>A	Phase 1, open label, dose-escalating	Gene therapy	Recruiting	na	NCT02161380
rAAV2/2-ND4 (GS010)	LHON	Phase1/2 safety, open label, dose escalating	Gene therapy	Active/not recruiting	na	NCT02064569

Allogenic HSCT	MNGIE	Phase 1 safety study	Production of TP	Recruiting	na	NCT02427178
COMPLETED STUDIES						
KH176	MELAS, MIDD, mitochondrial myopathies, MDs	Phase 2, randomized, double blind, placebo-controlled, crossover	Antioxidant	Completed	Twice daily oral 100mg KH176 was well-tolerated and appeared safe. Primary outcome (gait measures) did not reach statistical significance, but possible positive effects on alterness and mood were noted.	NCT02909400
RTA 408 (omaveloxolone)	Mitochondrial myopathy	Phase 2 randomized, double blind, placebo-controlled, dose-escalating	Antioxidant, NRF2 activator, NFkB inhibitor	Completed	Oral omaveloxone up to 160mg daily was well-tolerated and appeared safe. Primary (peak cycling exercise workload) and secondary (6-minute walk test) did not achieve statistical significance, but improvements in exploratory endpoints (lowering heart rate and lactate production during submaximal exercise) were reported.	NCT02255422
MTP-131 (Elamipretide)	Mitochondrial myopathy	Phase 2, randomized, double-blind, placebo-controlled, crossover	Cardiolipin stabilization	Completed	na	NCT02805790
MTP-131 (Elamipretide)	MDs	Phase 2, open label	Cardiolipin stabilization	Completed	na	NCT02976038
CoQ10	Children with MDs with mtDNA mutations or specific OXPHOS complexes defects	Phase 3, randomized, double blind	OXPHOS/ROS	Completed	na	NCT00432744

Table 11.1 (continued)

Treatment	Disease	Design	Mechanism	Status	Outcome	Trial number
Idebenone	LHON	Phase 2, randomized, double blind, placebo-controlled	Antioxidant	Completed	Primary endpoint did not reach statistical significance; secondary outcomes significantly differ in a subgroup of patients with discordant acuity at baseline	NCT00747487
Idebenone	MELAS	Phase 2, randomized, double blind, placebo-controlled, dose-finding	Antioxidant	Completed	Primary endpoint did not reach statistical significance	NCT0887562
EPI-743	Leigh syndrome	Phase 2b, randomized, double blind, placebo-controlled	Antioxidant	Completed	na	NCT01721733
EPI-743	Pearson syndrome	Phase 2, open label	Antioxidant	Terminated	Results from other studies did not support continuation of this trial	NCT02104336
KH176	MELAS, LHON, Leigh, and other MDs	Phase 1, randomized, double blind, placebo-controlled, crossover	Antioxidant	Completed	Well tolerated with promising pharmacokinetic profile	NCT02544217
Bezafibrate	Mitochondrial myopathy (m.3243A>G)	Phase 2, open label	Mitochondrial biogenesis	Completed	na	NCT02398201
Curcumin	LHON	Phase 3, randomized, double blind, placebo-controlled	Antioxidant	Completed	na	NCT00528151

MTP-131	Mitochondrial myopathy	Phase 1/2 randomized, double-blind, placebo-controlled, dose-escalating	Cardiolipin stabilization	Completed	na	NCT02367014
RP103 (Cysteamine bitartrate delayed-release)	Childhood MDs including Leigh syndrome	Phase 2 open-label, dose-escalating	Cystine-depleting agent	Completed	Primary endpoint did not reach statistical significance; high percentage of serious Adverse Events (30.56%)	NCT0202386 6
RP103 (Cysteamine bitartrate delayed-release)	Childhood MDs including Leigh syndrome	Phase 2, long term open-label extension study	Cystine-depleting agent	Completed	na	NCT02473445
Medium chain triglycerides	MELAS	Phase 1, open label	Shift heteroplasmy	Completed	na	NCT01252979
L-Arginine	MELAS	Phase 2, open label	NO precursor	Completed	Improvement in aerobic capacity and muscle metabolism	NCT01603446
L-Arginine (IV)	MELAS	Phase 3, open label	NO precursor	Completed	Improvement of strok-like symptoms	JMA-IIA00023
L-Arginine (PO)	MELAS	Phase 3, open-label	NO precursor	Completed	Improved endothelial dysfunction	JMA-IIA00025
Arginine and citrulline	MELAS	Phase 1, open-label	NO precursor	Completed	na	NCT01339494
Lipoic acid	Mitochondrial myopathy	Pilot compassionate use study	–	Completed	na	NCT00004770
RG2133 (2′,3′,5′-tri-o-acetyluridine)	MDs	Phase 1, open label, dose escalating	–	Completed	na	NCT00060515
SPP-004 (5-Ala and SFC)	MDs, mainly to cranial nerve symptoms	Phase 2, randomized, placebo-controlled	–	Completed	na	JMA-IIA00200

Table 11.1 (continued)

Treatment	Disease	Design	Mechanism	Status	Outcome	Trial number
Taurine	MELAS	Phase 2/3 open-label	Taurine modification	Completed	na	UMIN000011908
Pyruvate	MELAS	Phase 2, randomized, placebo-controlled	NAD donor	Unknown	na	JMA–IIA00093
DCA (dichloroacetate)	MELAS	Phase 2, randomized, double blind, crossover	Lowering lactate levels	Terminated	Terminated because of peripheral nerve toxicity	NCT00068913
Cyclosporine	LHON acute phase	Phase 2, open label	Inhibition of mitochondrial PTP	Unknown	na	NCT02176733
rAAV2-ND4	LHON	Open label	Gene therapy	Completed	Improvement of visual acuity and enlargement of visual field	NCT01267422

MDs mitochondrial disorders, *MELAS* mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes, *MIDD* maternally inherited diabetes-deafness syndrome, *LHON* Leber hereditary optic neuropathy, *PDH* pyruvate dehydrogenase deficiency, *HSCT* hematopoietic stem cell transplantation, *MNGIE* mitochondrial neurogastrointestinal encephalomyopathy, *IV* intra-venous, *PO* oral, *PTP* permeability transition pore, *na* not available

MELAS Syndrome

MELAS is one of the most frequent maternally inherited mitochondrial disorders. The pathogenesis of this disorder is not completely understood and results from different factor. Energy failure due to faulty mitochondria is a common feature of MDs as well as the overproduction of ROS. Different approaches have been studied in order to reduce the oxidative stress in MDs and in particular in MELAS patients. Idebenone is a short-tail ubiquinone synthetic analog, which is more water-soluble compared to CoQ_{10} and act as antioxidant. Several studies have been conducted to assess the efficacy and safety of idebenone in Friedreich Ataxia (FA), and proven that idebenone is in fact well tolerated and may stabilize neurological features in this disorder (Mariotti et al. 2003; Di Prospero et al. 2007a, b; Meier et al. 2012; Lynch et al. 2010). A phase 2a, randomized, double blind, placebo-control, dose-finding study in patients with MELAS syndrome has recently been completed and showed that the primary endpoint did not reach statistical significance (NCT00887562). KH176 is a small molecule derived from Vitamin E and is a potent ROS scavenger. After the completion of a dose escalating clinical trial with KH176 in healthy individual that has demonstrated good tolerability and a promising pharmacokinetic profile (NCT02544217), a randomized trial studied 18 adults with the m.3243A>G mutation (NCT02909400). Twice daily oral 100mg KH176 was well-tolerated and appeared safe. Primary outcome (gait measures) did not reach statistical significance, but possible positive effects on alterness and mood were noted (Janssen et al. 2019). Moreover, bezafibrate is an activator of the transcription factor peroxisomal proliferator receptors (PPARs), that in turn when activated promote transcription of mitochondrial genes. Its efficacy is being evaluated in patients with m.3243A>G mutation (NCT02398201) and evidence of myopathy, but results are not available at the moment. In addition to energy failure and ROS accumulation, there has been growing evidence that nitric oxide (NO) deficiency play a central role in the pathogenesis of the stroke-like episodes (El-Hattab et al. 2016). Arginine is the substrate of nitric oxide synthase, which produces NO, therefore arginine is a promising treatment for MELAS patients. Multiple open-label trials have been conducted (NCT01603446, JMA-IIA00023, and JMA-IIA00025) and have shown the efficacy of chronic oral administration (Koga et al. 2006, 2007; Rodan et al. 2015) and acute intravenous administration (Koga et al. 2005, 2006) of arginine in patients with MELAS syndrome, although a placebo-controlled randomized clinical trial has not yet been conducted. Furthermore, preliminary evidence has been provided for the effect of citrulline in stroke-like episodes in MELAS patients (El-Hattab et al. 2016) and a Phase 1, open-label study has been conducted (NCT01339494), although data are not available yet. Other molecules investigated in clinical trials for patients with MELAS include pyruvate (JMA-IIA00093), taurine (UMIN000011908), and supplemental medium chain triglycerides (NCT01252979); however, those results have never been reported.

Leber Hereditary Optic Neuropathy (LHON)

LHON is a mitochondrial disorder characterized by painless, subacute visual loss affecting the central visual field in one eye, followed by similar symptoms in the

other eye typically with 2 or 3 months of delay. Three mtDNA mutations are commonly associated with LHON: m.3460G>A in MT-ND1, m.11778G>A in MT-ND4, or m.14484T>C in MT-ND6 (Yu-Wai-Man and Chinnery 1993). Many therapeutic approaches have been tested in patients with LHON, the majority of which are focused on the use of antioxidants. In particular, Idebenone has been evaluated in a clinical trial of LHON and, even if primary endpoints did not reach statistical significance, a possible beneficial effect has been shown in a subgroup of patients with discordant visual acuity at baseline (Klopstock et al. 2011) (NCT00747487). Its use has been recently approved for the treatment of this disease in Europe. MTP-131 (elamipretide) is a small molecule targeting inner mitochondrial membrane that has been demonstrated to correct excessive ROS and increase ATP synthesis in preclinical studies (Szeto 2014); this molecule has entered clinical trials for LHON (NCT02693119), as well as for mitochondrial myopathy and Barth Syndrome (NCT02367014, NCT02976038, NCT02805790). Although the preliminary results of phase I and II studies of this compound for mitochondrial myopathy were promising, the phase III study failed to reach primary endpoints. Further assessments of elamipretide for Barth syndrome are ongoing (NCT03098797). Curcumin, a derivate of the spice turmeric (*Curcuma longa*), has also displayed antioxidant properties and a trial has been completed in patients with LHON, but no results are available (NCT00528151). Lastly, AAV-mediated allotopic ND4 gene therapy has been attempted by three groups in patients with LHON (NCT02161380; NCT02064569; NCT02652767; NCT02652780). Curiously, the three teams have reported improvement in best corrected visual acuity in both treated and sham/untreated eyes in some subjects. These results were interpreted as possible transfer of the AAV-ND4 across the optic chiasm (Wan et al. 2016; Feuer et al. 2016; Vignal et al. 2018; Yang et al. 2016).

MNGIE

MNGIE (mithocondrial neuro-gastro-intestinal encephalomyopathy) is an autosomal recessive mitochondrial disorder characterized by severe gastrointestinal dysmotility, cachexia, progressive external ophthalmoplegia, myopathy, and peripheral demyelinating neuropathy (Hirano 1993). It is caused by autosomal recessive mutations in *TYMP* gene, encoding thymidine phosphorylase (TP). TP is a cytosolic enzyme that catalyzes the first step of thymidine and deoxyuridine catabolism. When the enzyme is deficient, thymidine and deoxyuridine accumulates and become toxic, leading to mtDNA instability. Two trials at Columbia University, USA, are currently recruiting to define natural history of the disease (NCT01694953) and to assess the safety of hematopoetic stem cell transplant (HSCT), as a mean to replace TP enzyme, for MNGIE patients (NCT01694953) (see also Sect. 11.4). Orthoptic liver transplantation has been performed on three MNGIE patients with early promising results and possible greater safety than HSCT) (D'Angelo et al. 2017; De Giorgio et al. 2016).

Primary Mitochondrial Myopathies

Primary mitochondrial myopathies, defined as genetically confirmed disorders of oxidative phosphorylation affecting predominantly skeletal muscle (Mancuso et al.

2017), have emerged as targets for novel potential therapies (Madsen et al. 2020; Karaa et al. 2020). Oral omaveloxone, a semi-synthetic oleanoic triterpenoid activator of Nrf2, up to 160mg daily was well-tolerated and appeared safe. Primary (peak cycling exercise workload) and secondary (6-minute walk test) did not achieve statistical significance, but improvements in exploratory endpoints (lowering heart rate and lactate production during submaximal exercise) were reported (Madsen et al. 2020). A phase 3 trial of MTP-131 (elampretide), a small peptide stabilizer of cardiolipin, has been completed but publication of results are pending (Karaa et al. 2020).

Heterogeneous Cohort of Patients with MDs
Many of the therapeutic interventions acting on common pathogenic pathways of MDs have been tested in non-homogenous cohort of patients or in different disorders. One example is EPI-743, a para-benzoquinone analog modified to exert a higher antioxidant effect compared with CoQ_{10} and idebenone (Enns et al. 2012). This molecule is supposed to enhance the biosynthesis of glutathione (GSH), which is an important cellular antioxidant. Two open label studies conducted independently in North America and in Italy showed promising results in a cohort of patients with various mitochondrial diseases and with Leigh syndrome, respectively (Blankenberg et al. 2012; Martinelli et al. 2012). As a result, a randomized clinical trial has started in patients with Leigh syndrome (ClinicalTrials.gov NCT01721733) as well as in patients with FA (NCT01728064), and in acutely ill patients (90 days of end-of-life care) (NCT01370447). A clinical trial on Pearson Syndrome has been terminated because results of other studies have not supported continuation (NCT02104336). EPI-743 has also been studied in an open-label trial of patients with LHON with favorable outcome (Sadun et al. 2012).

11.4   Emerging Therapies

In the past few years, many potential treatments have been proposed for mitochondrial disorders. These approaches act on different mechanisms and can be broadly divided in "non-tailored strategies", acting on common pathways thus in theory relevant to different MDs, and "disease-tailored" strategies (Viscomi 2016). Examples of these strategies are summarized in Table 11.2. Components of the first group are, for instance, strategies aiming at: (1) activation of mitochondrial biogenesis; (2) regulation of mitophagy and mitochondrial dynamics; (3) bypass of OXPHOS defects; (4) mitochondrial replacement therapy (MRT). Part of the second group includes: (5) scavenging of specific toxic compounds; (6) supplementation of nucleosides; (7) cell replacement therapies; (8) gene therapy; (9) shifting heteroplasmy; and (10) stabilizing mutant tRNAs. Some of these approaches have been proven effective only in preclinical models while others have already been successfully applied in anecdotic patients with MDs (Table 11.2).

Table 11.2 Examples of experimental therapies in mitochondrial disorders

Strategy	Method	Model	References
NON-TAILORED			
Activation of mitochondrial biogenesis	Nicotinamide riboside and PARP1	*Sco2* knockout/ knockin mouse, Deletor mouse model	Khan et al. (2014) and Cerutti et al. (2014)
	AICAR	Patients fibroblasts and mouse models of COX deficiency	Viscomi et al. (2011)
	Bezafibrate	Patients fibroblasts, cybrids, mouse models of COX deficiency, Deletor mouse model	Bastin et al. (2008), Noe et al. (2013), Hofer et al. (2014), Yatsuga and Suomalainen (2012), and Dillon et al. (2012)
	Resveratrol	*In vitro* models, Drosophila models, human fibroblasts	Lopes Costa et al. (2014), Mizuguchi et al. (2017), and De Paepe et al. (2014)
	Retinoic acid	Cybrids (m.3243A>G)	Chae et al. (2013)
	Endurance exercise	Mouse models of COX deficiency, mtDNA mutator mice, patients with MDs	Safdar et al. (2011), Jeppesen et al. (2009), Zeviani (2008), and Rowe et al. (2012)
Regulating mitophagy and mitochondrial dynamics	Rapamycin	*in vitro* models and *Ndufs4*$^{-/-}$ mouse model of Leigh syndrome; *Tk2*$^{H126N/H126N}$ mouse model	Johnson et al. (2013) and Siegmund et al. (2017)
	P110	*In vitro* models	Qi et al. (2013)
Bypassing OXPHOS blocks	Ndi1 (Complex I defect) and AOX (Complex III and IV defects)¡	*In vitro* models and Drosophila models	Perales-Clemente et al. (2008), Sanz et al. (2010), Dassa et al. (2009), and Fernandez-Ayala et al. (2009)
Mitochondrial replacement therapy	Oocyte nuclear genetic material transfer	Non-human primates; healthy subjects and patients with MDs	Tachibana et al. (2009, 2013), Craven et al. (2010), Paull et al. (2013), Kang et al. (2016), and Zhang et al. (2017)
DISEASE-TAILORED			
Scavenging of specific toxic compounds	N-acetyl cysteine and metronidazole	*Ethe1*$^{-/-}$ mouse model of EE; patients with EE	Viscomi et al. (2010)
	Hemodialysis	Patients with MNGIE	Spinazzola et al. (2002)

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Table 11.2 (continued)

Strategy	Method	Model	References
Supplementation of nucleotides/nucleosides	Deoxycytidine and deoxythymidine monophosphates; deoxycytidine and deoxythymidine	$Tk2^{H126N/H126N}$ mouse model; patients with TK2 deficiency	Garone et al. 2014 and López-Gómez et al. (2017)
	Deoxycytidine or tetrahydrouridine	Thymidine-induced mtDNA depleted cells and $Tymp/Upp1$ knockout murine model of MNGIE	Camara et al. (2014)
	Deoxyguanosine	dGK deficient human fibroblasts	Camara et al. (2014)
Cell replacement therapies	Platelets or erythrocyte-encapsulated thymidine phsphorylase	Patients with MNGIE	Lara et al. (2006) and Bax et al. (2013)
	Allogenic HSCT	Patients with MNGIE	Hirano et al. (2006) and Halter et al. (2015)
	Liver transplantation	One patient with EE	Dionisi-Vici et al. (2016)
Gene therapy	AAV-mediated gene therapy	$Ant1^{-/-}$ mouse model; harlequin mouse; mouse model of EE; mouse model of MNGIE; mpv17 knockout mouse model	Flierl et al. (2005), Bouaita et al. (2012), Di Meo et al. (2012), Torres-Torronteras et al. (2014), and Bottani et al. (2014)
	Allotopic expression of mtDNA encoded proteins	Human fibroblasts (mutations in ND1, ND4, and ATP6 genes) and a mouse model of LHON	Bonnet et al. (2007, 2008), Kaltimbacher et al. (2006), and Ellouze et al. (2008)
	CRIPR/Cas9	iPS cell model of CoQ_{10} deficiency (c.483G > C in $COQ4$ gene)	Romero-Moyà et al. (2017)

Table 11.2 (continued)

Strategy	Method	Model	References
Shifting heteroplasmy	Mitochondrial-targeted restriction endonucleases	Cybrids (m.8993T>C) Artificial mammalian oocytes and the NZB/BALB heteroplasmic mouse model	Srivastava and Moraes (2001), Tanaka et al. (2002), Alexeyev et al. (2008), Bayona-Bafaluy et al. (2005), Bacman et al. (2007, 2010, 2012), and Reddy et al. (2015)
	mZFNs	Cybrids (m.8993T>C, mtDNA common deletion)	Minczuk et al. (2008) and Gammage et al. (2014)
	TALENs	Cybrids (mtDNA common deletion, m. 14459G>A) Artificial mammalian oocytes and the NZB/BALB heteroplasmic mouse model	Bacman et al. (2013) and Reddy et al. (2015)
Stabilizing mutant tRNAs	Overexpressing cognate and non-cognate aminoacyl mt-tRNA synthetase	*In vitro* yeast and human cell line models	De Luca et al. (2006, 2009), Li and Guan (2010), Rorbach et al. (2008), Perli et al. (2014), and Horning-Do et al. (2014)

MDs mitochondrial disorders, *EE* ethylmalonic encephalopathy, *MNGIE* mitochondrial neurogastrointestinal encephalomyopathy, *HSCT* hematopoietic stem cell transplantation, *iPS* induced pluripotent stem, *ZFNs* zinc finger endonucleases, *TALENs* transcription activator-like effectors nucleases, *CRISPR* clustered regularly interspaced palindromic repeat

11.4.1   *Activation of Mitochondrial Biogenesis*

Energy failure is a hallmark of mitochondrial diseases and various therapeutic interventions have been used to stimulate mitochondrial biogenesis. Although these interventions do not fix the underlying cause of the disease, increasing the mitochondrial mass might increase energy production, thus ameliorating the phenotype. There is increasing evidence in *in vitro* studies and animal models that increased mitochondrial biogenesis might be beneficial in many mitochondrial diseases. Interestingly, a recent observation suggested that increased mitochondrial content influences incomplete penetrance in LHON patients and that mitochondrial biogenesis could be used as a therapeutic strategy in this group of patients (Giordano et al. 2014). The biological pathway that controls mitochondrial biogenesis is complex and relies mostly on the peroxisome proliferator-activated receptor gamma (PPAR γ) coactivator 1α (PGC1α). PGC1α interacts with several transcription factors, including nuclear respiratory factors 1 and 2 (NRF1 and NRF2) and the

peroxisome proliferator-activated receptors α, β, and γ. Once activated, NRFs increases the transcription of OXPHOS genes and PPARs increase the expression of genes related to fatty acid oxidation (FAO) (Scarpulla 2008). Besides, PGC1α activity is increased by deacetylation and phosphorylation. Importantly, two enzymes responsible for these modifications, deacetylation by Sirt1 and phosphorylation by AMPK, can be modulated by drugs (Puigserver and Spiegelman 2003) and have been tested in preclinical models. Different agents used with this purpose are listed below.

Sirt1 is a nuclear deacetylase that utilizes NAD^+ to deacetylate residues of acetyl-lysine of proteins. Notably, Sirt1 is activated by increased cellular levels of NAD^+. This increase can be achieved by providing NAD precursor, such as nicotinamide riboside, or inhibiting NAD consuming enzymes, such as poly(ADP) ribosylpolymerase1 (PARP1). These approaches have been tested in animal models of mitochondrial myopathies with beneficial effects (Cerutti et al. 2014; Khan et al. 2014).

5-aminoimidazole-4-carboxamide ribonucleotide (AICAR), an adenosine monophosphate analog, is an agonist of AMPK that has been used to increase the respiratory chain complex activities in three mouse models of COX deficiency (Surf$^{-/-}$, Sco2^{KOKI}, and ACTA-Cox15$^{-/-}$) with striking improvement of motor performances only in the Sco2 model (Viscomi et al. 2011).

Bezafibrate, a pan-PPAR activator, was tested in fibroblasts of patients with different MDs and was able to stimulate PGC1α and improve the mitochondrial respiratory chain defects (Bastin et al. 2008). These findings were subsequently confirmed by *in vivo* studies in mouse models of COX-deficiency (Noe et al. 2013; Hofer et al. 2014). However, studies on other mouse models did not show induction of mitochondrial biogenesis or increased mitochondrial respiratory chain enzyme activities (Viscomi et al. 2011; Yatsuga and Suomalainen 2012; Dillon et al. 2012). PPAR-δ agonists have also been proposed as potential therapeutic agents for primary mitochondrial myopathies.

Resveratrol has also been described as an activator of mitochondrial biogenesis in animal models and in human fibroblasts (Lopes Costa et al. 2014; Mizuguchi et al. 2017), although its mechanism of action is still unknown and it did not appear to increase OXPHOS activities in another study on human fibroblasts (De Paepe et al. 2014).

Retinoic acid has been used to stimulate the retinoid X receptor-alfa (RXRalfa) in cybrid containing the m.3243A>G mutation, ameliorating the respiratory chain defect (Chae et al. 2013).

Endurance training has also been used as an activator of mitochondrial biogenesis and has been reported to be beneficial and safe in the mtDNA *mutator* mice (Safdar et al. 2011), and in patients with MDs (Jeppesen et al. 2009; Zeviani 2008). Endurance training seems able to regulate not only PGC1α but also PGC1β, AMPK, and the hypoxia inducible factors (HIFs) (Rowe et al. 2012).

11.4.2 Regulating Mitophagy and Mitochondrial Dynamics

Mitophagy is the selective elimination of dysfunctional mitochondria, a physiological process fundamental for maintaining normal mitochondrial function (Kim et al. 2007; Ashrafi and Schwarz 2013). This process is under the regulation of various pathways. One way of targeting mitophagy is via mTOR inhibition, which can be achieved by rapamycin. This approach has been investigated in a mouse model of Leigh syndrome (Ndufs4$^{-/-}$) (Johnson et al. 2013) and in a knock-in mouse model of mtDNA depletion syndrome, (Siegmund et al. 2017) and appeared to ameliorate the clinical phenotype and life-span of the treated mice, even though the biochemical defect was not rescued.

The balance between mitochondrial fusion and fission also contributes to the maintenance of mitochondrial function and can theoretically be regulated by specific modulators. An inhibitor of the mitochondrial fission protein dynamin-related protein1 (DRP1), for example the selective inhibitor P110, could potentially decrease pathological hyper-fragmentation observed in some MDs (Qi et al. 2013).

11.4.3 Bypassing OXPHOS Blocks

The use of single-peptide enzymes derived from yeast or low eukaryotes to bypass mitochondrial respiratory chain defects has been tested in *in vitro* and in vivo models. In particular, Ndi1 substitutes complex I in yeast and transfers electron to CoQ, without pumping protons across the membrane. AOX is present in lower eukaryotes and bypasses complex III and IV transferring electrons from CoQ. Expression of these enzymes has been used to bypass complex I deficiency (Perales-Clemente et al. 2008; Sanz et al. 2010) and Complex III-IV deficiencies in human cells and drosophila (Dassa et al. 2009; Fernandez-Ayala et al. 2009), but not in mammals *in vivo*.

11.4.4 Mitochondrial Replacement Therapy

Mitochondrial DNA mutations are transmitted maternally and can cause fatal or severe disorders in children (Schon et al. 2012). Moreover, these mutations are relatively common with an estimated 12,423 women at risk for transmitting mtDNA pathogenic mutations and 778 affected children per year in the United States (Gorman et al. 2015). Prenatal and preimplantation diagnoses are currently the only options available to women carrying mtDNA mutations who want to give birth to healthy children genetically related to them (Richardson et al. 2015). These techniques can accurately predict the risk of the embryo of carrying a high mtDNA mutation load. However, they cannot be applied to women with homoplasmic or

nearly-homoplasmic mutations. Mitochondrial replacement therapy is a promising new reproductive technique that prevents the transmission of mtDNA mutations. It consists in combining nuclear DNA (nDNA) from a woman with a mtDNA mutation with the mitochondria of a healthy donor. This can be achieved either transferring nuclear genetic material between the oocyte of the woman carrying the mutation and the oocyte of a healthy donor or between embryos (pronuclear transfer). These techniques have been first performed in non-human primates (Tachibana et al. 2009), and then in human embryos and oocytes of healthy individuals (Craven et al. 2010; Paull et al. 2013; Tachibana et al. 2013) and women with mtDNA mutations (Kang et al. 2016) with success. Optimization of these techniques is *ongoing* and long-term efficacy and safety still under examination. A reversion to the original mtDNA after MRT, for example, has been recently reported from different groups (Yamada et al. 2016; Hyslop et al. 2016; Kang et al. 2016) pointing towards the necessity of additional studies to evaluate the compatibility of donor mtDNA haplogroups. Moreover, there is a fervent debate on the ethical issues concerning manipulating oocytes. Nonetheless, 2 years after the approval of the UK parliament, the UK Human Fertilisation and Embryology Authority (HFEA) authorized the use of mitochondrial replacement on a case-by-case basis in 2016. The authorities have not yet approved mitochondrial replacement techniques in the United States, even if there is evidence that women carrying mtDNA mutations and oocyte donors would support the development of these procedures (Engelstad et al. 2016). The first successful mitochondrial replacement therapy via oocyte chromosomal spindle transfer has been reported; a woman carrier of the m.8993T>G mutation with a prior history of four miscarriages and 2 children who died of Leigh syndrome gave birth to a healthy boy with neonatal mtDNA mutation loads 2.36–9.23% in tested tissues (Zhang et al. 2017).

11.4.5 Scavenging of Specific Toxic Compounds

Ethylmalonic encephalopathy (EE) is a devastating disorder of infancy due to ETHE1 mutations. ETHE1 encodes a mitochondrial sulfur dioxygenase (SDO) involved in the elimination of H_2S. Accumulation of H_2S, produced by the catabolism of amino acids and by the anaerobic flora of the intestine, is toxic and leads to inhibition of COX activity and to endothelial damage. N-acetyl cysteine is a precursor of glutathione and can be used to buffer intracellular H_2S. Metronidazole is an intestinal antibiotic active against anaerobic bacteria that produce H_2S.The use of metronidazole and N-acetyl cysteine in a mouse model of ethylmalonic encephalopathy (*Ethe1$^{-/-}$*) prolonged the lifespan and ameliorated the clinical phenotype of this model. Moreover, the administration of these compounds in a cohort of patients with EE was able to improve some of the clinical features of the disease (Viscomi et al. 2010). This treatment has not been tested in clinical trials yet.

In MNGIE, hemodialysis has been used to remove these toxins but was not effective in decreasing thymidine or deoxyuridine levels (Spinazzola et al. 2002).

11.4.6 Supplementation of Nucleotides/Nucleosides

Supplementation of deoxyribonucleotides and deoxyribonucleosides has been exploited in *in vitro* and *in vivo* models of mitochondrial deoxynucleotide triphosphate (dNTP) pool unbalance. Mitochondrial dNTP pool unbalance causes mtDNA instability and consequent mtDNA depletion, multiple deletions, and point mutations. Different enzymes are involved in the maintenance of dNTP pools, such as thymidine kinase 2 (TK2), deoxyguanosine kinase (dGK), and thymidine phosphorylase (TP).

Thymidine kinase 2 (TK2) is a mitochondrial matrix protein that phosphorylates thymidine and deoxycytidine nycleosides to generate deoxythymidine and deoxycytidine monophosphate (dTMP, dCMP), which are then converted to dNTPs, fundamental for mtDNA synthesis. Recessive mutations in *TK2* gene cause dNTP pool unbalance and mtDNA instability. The consequent clinical phenotypes range from a severe infantile neuromuscular form to adult-onset chronic progressive external ophthalmoplegia. Promising results were obtained in a Tk2 knockin mouse model (Tk2$^{H126N/\ H126N}$) with oral administration of deoxycytidine and deoxythymidine monophosphates and subsequently deoxycytidine and deoxythymidine; both treatments increased mtDNA levels and mitochondrial respiratory chain enzyme activities, and prolonged the lifespan of the homozygous mutant mice (Garone et al. 2014; Lopez-Gomez et al. 2017). In 2019, a compassionate use (expanded access) study of 16 patients demonstrated safety and improved survival in early onset TK2 deficiency patient (onset <2 years-old) as well as well as motor functions in all forms of TK2 deficiency relative to the natural history studies (Dominguez-Gonzalez et al. 2019).

Depletion of mtDNA has been corrected in vivo in a Tymp knockout mouse model of MNGIE disease by administrating deoxycytidine or tetrahydrouridine (Camara et al. 2014). In the same study, the addition of deoxycytidine and tetrahydrouridine to a cell model of MNGIE disease (dThd-induced mtDNA depleted fibroblasts) was also able to prevent mtDNA depletion. mtDNA depletion was also corrected in dGK deficient human fibroblasts by adding deoxyguanosine (Camara et al. 2014).

11.4.7 Cell Replacement Therapies

Cell replacement has been explored in different mitochondrial disorders as a method to deliver a specific protein when deficient. For instance, platelets (Lara et al. 2006) or erythrocyte-encapsulated thymidine phsphorylase (Bax et al. 2013) have been transfused in patients with MNGIE disease with temporary reduction of thymidine and deoxyuridine levels, but currently there is no evidence of the sustained effect of these approaches.

Allogenic hematopoietic stem cell transplantation (HSCT) has shown potential long-term effects in treating MNGIE patients (Hirano et al. 2006) being able to restore TP function and improve biochemical and clinical manifestation of the disease. This procedure albeit has been associated with high mortality. A retrospective study (Halter et al. 2015) evaluated the experience of HSCT in MNGIE patients and underlined the effectiveness of this treatment and the importance of balancing risks and benefits of this procedure. A consensus statement for future transplants in MNGIE patients has been published (Halter et al. 2011) and HSCT should be recommended in selected patients with optimized transplant conditions (Halter et al. 2015).

Liver transplantation has been recently performed in an infant with EE due to ETHE1 mutations (Dionisi-Vici et al. 2016). The patient showed progressive improvement of the neurological function and normalization of the biochemical abnormalities. Liver transplantation can replace the deficient enzyme and clear the toxic compounds that accumulate in this disorder, constituting a feasible therapeutic option in patients with EE.

11.4.8   Gene Therapy

Nuclear DNA Defects
Adeno-associated viruses (AAV) are ideal candidates as viral vectors for gene therapy, given their low risk of insertional mutagenesis, and are currently the most widely used. AAV-mediated gene therapy has been performed in different mouse models of nuclear-encoded MDs. The first animal model was a Ant1$^{-/-}$ treated with muscle injection of AAV2 (Flierl et al. 2005). AAV2 vector targeted to retina was used to express AIF in the eye of the Harlequin mouse and restore complex I deficiency (Bouaita et al. 2012). A liver specific AAV2,8 serotype was used in a mouse model of EE and was able to dramatically improve the clinical course and the biochemical abnormalities of mutant mice (Di Meo et al. 2012). This study demonstrated that restoring ETHE1 activity selectively in the liver was sufficient to correct the enzymatic defect and led to the hypothesis that liver transplant could be used in patients with EE. Similarly, the same hepatotropic vector was used in a mouse model of MNGIE disease and was proven successful (Torres-Torronteras et al. 2014), suggesting that liver transplantation could be an option also in MNGIE patients (Boschetti et al. 2014). The first transplanted patient was described in 2016 (De Giorgio et al. 2016). The biochemical abnormalities rapidly normalized after the transplant, and clinical conditions remained stable after 400 days. AAV2,8 vector was also used to express the wild-type MPV17 protein in a mpv17 knockout mouse model of mtDNA depletion and hepato-cerebral syndrome (Bottani et al. 2014). The vector was able to rescue the mtDNA depletion and prevent liver steatosis induced by ketogenic diet in this model.

mtDNA Defects

Even more challenging is delivering gene therapy into mitochondria. One attempted approach is to allotopically express recombinant mtDNA encoded proteins containing a mitochondrial targeting sequence (MTS) in the nucleus. This approach has been tried in fibroblasts carrying mutations in ND1, ND4, and ATP6 genes (Bonnet et al. 2007, 2008; Kaltimbacher et al. 2006) and in an animal model of LHON (Ellouze et al. 2008). Despite the controversial preclinical results, clinical trials have started in patients with LHON (NCT01267422, NCT02064569 NCT02161380) and one recently completed clinical trials has shown promising results (Wan et al. 2016) (See Sect. 11.3).

11.4.9   Shifting mtDNA Mutation Heteroplasmy

Pathogenic mtDNA mutations are usually heteroplasmic, requiring a minimum critical mutation load to cause mitochondrial dysfunction. Shifting heteroplasmic levels in order to reduce the amount of mutated DNA below this threshold, therefore, has been used as a therapeutic approach. This can be achieved with different techniques: mitochondrial-targeted restriction endonucleases (Srivastava and Moraes 2001), zinc finger endonucleases (ZFNs) (Minczuk et al. 2008), transcription activator-like effectors nucleases (TALENs) (Bacman et al. 2013), and CRISPR (clustered regularly interspaced palindromic repeat)/Cas9. Restriction endonuclease *Sma*I has been used in cybrids carrying the m.8399T>G mutation and was able to reduce the mutation load and increase ATP levels (Tanaka et al. 2002; Alexeyev et al. 2008). Restriction endonucleases have been exploited also in heteroplasmic mouse models, using AAV vectors, with promising results (Bayona-Bafaluy et al. 2005; Bacman et al. 2007, 2010, 2012). The main limitation of this approach is that requires the generation of a suitable restriction site by the mtDNA mutation. The introduction of ZFNs and TALENs overcomes this limitation. ZFNs are engineered mitochondrially targeted heterodimeric zinc finger nucleases conjugated to the restriction enzyme *Fok*I. Each zinc finger domain recognizes three nucleotides, so arranging zinc finger modules appropriately allow for recognition of virtually any DNA sequence. Expression of mtZFNs in cybrids was able to reduce the mutant mtDNA and restore mitochondrial function (Gammage et al. 2014). TALENs also work as heterodimers, requiring two monomers to bind close DNA sequence in order to allow the *Fok*I nuclease to dimerize and cleave DNA, as for the mtZFNs. Reengineered TALENs targeted to different mtDNA point mutations and deletion (MitoTALENs) were able to permanently reduce the mutation load in patient-derived cells (Bacman et al. 2013). The CRISPR/Cas9 system, another endonuclease-based system, has been reported to be more effective than TALENs and has been demonstrated to rescue mitochondrial and skeletal muscle impairment in an iPS cell model of CoQ$_{10}$ deficiency due to a mutation in the *COQ4* gene (Doudna and Charpentier 2014; Romero-Moya et al. 2017). A possible limitation to the use of ZNFs and MitoTALENs in clinical practice is that AAV vectors usually are able to

fit smaller constructs. Moreover, the risk of a rapid reduction in mtDNA copy numbers of inducing a potential mtDNA depletion syndrome remains a limitation for the potential clinical application of these approaches. Lastly, mitochondria-targeted restriction endonucleases and TALENs have also been used in the selective elimination of mtDNA mutations in the germline of the heteroplasmic mouse model and artificial mammalian oocytes as a potential approach for preventing transmission of mtDNA mutations (Reddy et al. 2015).

11.4.10   Stabilizing Mutant tRNAs

The majority of the mtDNA mutations are localized to tRNA genes. It is not surprising therefore that several therapies have been targeting mt tRNAs. In particular, tRNA synthetases are enzymes that catalyze the attachment of amino acids to their cognate tRNA during protein synthesis. Many studies indicated that overexpressing cognate and non-cognate aminoacyl mt-tRNA synthetase can stabilize mt-tRNAs and attenuate the detrimental effect of the mutation in yeast and human cell lines (De Luca et al. 2006, 2009; Li and Guan 2010; Rorbach et al. 2008; Perli et al. 2014; Hornig-Do et al. 2014).

11.5   Conclusions

Remarkable progress has been made in the past years in mitochondrial medicine. Many potential therapeutic approaches for MDs have been proposed and are now at different stages of development. Translating preclinical studies to bedside remains challenging and well-controlled trials of high quality are necessary to define the efficacy of potential therapies already in use and novel drugs (Pfeffer et al. 2013). Based on the knowledge acquired with the previous studies, these future trials may overcome the challenges posed by this heterogeneous group of disorders in the context of multicenter collaborations, by selecting numerous subgroups of homogeneous patients and by selecting outcome measures that are objective and relevant to patient care and quality of life. Undoubtedly, there is a need for evidenced-based guidelines in the treatment of mitochondrial patients and the development of more effective therapies is an exciting perspective for the near future.

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