Current progress in the therapeutic options for mitochondrial disorders

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Short title: Treatment of mitochondrial disorders

Summary

Mitochondrial disorders manifest enormous genetic and clinical heterogeneity - they can appear at any age, present with various phenotypes affecting any organ, and display any mode of inheritance. What mitochondrial diseases do have in common, is impairment of respiratory chain activity, which is responsible for more than 90% of energy production within cells.

While diagnostics of mitochondrial disorders has been accelerated by introducing Next-Generation Sequencing techniques in recent years, the treatment options are still very limited. For many patients only a supportive or symptomatic therapy is available at the moment. However, decades of basic and preclinical research have uncovered potential target points and numerous compounds or interventions are now subjects of clinical trials. In this review, we focus on current and emerging therapeutic approaches towards the treatment of mitochondrial disorders. We focus on small compounds, metabolic interference, such as endurance training or ketogenic diet and also on genomic approaches.

Keywords: mitochondria, OXPHOS, mitochondrial diseases, gene therapy, mitochondrial donation

Introduction

Mitochondria are ancient organelles fundamental for normal physiology and health (Henze and Martin, 2003). They are responsible for various aspects of cellular metabolism. The oxidative phosphorylation system (OXPHOS), localized to the inner mitochondrial membrane, generates approximately 90% of cellular energetic currency, adenosine triphosphate (ATP) (Rich, 2003). In addition, mitochondria are involved in a plethora of other functions – these include tricarboxylic acid cycle (i.e. Krebs cycle), urea cycle, gluconeogenesis and ketogenesis, enzymes of calcium signalling, adaptive thermogenesis, ion homeostasis, fatty acid oxidation, amino acid metabolism, lipid metabolism as well as physiological production of reactive oxygen species (ROS). Mitochondria also host individual steps from the biosynthesis of steroids, haem and iron–sulphur clusters and play a role in programmed cell death (Voet et al., 2013). Moreover, mitochondrial structure and function are under the control of two genomes, nuclear and mitochondrial. Unlike the nuclear genome, mitochondrial DNA (mtDNA) is maternally inherited and exists in up to several thousand copies per cell, depending on the cell type (Sciacco et al., 1994, Taylor and Turnbull, 2005). Most of the sequence is coding and lacks an intron–exon structure, with the majority of genes located on one strand of the DNA molecule. Vast majority of the 1 500 mitochondrial proteins is encoded by nuclear genes. Mitochondrial DNA codes only for 13 structural subunits of OXPHOS system and a set of transfer and ribosomal RNAs that are essential for translation and mtDNA replication.

Mitochondrial disorders present the most heterogeneous group of metabolic diseases (Smeitink, 2003, Thorburn, 2004, Mayr et al., 2015). While individual mutations are very rare, as a whole group, they are among the most common forms of inborn errors of metabolism and inherited neurological disorders; it is estimated, that they affect at least 20 individuals per 100 000 people (Gorman et al., 2016). To date, variants in more than 300 nuclear genes have been reported as disease causing (Stenton and Prokisch, 2018, Stenton and Prokisch, 2020). The analysis of monogenic mitochondrial diseases has considerably enhanced general knowledge of the cellular pathophysiology of mitochondrial (dys)function. However, the diagnosis of mitochondrial diseases is challenging due to the large number of genes involved. Hence, in some genes, the mutations give rise to many common clinical syndromes. Unfortunately, the reverse is also true, some of the mitochondrial disease syndromes can have a very multifarious genetic background. As an example, Leigh syndrome, a severe neurological disorder has been reported to be caused by mutations in 75 genes located across the mitochondrial and nuclear genomes (Lake et al., 2016). The phenotypic spectrum of mitochondrial disorders is thus extremely broad and the clinical symptoms can affect any single tissue or organ or their combination at any age of onset with any mode of inheritance (Munnich and Rustin, 2001).

The most commonly and severely affected tissues in mitochondriopathies are those with a high energy demand, such as the brain, retina, kidney, liver and skeletal or cardiac muscle. Hence, patients present with neurodegenerations, in many cases in combination with muscle weakness, cardiomyopathy, optic atrophy, or liver failure (Gorman et al., 2016, Suomalainen and Battersby, 2018). This frequently makes mitochondriopathy a multisystemic disorder. Although, most mitochondrial disorders are progressive, there are many which manifest with a more stable course over decades, or even see amelioration of their symptoms (Koene and

Smeitink, 2011). The variety of possible clinical phenotypes is summarised in Figure 1 alongside the affected organs. Moreover, mitochondrial impairment is often observed in association with common disease, very often Parkinson's, Alzheimer's and Huntington's disease.

Within the last decades, enormous progress in understanding the molecular basis of mitochondrial disorders resulted in efficient screening and diagnosis. Unfortunately, this has not been reflected in the increased success in treatment of mitochondrial diseases. The number of approved drugs suitable for the majority of mitochondrial diseases is so far very restricted (Lightowlers et al., 2015). Still, numerous therapeutic options to interfere with disease progression have been explored in the past and some show promising results. They target various levels, ranging from lifestyle interventions through metabolic bypass to genome editing (Figure 2).

In this review, we summarize currently available treatment approaches with focus on small compounds (see Figure 3 for their molecular targets), manipulation of genomes and metabolic interference, such as endurance training or ketogenic diet. This review focuses on treatments which are currently already being evaluated in clinical trials, as well as the underlying preclinical research directed the bellow-mentioned topics. Numerous other approaches were explored on patient derived cell-cultures or different animal models, but they are outside of the scope of this review.

Small molecules

The variety of phenotypes connected with mitochondrial diseases complicates the situation. The first choice in suspected mitochondrial disorders, is often a cocktail of nutritional supplements and vitamins, typically provided to the patient for a limited period of time (Chinnery and Turnbull, 2001, Koene and Smeitink, 2011, Pfeffer et al., 2012). The exact composition varies among clinical centres and is also dependent on the specific needs of individual patients. Most commonly the cocktail includes L-arginine (for metabolic strokes), coenzyme Q_{10} (in the form of ubiquinol or ubiquinone), creatine (to facilitate recycling of ATP), and L-carnitine (as an antioxidant and energy source) (Enns, 2014).

Cofactors

Mitochondria and especially the OXPHOS require organic and inorganic cofactors for their proper function. Many of the organic cofactors do have their precursors in vitamins such as biotin, niacin, riboflavin, or thiamine, while others are non-vitamin related (Coenzyme Q_{10}, CoQ in short, or haem). In some cases, the genetic cause lies in enzymes involved in biosynthetic processes e.g. converting vitamins into the active cofactors or in CoQ biosynthesis. Here, supplementation can bypass a genetic defect and make such defects amenable to the treatment (Figure 3). In that regard, they can be classified as treatable inborn errors of metabolism (Balasubramaniam et al., 2019). However, the same compounds can be also administered to other mitochondrial patients with the aim of improving flux through energy

generating pathways. Since these cofactors are not connected to any harmful side-effects (Distelmaier et al., 2017), their administration can be considered as relatively safe and they can be involved in initial medication of suspected mitochondrial patients. Most notable examples of cofactors and their therapeutic use in mitochondrial disorders will be discussed in the following sections.

Coenzyme Q_{10}

Coenzyme Q_{10} is a crucial lipid-soluble electron carrier within OXPHOS and a potent antioxidant. The CoQ biosynthetic pathway consists of ten consecutive enzymatic reactions, with mutations described in each enzyme from this pathway (Awad et al., 2018). For these patients, CoQ supplementation represents a rational therapy approach, since the supplementation with CoQ may overcome its insufficient natural production (Hargreaves, 2014, Awad et al., 2018, Potgieter et al., 2013). However, universal benefit of CoQ supplementation is inconsistent. In some cases the patients with CoQ synthesis deficiency responded well to CoQ supplementation with significant improvement of the disease course (Horvath, 2012, Caglayan et al., 2019), but for example in patient with encephalomyopathy due to *COQ7* mutation the response to CoQ treatment was poor and alternative approaches had to be explored (Kwong et al., 2019).

Regarding the general benefit of CoQ supplementation for different types of mutations, randomized double-blinded trial did not reveal the beneficial effect on any of the clinically relevant monitored variables (Glover et al., 2010). On the other hand, some case studies offer a more optimistic picture. For example, CoQ, pyruvate, and a β-adrenergic receptor blocker was successfully introduced in a paediatric patient with a mutation in acyl-CoA dehydrogenase 9 (*ACAD9*) leading to a complex I defect (Kadoya et al., 2019). Unassisted CoQ was also administered to patients with *UQCRFS1* variants causing complex III deficiency (Gusic et al., 2020). One of the key factors for the successful usage of CoQ as a therapy for mitochondrial diseases is the timing of CoQ administration (Horvath, 2012). Nevertheless, the most crucial is the bioavailability of the CoQ, which is limited and varies in different organs. This fuelled investigation of its artificial analogues with better solubility and thus bioavailability (Acosta et al., 2016).

Idebenone

Idebenone is one of the synthetic CoQ analogues used to treat mitochondrial disorders. Idebenone is a drug originally developed to treat Alzheimer's disease and similar cognitive defects (EMA, 2008). Later, clinical trials examined the use of Idebenone for patients with Friedreich's ataxia and Duchenne muscular dystrophy, however, usage in any of these settings has either not been successful or approved (Buyse et al., 2003, Di Prospero et al., 2007, Tonon and Lodi, 2008). Despite this, Idebenone was approved as a treatment for Leber's hereditary optic neuropathy (LHON) in Europe. In LHON patients, it shows discontinuation of further visual impairment (Klopstock et al., 2011, Klopstock et al., 2013, Rudolph et al., 2013, Lyseng-

Williamson, 2016). LHON syndrome is a common mitochondrial disease, characterized by a bilateral visual loss caused by defective complex I. In more than 95% of cases, it is caused by one out of three mtDNA mutations in *ND4* (m.11778G>A), *ND6* (m.14484T>C), or *ND1* (m.3460G>A) genes (Yu-Wai-Man et al., 2002). Idebenone can bypass malfunctioning complex I and feed electrons directly to complex III. It is also recapturing electrons outside the inner mitochondrial membrane to prevent reactive oxygen species production. For its antioxidant properties, Idebenone supplementation was successfully implemented also in a patient harbouring a mutation in *TXN2*, coding for thioredoxin 2, a crucial mitochondrial antioxidant enzyme (Holzerova et al., 2016). Idebenone can easily be administered with fatty food and it is well absorbed into cells. However, less than 1% is able to pass through liver into circulation. This can be the main reason for its limited utility outside LHON cases.

Thiamine

Thiamine (vitamin B_1) intake is beneficial in patients with a defective thiamine metabolism and cerebral thiamine transport through cellular and mitochondrial membranes (Ortigoza-Escobar et al., 2016). It was reported to prevent further neurological deterioration and metabolic crisis caused by mutations in thiamine transport related genes (Ortigoza-Escobar et al., 2016, Haack et al., 2014, Pomahačová et al., 2017). The identification of defects in thiamine uptake in infancy or early childhood may improve the prognosis, because that is largely dependent on the prompt initiation of thiamine treatment (Pérez-Dueñas et al., 2013). Early diagnosis may be achieved via biomarkers such as a decreased concentrations of free-thiamine in cerebrospinal fluid or of thiamine pyrophosphate in blood and muscle (Marcé-Grau et al., 2019). Thiamine supplementation also helps to correct some other metabolic disorders, such as maple syrup urine disease and Leigh syndrome (Jauhari et al., 2017) as well as mitochondrial myopathy (Sato et al., 2000). The derivatives of thiamine play a role in several metabolic pathways, above all in the enzymatic reactions of sugar and amino acids catabolism or alcoholic fermentation. Thiamine also enhances the activity of pyruvate dehydrogenase, therefore increases production of reduced cofactors NADH and $FADH_2$ (Gray et al., 2014).

Riboflavin

Another member of the vitamin B family is riboflavin, vitamin B_2. It is a water-soluble precursor for flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD). Riboflavin or its derivatives take part in more than 100 enzymatic reactions including OXPHOS, fatty acid β-oxidation or the Krebs cycle (Lienhart et al., 2013). Riboflavin is synthetized in bacteria, plants and fungi; however, this ability has been lost in higher eukaryotes (Kaiser et al., 2002), therefore it must be obtained from food (Phillips and Shephard, 1993). Supplementation is recommended for patients with impaired flavoproteome, where it is supposed to modulate mitochondrial electron transfer flux (Balasubramaniam et al., 2019). As an example, the riboflavin therapy was effective and mitigated symptoms in a deficiency of ACAD9, an FAD-containing enzyme (Haack et al., 2010, Repp et al., 2018), as well as in the case of FAD synthase deficiency (Olsen et al., 2016, Muru et al., 2019). Likewise, riboflavin

supplementation was successfully used in multiple acyl-CoA dehydrogenase deficiency, typically caused by mutations in one of two genes coding for electron transferring flavoprotein *ETFA* or *ETFB* (Olsen et al., 2007, Olsen et al., 2016).

Niacin

The last example from the vitamin B family is vitamin B_3, which serves as a precursor of nicotinamide adenine dinucleotide (NAD^+). It exists in several forms: nicotinic acid, nicotinamide (collectively termed niacin), or nicotinamide riboside (NR). NAD^+ (or NADH if reduced) acts as a coenzyme in redox reactions, many of them occurring in substrate catabolism leading to the synthesis of ATP, or as a donor of ADP-ribose (Billington et al., 2006). It was shown that vitamin B_3 supplementation might have a beneficial outcome in case of disruption of NAXE (also known as APOA1BP) (Kremer et al., 2016, Trinh et al., 2020). NAXE is an epimerase for NAD(P)HX that is a toxic metabolite. In one patient, the combination of vitamin B_3 and CoQ supplementation brought continuous motor and cognitive improvement (Trinh et al., 2020).

Targeting NAD^+ metabolism was also attempted in general mitochondrial myopathies. In two mouse models of mitochondrial myopathy – deletor mouse (Khan et al., 2014) and *Sco2* KO/KI mouse (Cerutti et al., 2014) – supplementation with NR delayed symptoms of the disease and improved OXPHOS function. Mouse studies favoured NR as the optimal form of boosting NAD^+, since it does not inhibit sirtuins (Belenky et al., 2007). However, human studies to date used either nicotinamide or nicotinic acid, probably for their proven safety record in humans. Initial case report dating to 1996 described nicotinamide treatment in MELAS (Mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes, m.3243A>G) patient, which boosted NAD^+ levels and decreased blood and urine lactate (Majamaa et al., 1996). A recent study in genetically varied group of patients with mitochondrial myopathy, then demonstrated systemic NAD^+ deficiency in patients, which was corrected by nicotinic acid supplementation. During a 10-month follow-up their mitochondrial biogenesis was increased and partial correction of muscle metabolome as well as increase in muscle strength were observed (Pirinen et al., 2020).

Metal ions

Several inorganic metal ions (e.g. copper, iron, magnesium, molybdenum, and zinc) also belong to the cofactor group. For cytochrome *c* oxidase (COX) biogenesis, several mutations have been described in genes coding for proteins involved in copper delivery to the enzyme (*SCO1, SCO2, COA6*). In such cases, copper supplementation may also represent rational therapy. However, beneficial effect to *SCO2* patient is limited to one described case-study using copper-histidine (Freisinger et al., 2004). On the other hand, recent preclinical studies identified Elesclomol as a highly efficient copper transporting pharmaceutical agent, which corrects COX deficiency in *SCO2* patient cells (Soma et al., 2018). Since Elesclomol displayed favourable pharmacological

profile in other clinical trials (O'Day et al., 2013), it may be considered for trial in human SCO2 defects.

Nucleoside bypass therapy

Nucleoside bypass therapy represents a specific type of a small molecule treatment. It is relevant for patients with Thymidine kinase 2 (TK2) deficiency. TK2 is responsible for phosphorylation of thymidine and deoxycytidine in order to produce deoxythymidine and deoxycytidine monophosphate (dTMP and dCMP), precursors of mitochondrial dNTPs. The lack of dNTPs then results in mtDNA instability and causes mtDNA depletion or multiple deletions. In preclinical mouse studies, supplementation of dTMP and dCMP delayed the disease onset, prolonged life span, and led to restoration of mtDNA levels as well as activities of OXPHOS complexes (Lopez-Gomez et al., 2017, Garone et al., 2014, Blázquez-Bermejo et al., 2019). First human trial was initiated as compassionate use program, based on the recommendations from 232nd European Neuromuscular Centre workshop (Saada, 2019, ENMC. et al., 2017). Compared to a natural course of the disease of untreated TK2 patients, first results indicate improved clinical measures for patients with early onset of symptoms. To the contrary, for those with the late onset of the disease, nucleoside supplementation only led to a stabilisation of current symptoms (Domínguez-González et al., 2019). These results are now further elaborated in subsequent open-label long term clinical trial (NCT03639701).

In principle, analogous approach can be used in patients with deoxyguanosine kinase deficiency, enzyme responsible for proper function of mitochondrial purine nucleoside salvage pathway (El-Hattab et al., 2017).

Antioxidants (Redox modulators)

Mitochondrial disorders are often associated with increased levels of reactive oxygen species and *vice versa.* The elevation of oxidative stress can be caused either by a defective respiratory chain that is prone to leak electrons, or by a malfunction in the natural ROS removal. For a long time, ROS used to be seen only as dangerous agents that attack proteins, lipids and nucleic acids. However, in the last two decades, the importance of ROS in several signalling pathways has been described (Jadiya and Tomar, 2020). Antioxidants in the broadest sense can correct the potential disbalance between ROS production and removal in mitochondrial patients (Holzerová and Prokisch, 2015). Due to their nature, antioxidant is 'any substance that delays, prevents, or removes oxidative damage to a target molecule' (Halliwell and Gutteridge, 2015). The naturally-derived antioxidants include vitamin C (ascorbic acid) and E (tocopherol), glutathione or CoQ (Marshall, 2014). Based on their properties, artificial antioxidants have been produced, e.g. Trolox (analogue of vit. E), Idebenone and MitoQ (analogues of CoQ), or SkQ (a cationic derivative of the plant plastoquinone). Also, compounds such as N-acetylcysteine and α-lipoic acid are able to boost the glutathione biosynthesis (Magalhães et al., 2016). The group of antioxidant drugs also includes L-carnitine, resveratrol, vitamins K_1 and K_3 (menadione), or commercially produced EPI-743 (a para-benzoquinone analogue), RP103

(cysteamine bitartrate, a promoter of glutathione synthesis) (Enns, 2014, Russell et al., 2020) or KH176 (derivative of water-soluble form of vitamin E) (Koene et al., 2017, de Haas et al., 2017).

While some of these molecules are trialled for other conditions with supposed mitochondrial involvement (e.g. MitoQ and Alzheimer's disease (Russell et al., 2020)), use in mitochondrial patients mostly lacks reliable evidence of a therapeutic effect. Some trials (NCT02023866 for RP103) were terminated for lack of efficacy, others, such as phase IIA clinical trial of KH176 in adult m.3243A>G patients (NCT02909400) did not achieve their primary outcome. However, KH176 testing still proved its safety and achieved positive effect on alertness and mood (Janssen et al., 2019). These effects are now further explored in a follow-up phase IIB trial, again on m.3243A>G patients (NCT04165239). Successful was also the use of Idebenone in TXN2 patient, where it acted as an antioxidant (Holzerova et al., 2016). This is further discussed in the „*Idebenone*" section above.

Elamipretide

Elamipretide (also known as SS-31 peptide, Bendavia, or MTP-131) represents specific example of a compound falling under the antioxidants umbrella. Chemically, it is cell-permeable mitochondria-targeted antioxidant tetrapeptide with an alternating aromatic-cationic structure. It targets cardiolipin on the inner mitochondrial membrane and improves coupling of the electron transport chain and thus phospholipid-dependent bioenergetics (Zhao et al., 2004). Elamipretide was proposed to have beneficial effects in numerous conditions. For example, neuroprotective effects of Elamipretide were demonstrated on mouse model of Alzheimer's disease where it caused reduction of synaptic activity and Aβ levels (Reddy et al., 2017). Recent clinical research demonstrated significant improvement of mitochondrial function in cardiac cells through enhanced oxygen flux, CI and CIV activity and coupling of the supercomplexes after Elamipretide treatment (Chatfield et al., 2019). Use of Elamipretide showed cardioprotective effects and reduced myocardial infarction in experimental models of ischemia-reperfusion injury (Cho et al., 2007), as well as in human biopsies following simulated ischemia reperfusion (Wijermars et al., 2016).

Elamipretide has also been tested in several consecutive clinical trials for patients with mitochondrial myopathy. Trials involved approximately 30 patients with diverse myopathies either due to nuclear or mtDNA mutations. While in a five day dose-escalation trial they managed to achieve improvement in a 6-minute walk test (primary outcome) (Karaa et al., 2018), this was narrowly missed in four week crossover trial (Karaa et al., 2020). Nevertheless, secondary outcomes, such as reported fatigue were significantly improved (Karaa et al., 2020), and neither of the studies reported significant side effects. This prompted phase III trial for Elamipretide (MMPOWER-3, NCT03323749), however, this was recently terminated, since double blind portion of the trial did not meet the primary end points. Yet another clinical trial of Elamipretide for mitochondrial diseases (NCT02693119) evaluates its utility for LHON patients.

Agents enhancing mitochondrial biogenesis

Another approach is to increase mitochondrial population utilizing inducers of mitochondrial biogenesis, which was effective in animal models of mitochondrial disorders (Cerutti et al., 2014, Peralta et al., 2016). General inducers of mitochondrial biogenesis include epicatechin, RTA 408 (omaveloxolone, potentially beneficial in case of Friedreich's ataxia), or bezafibrate. The last one is a PPAR agonist that can affect the PPAR/PCG-1α pathway (Wu et al., 2020) leading to an increased mitochondrial mass, OXPHOS capacity, energy production and improvement in a supramolecular assembly and stability of OXPHOS complexes. Indeed, bezafibrate has shown promise as a disease modifying pharmaceutical agent in preclinical studies using both animal models (Dillon et al., 2012) and cell lines (Hofer et al., 2014). In patients with impairment in ACAD9, bezafibrate shows increase in complex I activity followed by improved respiration in patient cell lines (Repp et al., 2018). In a recent study, the effect of bezafibrate has been examined on mitochondrial function in fibroblasts derived from a patient carrying a dominant negative dynamin-1-like protein *DNM1L* mutation. It improved mitochondrial morphology, although causing a mild increase in ROS production at the same time (Douiev et al., 2020). Bezafibrate also controls the expression of many fatty acid oxidation (FAO) genes, thus is commonly used to treat dyslipidemia, and as a potential treatment for FAO disorders. It showed beneficial response in six patients with the myopathic form of carnitine palmitoyltransferase 2 (CPT-2) deficiency, where it markedly upregulated CPT-2, increased oxidation rates of the long-chain fatty acids, decreased muscle pain and increased physical activity in all treated patients (Bonnefont et al., 2010). Unfortunately, bezafibrate improved neither FAO nor exercise tolerance in patients with CPT-2 and very long-chain acyl-CoA dehydrogenase deficiencies, possibly due to the high plasma insulin in patients, which markedly inhibited lipolysis and masked effects of bezafibrate (Ørngreen et al., 2014). Recent open-label clinical trial on six patients with mitochondrial myopathy caused by the m.3243A>G *MTTL1* mutation, shows expected induction of FAO, but minimal impact on markers of mitochondrial biogenesis (Steele et al., 2020). Importantly, in the same group of patients, they observed reduction in the number of complex IV-immunodeficient muscle fibres together with independent improvement of cardiac function. However, this was accompanied by an increase in serum biomarkers of mitochondrial disease and dysregulation of fatty acid and amino acid metabolism, indicating necessity for careful consideration of all outcomes for a long-term therapy (Steele et al., 2020).

Rapamycin

Rapamycin (also known as sirolimus) has a general effect on cellular fitness and thus can act in a similar fashion as agents enhancing mitochondrial biogenesis. Biochemically, rapamycin is a cellular inhibitor of mTOR (mammalian target of rapamycin) that has a broad effect on cell growth, metabolism, autophagy, lipid synthesis, transcription, translation, mitochondrial biogenesis, cell proliferation, and cell survival (Kaur and Sharma, 2017).

There is accumulating body of preclinical evidence for the utility of rapamycin in treatment of mitochondrial disorders. For example, at cellular level it was shown that rapamycin can activate

mitophagy selectively in cells with pathogenic mtDNA mutation (Dai et al., 2014). Rapamycin was also demonstrated to ameliorate disease outcomes in four mouse models of mitochondrial disorder (Johnson et al., 2013, Khan et al., 2017, Siegmund et al., 2017, Ferrari et al., 2017, Civiletto et al., 2018). Moreover, administration of rapamycin in Deletor (Khan et al., 2017) and *Ndufs4* KO (Leigh syndrome model) mice (Johnson et al., 2013) resulted in prolongation of lifespan. As a cautionary note, this is a general effect of the drug observed also in various models of ageing, so while beneficial, it may not be specific for models of mitochondrial dysfunction (Johnson and Kaeberlein, 2016, Garone and Viscomi, 2018). Although the precise mechanism of rescue remains to be elucidated, it seems that the stimulation of mitochondrial turnover, increase of autophagic flux and upregulation of lysosome biogenesis are probably responsible for rescuing mitochondrial disease phenotype via a promotion of metabolic reprogramming (Civiletto et al., 2018).

Just recently, two paediatric patients have been treated with a rapamycin analogue everolimus. A patient with Leigh syndrome showed sustained benefit, while clinical course of a patient with MELAS followed the known natural progression of the disease and resulted in an early death (Sage-Schwaede et al., 2019). Based on the preclinical observation, rapamycin is currently tested in paediatric patients with Leigh or Leigh-like syndrome (NCT03747328). Further trials might benefit from other molecules targeting mTOR pathway, but with different mechanisms of action than rapamycin (Lamming et al., 2013).

Additional small compounds

Next to the already mentioned compounds, several other drugs might be used to treat mitochondrial disorders. Examples are: (1) arginine or citrulline as precursors of nitric oxide whose lack causes stroke-like episodes; (2) creatine as a precursor of phosphocreatine serving as a source of high energy phosphate in metabolism; (3) succinate, a substrate of complex II (Parikh et al., 2009, Kerr, 2013, El-Hattab et al., 2017). More recently, also the synthetic cell-permeable analogue of succinate (NV189), has been shown to improve electron transport, membrane potential and ATP production in Leigh syndrome cell culture model (Ehinger et al., 2016). Second generation of succinate prodrugs (NV354) is currently being explored with the aim to bring them to clinical testing.

Some mitochondrial treatments aim to enhance OXPHOS function through increasing substrate availability. Dichloroacetate (DCA) is a structural analogue of pyruvate and is able to activate the pyruvate dehydrogenase through the inhibition of pyruvate dehydrogenase kinase. Therefore, DCA increases flux of pyruvate into mitochondria and boosts mitochondrial respiration and generation of ATP. DCA is generally well-tolerated in individuals with congenital lactic acidosis (Abdelmalak et al., 2013) and beneficial for alleviating lactic acidosis in mitochondrial diseases (Barshop et al., 2004). Since it causes peripheral nerve toxicity, DCA has not been shown as an efficient treatment in controlled clinical trial for MELAS patients (Kaufmann et al., 2006). A randomized controlled trial has been initiated recently, based on clinical and molecular genetic criteria in children with pyruvate dehydrogenase complex deficiency, i.e. the most common cause of congenital lactic acidosis. According to the authors

of this clinical trial, DCA possesses an ability to increase both the catalytic activity and stability of the enzyme complex (NCT02616484). Moreover, a recent study also identified phenylbutyrate as an induced of PDH complex activity. Interestingly, effects of phenylbutyrate and DCA seem to be additive, suggesting a potential therapeutic direction (Ferriero et al., 2015). Phenylbutyrate may also substitute DCA in future trials, since it causes less harmful side effects than DCA (Stacpoole et al., 2019).

Metabolic approach

Endurance training

Mitochondrial disorders frequently lead to a muscular weakness, mitochondrial myopathy and low exercise capability (Taivassalo et al., 2003). This fuelled research into possible use of endurance exercise as a tool to modulate progress of the disease (Ziaaldini et al., 2017). At the molecular level, exercise works through stimulation of PGC-1α and PGC-1β co-activators, which regulate mitochondrial biogenesis and oxidative metabolism (Handschin and Spiegelman, 2006). PGC-1α/β upregulation then improves OXPHOS function in cells to compensate for respiratory chain defects (Srivastava et al., 2009). In patients, endurance exercise may delay progressive deterioration of their condition and has been shown to improve exercise capacity over time. It was reported that endurance training improved mitochondrial function in muscle biopsies through an increase in total content of mitochondria and their increased activity was visualized by staining of the CIV activity. Consequently, it facilitated improvement in maximal oxygen uptake, peripheral muscle strength, not to mention the overall improvement of clinical symptoms (Taivassalo et al., 2006, Safdar et al., 2016).

Preclinical evidence comes from several mouse models of mitochondrial dysfunction. For example, studies on mtDNA mutator mice demonstrated improvement of progeroid phenotype upon exercise training (Safdar et al., 2011). This was further elaborated in subsequent work, where depletion of NAD^+ and carnitine in the brain was observed and this was partially improved by training (Clark-Matott et al., 2015). Second studied model was Harlequin mouse (*Hq*), with complex I deficiency due to reduced levels of AIF assembly factor (Bénit et al., 2008). Training program in these animals was started after the first signs of ataxia, at that time also myopathy was already present, which is a good simulation of the patient's situation. Aerobic and resistance training improved aerobic fitness, muscle strength and activity of OXPHOS complexes I, II and V in *Hq* mice. Training also slightly improved levels of proteins TFAM and PGC-1 (Fiuza-Luces et al., 2019).

Human studies have demonstrated that endurance training is safe and beneficial for the patients with metabolic myopathies caused by a large-scale mtDNA deletions (Porcelli et al., 2016, Taivassalo et al., 2006). Skeletal muscle function was also improved in patients with cardiomyopathy due to m.3243A>G (Bates et al., 2013). Maximal oxygen uptake (VO_{2max}), activity of citrate synthase and mtDNA quantity in muscle tissue increased after 12 weeks of exercise (Jeppesen et al., 2006). However, another smaller study showed that mutation load in mtDNA did not change during or after a training program (Jeppesen et al., 2009). A recent study with 12 patients with mtDNA mutations or deletions and with different clinical symptoms

introduced special training program composed of aerobic training, resistance training and inspiratory muscle training for 8 weeks. This scheme was well tolerated and produced significant benefits in numerous indicators of their physical capacity, including aerobic power, muscle strength, and inspiratory muscle power (Fiuza-Luces et al., 2018)

In conclusion, endurance or resistant training seems to be favourable for the majority of patients with mitochondrial disorder, especially for those with mtDNA mutations or deletions (Murphy et al., 2008, Fiuza-Luces et al., 2018). Unfortunately, about 20% of those patients are suffering from exercise intolerance (Mancuso et al., 2012) and thus enrolment into exercise scheme has to be closely monitored.

Ketogenic diet

Ketogenic diet (KD), due to its numerous positive effects on metabolic and neurodegenerative disorders, became a promising therapeutic option. The idea of ketogenic agent administration in order to induce and sustain therapeutic ketosis has been present for decades (Miller and Dymsza, 1967). Generally, the ketogenic diet is a high-fat, low-protein, low-carbohydrate diet that has been employed for anticonvulsant effects on epileptic seizures (Zhang et al., 2018). The hallmark feature of KD treatment is the increased production of ketone bodies by the liver, reduced blood glucose and stimulation of mitochondrial β-oxidation followed with increased mitochondrial function. KD forces a switch to predominant metabolism of ketones rather than glucose, and enhances conversion of ketone bodies such as beta-hydroxybutyrate and acetoacetate into acetyl-CoA to support ATP production (Yudkoff et al., 2007). Today KD is used as treatment option in numerous conditions with suspected mitochondrial involvement, such as Huntington disease (Ruskin et al., 2011), Alzheimer`s disease (Van der Auwera et al., 2005), or Parkinson`s disease (Hartman and Vining, 2007).

Original suggestions for use of ketones in mitochondrial disease is derived from studies on cybrid cell lines. Here, ketone bodies in culture medium caused heteroplasmy shift towards wild-type mtDNA in Kearns–Sayre syndrome (Santra et al., 2004) and LHON with m.13094 T > C heteroplasmic mutation (Emperador et al., 2019). In both cases, heteroplasmy shift was connected with improvement of mitochondrial function. *In vivo* animal studies then demonstrated, that in healthy mice models, KD administration increased longevity and health-span (Roberts et al., 2017) and its beneficial effects were also demonstrated on models of mitochondrial dysfunction. Thus, KD improved assembly and activity of impaired complexes, normalized mitochondrial morphology and ultrastructure and normalized metabolomic profile in deletor mice (Ahola-Erkkilä et al., 2010) as well as in *Bcs1l* KO model (Purhonen et al., 2017). KD also increased mitochondrial biogenesis in brain of mice with mutated mtDNA repair enzyme UNG1 (Hasan-Olive et al., 2019). However, as a cautionary note, despite increased biogenesis, KD in this model ultimately led to aggravated neurodegeneration and mitochondrial deterioration (Lauritzen et al., 2016).

Since ketone bodies are metabolized to acetyl-CoA, they may at least partially bypass complex I, which led to a suggestion, that patients with complex I defects may benefit most from KD. Indeed, there are numerous case reports (reviewed e.g. in (Scholl-Bürgi et al., 2015)), which

demonstrated improvement in clinical symptoms (typically seizures) but without a long-term follow-up. Utility of KD for other types of mitochondrial disease remains to be elucidated. Of interest is a study on patients with mitochondrial myopathies treated with modified Atkins diet, which led to muscle pain and selective lysis of abnormal muscle fibres, ultimately causing premature termination of that trial. However, two years of follow-up demonstrated improvement of muscle strength, suggesting activation of muscle regeneration (Ahola et al., 2016). While KD may show substantial promise in preclinical models, its use in human setting has therefore to be closely evaluated.

Hypoxia

Rather unexpected therapeutic option for mitochondrial diseases is also chronic hypoxia. Beneficial effects of chronic continuous normobaric hypoxia with 11% of oxygen were observed for mice with *Ndufs4* knockout as well as frataxin (*Fxn*) knockdown. For *Ndufs4*$^{-/-}$ mice, hypoxia corrected biochemical defect as well as neurological phenotype and led to dramatic increase in overall survival (Ferrari et al., 2017, Jain et al., 2016). To the contrast, in frataxin shFXN model it alleviated ataxia phenotype, but without concomitant effect on survival (Ast et al., 2019). Importantly, in both cases mild hyperoxia aggravated neurological phenotype. Molecular basis for beneficial effect of hypoxia still has to be elaborated, but it was demonstrated, that in *Ndufs4*$^{-/-}$ mice, it is independent form the action of HIF signalling pathway. Rather, it seems that hyperoxia in the brain of *Ndufs4*$^{-/-}$ animals is actually responsible for the development of pathology (Jain et al., 2019). Yet another physiological rationale for beneficial effect of hypoxia offers the observation on the same *Ndufs4*$^{-/-}$ mice, have impaired ability to induce hypoxic pulmonary vasoconstriction (HPV) response, which matches perfusion to ventilation in lungs. However, HPV response was restored after three weeks under normobaric hypoxia. Thus, under hypoxic conditions *Ndufs4*$^{-/-}$ mice can better regulate their blood oxygenation (Schleifer et al., 2019).

Overall the whole phenomenon of therapeutic use of hypoxia for mitochondrial diseases is rather obscure and its utility for the whole spectrum of disease genes cannot be granted. An interesting recent study on cellular model looked for the selective essentiality of individual genes under hypoxia and hyperoxia. It identified that hypoxia can buffer the loss of most complex I genes, genes from FeS cluster biosynthesis, CoQ biosynthesis and from pyruvate dehydrogenase complex (Jain et al., 2020). It can be anticipated, that patients with mutations in genes from these pathways may benefit from hypoxia treatment, while it can be neutral or even damaging for others. All in all, while hypoxia may represent an elegant treatment option, at the moment it is still limited to preclinical evidence and no human studies have been reported, yet.

Organ transplantation

Rather niche invasive approach to treatment of mitochondrial disease is represented by organ transplants. This strategy is also utilised for other inborn errors of metabolism and can serve well in conditions, where enzyme dysfunction leads to accumulation of toxic compound, which

can be cleared by the allogenic transplanted organ (Tan et al., 2019). Best example is mitochondrial neurogastrointestinal encephalomyopathy (MNGIE), caused by mutations in thymidine phosphorylase (*TYMP*). This leads to an accumulation of thymidine and deoxyuridine and secondary mtDNA depletion. Haematopoietic stem cell transplantation (HSCT) has been attempted in sizeable cohort of MNGIE patients and led to an improvement of both biochemical parameters and clinical symptoms (Halter et al., 2015). Rather high mortality associated with HSCT in MNGIE patients led to exploration of other strategy, namely liver transplantation, since liver has high expression of thymidine phosphorylase. Three reports to date cover six cases and all presented with improvements in biochemical and clinical characteristics at one-year follow-up (Madhok et al., 2019, D'Angelo et al., 2017, De Giorgio et al., 2016, Kripps et al., 2020).

Liver transplantation has also been attempted in patients with ethylmalonic encephalopathy caused by mutations in *ETHE1* gene coding for mitochondrial sulphur dioxygenase. It is associated with toxic accumulation of hydrogen sulphide which inhibits cytochrome *c* oxidase (CIV) and leads to a progressive neurological disorder (Tiranti et al., 2009). In all three cases reported so far, liver transplant led to improvement in neurological phenotype (Dionisi-Vici et al., 2016, Tam et al., 2019), but in one case this was not corroborated by improved biochemical characteristics, suggesting continued risk for the patient (Tam et al., 2019).

Overall, tissue transplants seem to be a perspective approach, effectively serving as an enzyme replacement therapy. With increasing confidence in their outcome, they can be considered for mitochondrial patients. Still, better outcomes can be expected if they are performed at early disease stage, before profound target tissue damage by toxic metabolites takes place.

Genomic approach

Genome editing

Unlike the above-mentioned types of treatment, genome editing techniques might solve the actual cause of the mitochondrial disorders and prevent a transmission to the offspring. There are several approaches, tailored for different types of disorders: (1) gene replacement, which is effective in monogenic disorders; (2) gene addition which may help in complex disorders such as heart failure and cancer; (3) gene expression alteration by RNA interference resulting in a removal of a target malfunctioning RNA; (4) precision genome editing via zinc finger endonucleases (ZFNs), TALENs (transcription activator-like effectors nucleases), or CRISPR (clustered regularly interspaced palindromic repeat)/Cas9 systems (Baker, 2012, Hsu et al., 2014, Wang and Gao, 2014). Gene editing approaches for mitochondrial diseases of nuclear origin can follow strategies for any other genetic disorder. Thus, the CRISPR/Cas9 editing system was reported in a model of CoQ deficiency, where it successfully corrected a mutation in the *COQ4* (Romero-Moya et al., 2017). In contrast, mtDNA mutations require unorthodox approaches, driven by complexities of mtDNA genetics, as well as accessibility of mtDNA for genetic tools. These aspects will be discussed below.

In the case of mitochondrial disorders, LHON syndrome is a promising target of a gene therapy (Karaarslan, 2019). Currently, LHON is one of the most common inherited optic neuropathies, occurring in 1 out of 30 000 patients, predominantly diagnosed in young men (Yu-Wai-Man et al., 2003). As mentioned already in the Idebenone section, only 3 mtDNA point mutations are responsible for 95% of LHON cases (Yu-Wai-Man et al., 2002). Therefore, a gene therapy approach is being investigated in ongoing clinical trials, currently in phase III. Patients with the most common mutation m.11778G>A (~70%) are injected into the vitreous cavity of a single eye with a wild type ND4 protein carried by an adeno-associated viral (AAV) delivery (El-Hattab et al., 2017). *ND4* transgene is modified to a nuclear DNA sequence to ensure consistency with nuclear translation and carries a mitochondrial targeting sequence (COX10 or ATP5MC1) to be transported into mitochondria after the expression in cytosol (Yang et al., 2016, Feuer et al., 2016). As reported in the clinical trials, patients' visual acuity improved and visual field was enlarged without observation of any undesirable side-effects (Wan et al., 2016, GenSight, 2019, Yang et al., 2016, Bouquet et al., 2019, Feuer et al., 2016). Rather surprisingly, improved visual function was observed also for the second, sham treated eye, counter to natural history of the disease. Recent data from non-human primates, demonstrating presence of AAV vector also in the non-treated eye may offer a clue for this observation (Moster et al., 2020).

Since there are many copies of mtDNA per cell, only a portion of them can carry pathological variant – an effect known as heteroplasmy (Gorman et al. 2016). Furthermore, levels of heteroplasmy may differ between individual cells and tissues in the body. If the percentage of heteroplasmy surpasses a certain level, called a threshold, a mitochondrial disorder can manifest. For some common mitochondrial diseases, the threshold level is known. Typically, a high proportion of mutated mtDNA (> 60%), including e.g. mtDNA deletions, is a prerequisite of cellular defects (Koene and Smeitink, 2011, Grady et al., 2014, Nuskova et al., 2020, Hejzlarová et al., 2015). Therefore, the various clinical presentations of mutations in mitochondrial DNA manifest as a direct consequence of the heteroplasmy level in the specific tissue (Stewart and Chinnery, 2015).

Genome editing systems are being developed to manipulate levels of mtDNA heteroplasmy. Briefly, the mutated mtDNA is targeted for removal, and is hence kept under the threshold level. Several of the currently available genome editing tools had been manipulated to target into mitochondria and partially or completely remove the mutant mtDNA. These tools can also be used to reduce the levels of mutant mtDNA in embryos during *in vitro* fertilization (Pereira and Moraes, 2017). Still, the current limitation of all of these approaches for clinical usage lays in the theoretical danger of rapid depletion of mtDNA (Hirano et al., 2018).

First of all, mitochondria-targeted restriction endonucleases (RE) have been tested in human-derived cell lines as well as in mouse models. RE are small enzymes able to recognise a unique DNA sequence and create double strand-breaks that lead to a rapid mtDNA removal (Bayona-Bafaluy et al., 2005). The specificity of the enzymes targets the mutated sequence and leaves the wild-type intact. However, the drawback of this approach lays in the finite number of available RE. Effectively, the only example of RE targeting human pathogenic mutation is SmaI and its isoschisomer XmaI, which cleave m.8993T>G mutation in *MT-ATP6* causing severe early-onset NARP (neuropathy, ataxia, and retinitis pigmentosa) or MILS (maternally inherited

Leigh syndrome) syndrome. The usage of this RE resulted in an increased ATP production in cybrid cells (Tanaka et al., 2002, Alexeyev et al., 2008). Mouse studies demonstrated *in vivo* utility of RE approach on polymorphisms between NZB and BALB mtDNA. Here, ApaLI and ScaI RE were delivered via viral vectors and both lead to a significant shift in mtDNA heteroplasmy in either muscle and brain, or liver tissue (Bayona-Bafaluy et al., 2005, Bacman et al., 2007)

Next, ZFNs and TALENs have been manipulated for mitochondrial localization. In contrast to RE, ZFNs and TALENs can be designed to target a specific sequence. First mitochondrially targeted ZFNs aimed pathogenic mtDNA with m.8993T>G point mutation mentioned above and a large "common deletion" (a 4977-bp deletion causing CPEO, Kearns-Sayre or Pearson's marrow pancreas syndromes). This study successfully decreased mutant mtDNA haplotype load without mtDNA depletion and with restoration of OXPHOS function in a cybrid cell model (Gammage et al., 2014). Next, mtZFN were used in mouse m.5024C>T $tRNA^{Ala}$ model, where mtZFNs delivered by AAV eliminated mutant mtDNA and reversed molecular and biochemical phenotypes in a heart (Gammage et al., 2018b). Another study demonstrated possible utility of mtZFNs in early mouse embryos to prevent transmission of mutated mtDNA through the germline (McCann et al., 2018).

Similarly, mitochondrial targeted TALENs (mitoTALENs) were tested in several patient-derived cell models of mtDNA mutation. MitoTALENs activity led to a reduction of mutated mtDNA (Bacman et al., 2013). In the next study, mitoTALENs were designed to target frequent point mutations m.8344A>G (causing MERRF – myoclonic epilepsy with ragged red fibers) and m.13513G>A (causing MELAS/Leigh syndrome). Both mitoTALENs were able to manipulate heteroplasmy and improve OXPHOS capacity. Also, TALENs targeting m.8344A>G were reduced in size for a better viral packaging with aim to increase its therapeutic potential (Hashimoto et al., 2015, Pereira et al., 2018). *In vivo* utility was demonstrated in mouse m.5024C>T $tRNA^{Ala}$ model, where AAV delivered mitoTALENs stably reduced load of mutant mtDNA in the affected tissues, muscle and heart, and this was accompanied by concurrent restoration of $tRNA^{Ala}$ levels (Bacman et al., 2018).

Most controversial at the moment is the possibility to adapt CRISPR based systems for mtDNA editing. Gammage et al. (Gammage et al., 2018a) claim that usage of CRISPR/Cas9 to manipulate mtDNA is questionable because of theoretical impossibility to transport so called guide RNA (gRNA) to mitochondria. CRISPR/Cas9 system is however dependent on the presence of gRNA, because it recognizes the targeted binding site. Still, mitochondrially targeted Cas9 (mitoCas9) was reported to specifically cleave mtDNA. Presumably, the mitoCas9 is able to bind the gRNA in the cytoplasm and transport it into the mitochondria (Jo et al., 2015). Moreover, the CRISPR/Cas9 system was able to edit mtDNA by knock-in strategy both in human cells and zebrafish. F_1 zebrafish offspring were found to harbour the designed mtDNA mutation (Bian et al., 2019). The newest development in the mitochondrial CRISPR system operates with stem loop element added to the gRNA that facilitates its transport into the organelle (Hussain et al., 2020).

Altogether, all gene editing techniques may present a potential individual-based therapy for the treatment of inherited mitochondrial diseases in the future.

Mitochondrial replacement therapy

Mitochondrial replacement therapy (MRT) presents relatively novel and effective technique in avoidance of inheritance of mtDNA mutations. If a mother is at risk of transmitting mutated mtDNA to her offspring, she can take the advantage of mitochondrial donation techniques. Either a maternal spindle transfer or pronuclear transfer is used, differing in the timing of the transfer, whether it is performed pre- or post-fertilization. In principle, the nuclear material (i.e. spindle or pronuclei) is removed from a mother's oocyte and transferred into a donor denucleated oocyte, where the healthy mitochondria are already located in cytoplasm (Rai et al. 2018). However, there is always a risk of the disease development, since a small amount of mutated mtDNAs are transferred from the recipient mother's mitochondria during the process. Unfortunately, if more than 5% of the mutant mtDNA is transmitted, a significant chance of disease development exists (Samuels et al., 2013). The preclinical studies and optimization were able to lower the mutant molecules to less than 2% (Hyslop et al., 2016). Thus, MRT techniques were successfully used in a mother carrying m.8993T>G mutation causing Leigh syndrome with a history of unsuccessful pregnancies. Her son born from MRT carried mutation load of 2.36-9.23% in tested tissues and was 7 months old at the time of publication. Long-term follow up is crucial (Zhang et al., 2017).

Another concern lays in the incompatibility of the nuclear and mitochondrial genomes. Concurrently, the outcome of a genome incompatibility in the case of introducing a novel mtDNA to a foreign nuclear genome is still questionable. Therefore, donor mothers with matching haplotype should be selected (Pan et al., 2019, Gómez-Tatay et al., 2017).

MRT usually undergoes thorough ethical reviews in countries considering its legalisation. This process had been very transparent in the United Kingdom, where MRT had been finally permitted in 2015 (Lightowlers et al. 2015; Gorman et al. 2016). Later, in 2017, the first clinic in the UK was licensed to provide this treatment (Rahman and Rahman, 2018). A current clinical trial will assess the outcome of mitochondrial donation on the first 75 children born (Russell et al., 2020). Ethical issues are still a matter of concern and for example in the USA, a National Academies of Sciences expert committee recommends to create male offspring to reduce the risk of disease transmission to the next generations (Reardon, 2016). Despite this, during the end of 2019, USA has taken a fairly restrictive approach to MRTs (Cohen et al., 2020). Specifically, in the Czech Republic, the legislature does not regulate the mitochondrial transfer, however, the potential usage of MRT must be approved by the State Institute for Drug Control.

In general, legal approach towards MRT is still developing. In addition to the United Kingdom, the Governments of Australia and Singapore are in the process of formal discussions aimed at MRT legalisation, but no formal decisions have been reached yet. Situation in other countries is even hazier. A recent study identified 21 countries where cytoplasmic transfer or nuclear transfer was available, namely Albania, Barbados, France, Georgia, Germany, Greece, India, Israel, Mexico, Northern Cyprus, Panama, Philippines, Portugal, Russian Federation, Serbia, Singapore, South Africa, Spain, Thailand, Turkey and Ukraine (Ishii and Hibino, 2018).

Clinical trial registries and the websites of clinics offering cross-border reproductive care were then identified in 16 countries: Albania, Canada, Czech Republic, China, India, Israel, Italy, Japan, Mexico, Northern Cyprus, Spain, Taiwan, Turkey, Ukraine, UAE and USA (Ishii and Hibino, 2018). On the other hand, other prerequisites for MRT, such as oocyte donation are not permitted in some of the listed countries (e.g. Germany, Turkey, UAE), which effectively prevents MRT in those countries. Because of restrictions in some countries and permissions in others, reproductive cross-border traveling is expected. In forthcoming laws, to prevent inheritance of modified genetic information, male-only selection of embryos for implantation could be preferable, but this raises ethical and moral issues.

Concluding remarks

Diagnosis of mitochondrial disorders has been accelerated by introducing Next-Generation Sequencing techniques in recent years. However, the treatment options are still very limited and for many patients only supportive or symptomatic therapies are available (Hirano et al., 2018). As discussed, most of the available therapies are in the phase of ongoing clinical trials and therefore still considered as an experimental treatment.

The potential for future development lays in discovery of new therapeutic options. The phenotypic and genotypic range of mitochondrial disorders (Fig.1) complicates therapeutic approaches. Evidently, the final decision for a specific treatment must reflect the cause of the disease. Principally, rare mitochondrial disorders demand ‘rare’ treatment tactics, hence personalized medicine. First of all, the metabolic approach could be favourable for some patients – endurance training for patients with mutations and deletions in mtDNA and ketogenic diet for patients with complex I deficiency. Luckily, drug development is a rapidly evolving field and many drugs currently undergoing approval process are in different phases of clinical trials. Yet, only 10 out of 49 clinical trials focussing on mitochondrial disease are currently in Phase III (Weissig, 2020). In principle, only Idebenone has been approved by U.S. Food and Drug Administration and European Medicines Agency specifically for treatment of mitochondrial disorders.

It is unlikely that there will be a drug to address a broad range of mitochondrial disorders. Yet, some underlaying biochemical principles or disease outcomes (such as accelerated glycolysis, increased ROS production, lack of a specific vitamin/cofactor) may be similar, revealing potential selective therapeutic directions as well. One promising example is represented by a current study of an engineered enzyme LOXCAT. It is able to extracellularly convert lactate and oxygen to pyruvate and water in order to manipulate lactate/pyruvate proportion and hence intracellular $NADH/NAD^+$ ratio. Since patient cells and tissues often show shift in $NADH/NAD^+$ balance, which is subsequently responsible for a 'reductive stress', correction of this disbalance may be therapeutically effective. LOXCAT technique has been submitted for a patent consideration (Patgiri et al., 2020).

Moreover, mitochondrial deficiency can also be a secondary defect in common diseases such as Parkinson’s, Alzheimer’s, ALS or cancer (Hadrava Vanova et al., 2020, Cedikova et al., 2016). These conditions may drive the necessary research associated with drug development

and allow later repurposing for primary mitochondrial diseases. Together with novel approaches including gene therapy or mitochondrial donation, potential cure is becoming available for an increasing number of patients. It is therefore important to recognize patients that could profit from any supplementation, such as vitamins or antioxidants, as early as possible, in order to maximize treatment benefits (Distelmaier et al., 2017).

Also, the change of attitude towards clinical trials will be required in the future, especially in the case of mitochondrial diseases. It is hardly acceptable to deny treatment to the patient in cases, where a functioning therapy exists, even if it is still considered as experimental. Good example here is nucleoside bypass therapy in *TK2* deficiency, where a successful treatment outcome can only be evaluated against natural disease progression and placebo-controlled trial will now hardly be approved. Therefore, the history of a successful compassionate use should be a sufficient proof for a drug approval. Altogether, the rare diseases require an innovative trial design (Abrahamyan et al., 2016, Rahman and Rahman, 2018). Simultaneously, due to clinical heterogeneity and limited number of patients manifesting the same phenotype, it is crucial to establish international consortia in order to find a treatment.

The future of personalised medicine lays in further adoption of gene therapy and mitochondrial replacement techniques despite all ethical controversies. Especially the MRT offers a possibility of having children for women with mtDNA mutation. Often, these women suffered from miscarriages and were forced to voluntary childlessness, oocyte donation or adoption. However, genetic counselling and preimplantation genetic diagnosis still represents option for parents with a child carrying any type of mutation to mitigate the chances of having another child affected by the same disorder.

Acknowledgements

This work was supported by Czech Health Research Council (16-33018A). Institutional support (Institute of Physiology) was provided by Czech Academy of Sciences (RVO:67985823). We acknowledge Kristýna Čunátová for her assistance in preparation of Figure 1.

Conflict of interest: Authors declare no conflict of interest.

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Figures

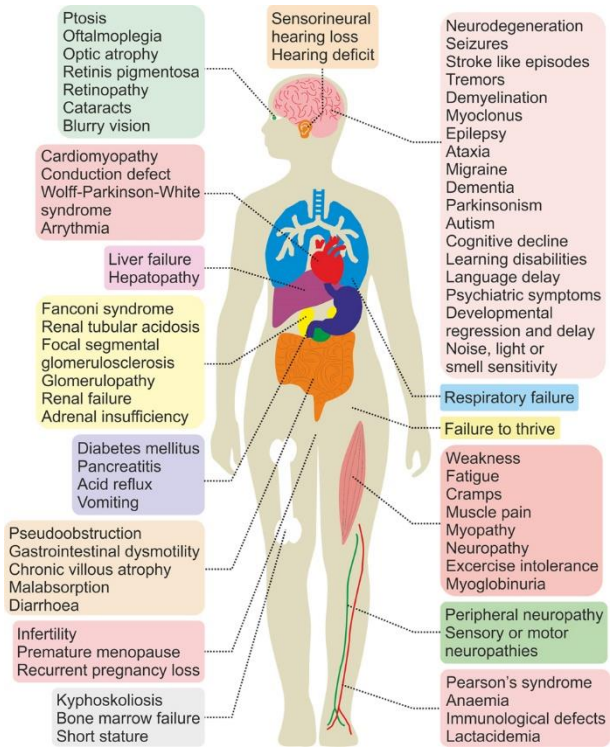

Figure 1: Clinical symptoms of mitochondrial diseases. Mitochondrial disorders can manifest with a variety of clinical symptoms. They vary from very mild ones to very severe, mostly neurological difficulties.

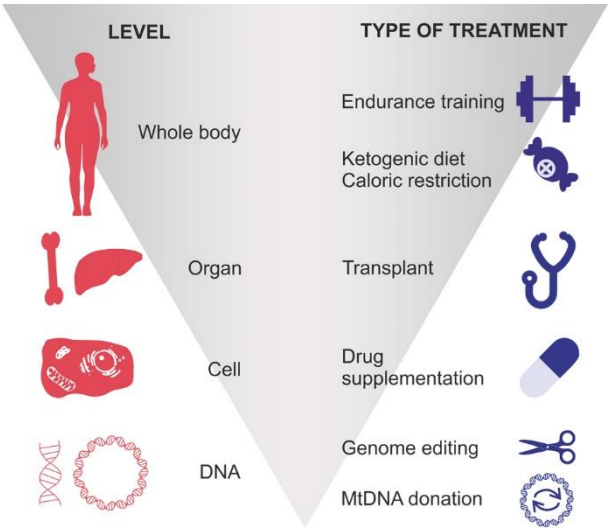

Figure 2: Strategies to mitigate mitochondrial disease pathology. Combating mitochondrial disorders can take place on different levels. Vast majority of the treatment option is symptomatic. However, new methods based on genome editing present the future of cure for these genetic disorders.

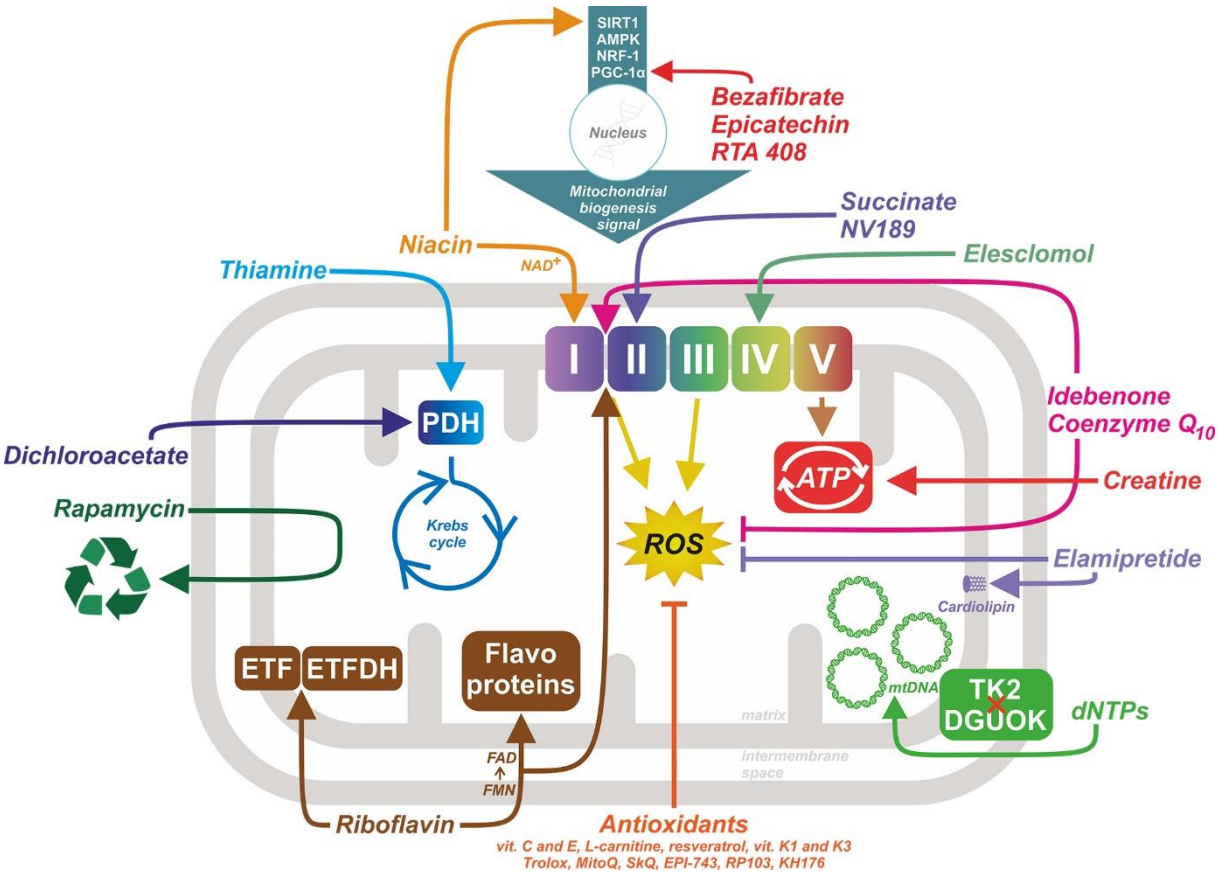

Figure 3: Targets of individual small molecules. Points of interference with biochemical pathways are indicated by arrows for individual small molecules. See text for further details.