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Therapies for Mitochondrial Disorders

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Cover Page Footnote

Firstly, I would like to thank my incredibility supportive, kind and knowledgeable supervisor Dr Anne Mulvihill, for all the encouragement and motivation that facilitated me in writing this article. Her unconditional praise and positive reassurance made me believe in myself and in my ability to complete this project in a timely manner. I am eternally grateful and privileged to have had Dr Anne Mulvihill as my supervisor, and for her overall guidance throughout my entire academic journey. This includes her encouragement towards my masters application, to study my passion for genetic and genomic counselling. In addition, I would like to thank my parents for their continuous support and review of this article. In particular, my father 'Roger Smyth' who helped me think of better ways of phrasing sentences, as well as teaching and me how to avoid grammatical error. Their unconditional support and positive regard meant a lot to me along the way. Lastly, I would like to thank Dr Anne Friel for inviting me to participate in the SUREJ article writing. This has been a wonderful opportunity that I am very grateful for, and my scientific writing has improved 100 fold throughout the journey.

Therapies for Mitochondrial Disorders

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Abstract

Mitochondria are cytoplasmic, double-membrane organelles that synthesise adenosine triphosphate (ATP). Mitochondria contain their own genome, mitochondrial DNA (mtDNA), which is maternally inherited from the oocyte. Mitochondrial proteins are encoded by either nuclear DNA (nDNA) or mtDNA, and both code for proteins forming the mitochondrial oxidative phosphorylation (OXPHOS) complexes of the respiratory chain. These complexes form a chain that allows the passage of electrons down the electron transport chain (ETC) through a proton motive force, creating ATP from adenosine diphosphate (ADP). This study aims to explore current and prospective therapies for mitochondrial disorders (MTDS). MTDS are clinical syndromes coupled with abnormalities of the ETC and OXPHOS, caused by pathogenic variants in mtDNA or nDNA. Many MTDS emerge from either homoplasmic or heteroplasmic mutations of the DNA, and include mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes. Current therapies include increasing mitochondrial biogenesis, the use of antioxidants, dietary approaches, and exercise. However, these are mostly symptomatic and supportive therapies. Future therapies comprise of personalised and precision medicine approaches which include gene, mitochondrial, cell, and *in utero*-based therapies. Obstacles towards discovering effective therapies include the rarity of MTDS, its pathogenic complexity and lack of clinical trials. Despite the lack of current curative therapies for MTDS, emerging therapies promise exciting and clinically meaningful therapies in the future.

Keywords: Mitochondrial diseases; Mitochondria; mtDNA; Oxidative phosphorylation; Therapies; ROS

1. Introduction

Mitochondria are cytoplasmic, double-membrane organelles in eukaryotic cells, derived originally from bacteria by endosymbiosis (Zeviani and Donato, 2004). Their main purpose is to synthesize adenosine triphosphate (ATP) which is crucial for eukaryotic life. They are also unique as organelles, because they contain their own genome (Zeviani and Donato, 2004; Annesley and Fisher, 2019).

1.1 Structure

The structure of mitochondria, as well as its mitochondrial protein and lipid arrangement, determines the capability of mitochondrial function in a cell. The efficiency of mitochondrial function is limited by the number of mitochondria in a cell, its inner elements, and its capability to obtain inputs and supply required products (Glancy, 2020).

Mitochondria consist of an inner lumen/matrix surrounded by a double membrane, as shown in **Figure 1**. This double membrane consists of an outer mitochondrial membrane (OMM) and an inner mitochondrial membrane (IMM), which separate the lumen of the mitochondria from the cytoplasm (Kühlbrandt, 2015).

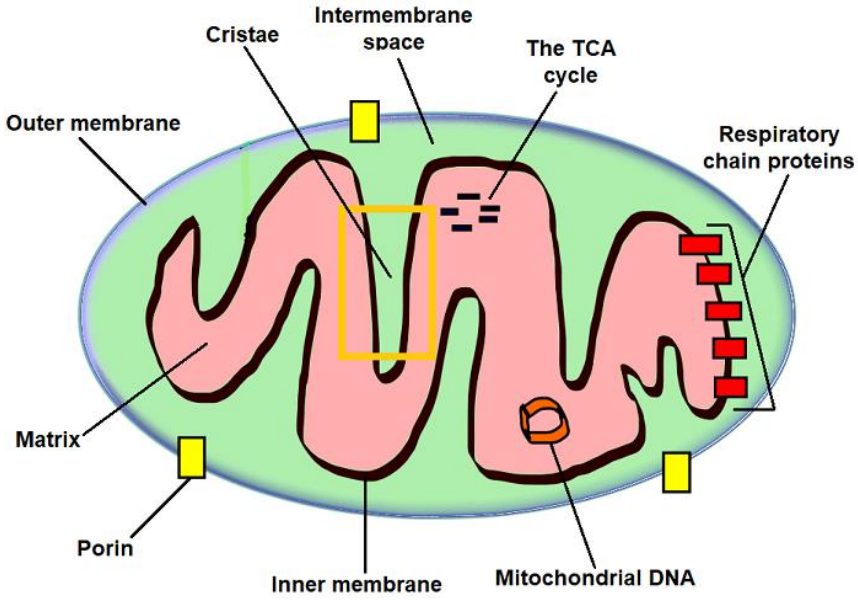

Figure 1. General structure of the mitochondria. The outer and inner membrane split the mitochondria into two sections, the matrix and the intermembrane space (Kühlbrandt, 2015). Porins in the outer membrane allow exchange of small metabolites between the cytosol and mitochondria (Endo and Sakaue, 2019). The tricarboxylic acid (TCA) cycle components and mitochondrial DNA are embedded within the matrix. Within the cristae folds are the machinery for oxidative phosphorylation (OXPHOS), the main mitochondrial function. The respiratory chain proteins are found on the edge of the matrix and are discussed in section 1.2.2.

1.1.1 The outer membrane (TOM)

TOM is porous to ions and small uncharged molecules < 5,000 Da, that pass through the pore-forming membrane proteins (porins), as shown in **Figure 1**. Due to TOM's porosity, no membrane potential exists across this membrane (Kühlbrandt, 2015; Supinski *et al.*, 2020).

Most mitochondrial proteins are made in the cytoplasm and are imported into the organelle. These larger proteins require special translocases to enter the mitochondria. Translocases consist of proteins in both the OMM and IMM (Hewitt *et al.*, 2013; Kühlbrandt, 2015).

The composition of the IMS is analogous to the cytosol in terms of ions and small molecules. As such, it acts as a functional barrier towards the passage of small molecules between the cytosol and matrix, whilst maintaining the protein gradient that drives OXPHOS. This is what allows for normal mitochondrial function (Cooper, 2000; Kühlbrandt, 2015; Suspinsky *et al.*, 2020).

1.1.2 The inner membrane (TIM)

TIM has many folds named cristae that extend into the matrix, shown in **Figure 1**. It contains the enzymes involved in energy conversion and reactive oxygen species (ROS) formation processes (Glancy, 2020). In addition, it acts as an anchor for elements of the electron transport chain (ETC) (Supinski *et al.,* 2020). It also contains ATP synthase, a channel that uses a proton motive force to drive ATP synthesis (Musante *et al.,* 2007).

The inner compartment, enclosed by TIM, is called the mitochondrial matrix, shown in **Figure 1**. The matrix has a large protein mass of up to 5000mg/ml and a pH of 7.9-8, creating the transmembrane electrochemical gradient that drives ATP synthesis (Kühlbrandt, 2015).

1.1.3 Mitochondrial genome

Mitochondria have their own genome comprised of circular deoxyribonucleic acid (DNA) molecules (Nass and Nass, 1963, cited by Kauppila *et al*., 2017, p. 57). The mammalian mitochondrial proteome comprises approximately 1,200 proteins, nearly all encoded by nuclear DNA (nDNA), which are translated in the cytosol and introduced into mitochondria (Kauppila *et al*., 2017). The performance of mitochondria relies on coordinated synthesis of nDNA and mitochondrial DNA (mtDNA) encoded proteins, for proper structure and function (Glancy, 2020; Supinski *et al.,* 2020).

Mitochondria are semi-autonomous, as the mtDNA encodes around 1% of the mitochondrial proteome, and these thirteen proteins are crucial for function. The mitochondrial genome is inherited from the mother who transmits her oocyte mtDNA to her offspring, and so forth (Zeviani and Donato, 2004).

Mutations in the nuclear genome can lead to mitochondrial disorders (MTDS), including the diminishing of mtDNA *e.g.,* mutations in the gene for the mtDNA polymerase γ (POLG) (Guerra *et al*., 2017). POLG is involved in a subunit of DNA polymerase gamma, the only DNA polymerase associated with replication and repair of mtDNA (Kanungo *et al.,* 2018). Mutations in mtDNA can have serious consequences for the mitochondria and its function. Heteroplasmy is where two or more differing mtDNA variants coexist inside a cell and is a frequent cause of mtDNA disorders. Homoplasmy is where all mtDNA within a cell are identical, either normal or mutant (Shalem and Riikka, 2020).

1.2 Functions of mitochondria

Mitochondria play a crucial role in the manufacture of metabolic energy in eukaryotic cells and are accountable for most of the valuable energy obtained from the degradation of carbohydrates and fatty acids (Cooper, 2000). This energy is in the form of ATP, an energy-rich compound that drives the main cellular functions including force generation, folding/breaking down of proteins and manufacturing/maintaining membrane potentials (Kühlbrandt, 2015).

Aside from ATP synthesis, the mitochondria have other functional roles including the manufacture of nicotinamide adenine dinucleotide (NADH) and guanosine triphosphate (GTP) in the TCA cycle (**Figure 2**), biosynthesis of amino acids, cellular signalling hubs and the production of phospholipids for membrane biogenesis (Kühlbrandt, 2015).

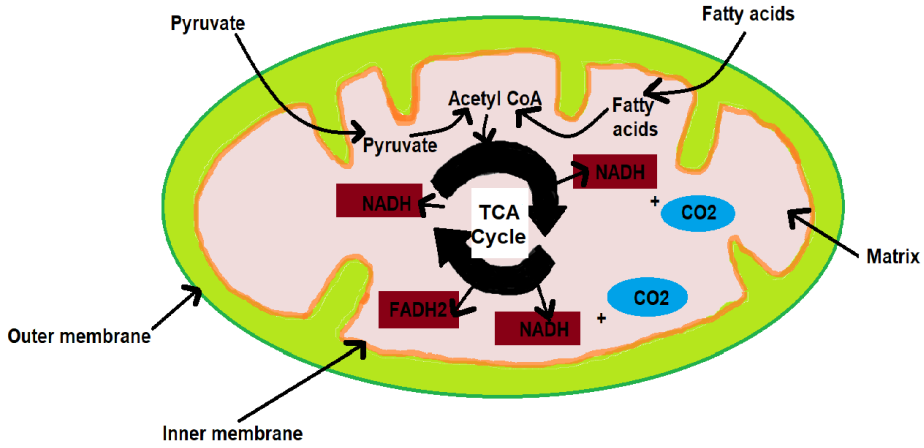

Figure 2. The TCA cycle. This occurs in the mitochondrial matrix and creates the reducing power used in OXPHOS, as well as metabolites used in other pathways. Macromolecules, such as fatty acids, must be broken down into acetyl coenzyme A (CoA) before entering the TCA cycle (Cooper, 2000).

1.2.1 Oxidative metabolism

The primary stages of glucose metabolism (glycolysis) occur in the cytosol, converting glucose to pyruvate, which is carried into mitochondria to complete oxidation to carbon dioxide (CO_2) (Blanco and Blanco, 2017). Primary oxidation of pyruvate to CoA, shown in **Figure 2**, is degraded to CO_2 through the TCA cycle (Cooper, 2000). The pyruvate dehydrogenase complex catalyses the conversion of pyruvate to acetyl CoA, making NADH and CO_2 when in the presence of CoA, nicotinamide adenine dinucleotide and triphenylphosphonium (Park *et al.*, 2018).

1.2.2 Respiratory chain complexes

Mitochondrial OXPHOS complexes of the respiratory chain, known as NADH, include ubiquinone oxidoreductase (complex I), succinate dehydrogenase (complex II), ubiquinone-cytochrome c oxidoreductase (complex III), cytochrome c oxidase (complex IV) and ATP synthase (complex V), shown in **Figure 3** (Sharma *et al.*, 2009).

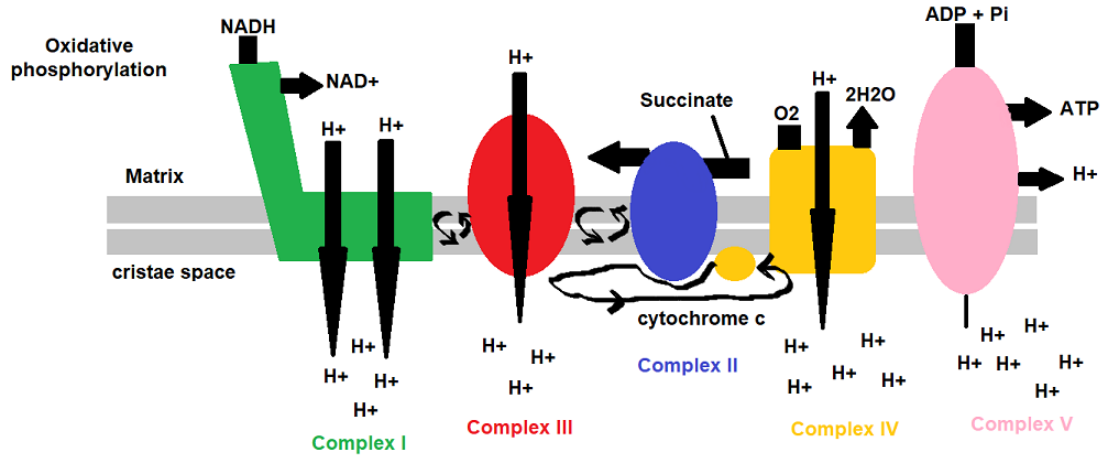

Figure 3. The five components of the mitochondrial ETC. Complex I oxidises NADH from the TCA cycle, then oxidised ubiquinone reduces ubiquinol via the acceptance of two electrons. In complex II, ubiquinol is re-oxidised, then transfers electrons to reduce oxygen to water in

complex IV. This releases redox energy, which delivers protons from the mitochondrial matrix to the periplasmic space, generating a proton-motive force across the IMM. Complex V uses this force (created by hydrogen ions) to generate ATP from adenosine diphosphate (ADP) and inorganic phosphate, constituting OXPHOS (Sharma *et al.,* 2009).

1.2.3 Mitochondrial fission and fusion

Mitochondria are dynamic organelles that are continually undergoing fusion and fission. Fusion combines elements between the mitochondrial contents of a cell, avoiding the complete loss of important elements (Youle and van der Bliek, 2012). It results in the creation of an individual mitochondrion from previous independent structures (Scott and Youle, 2010). Fusion is facilitated by mitofusion 1 and mitofusion 2 in the OMM and optic atrophy 1 (localised in the IMM), which functions to fuse internal/external membranes (Supinski *et al.,* 2020). Mitofusion 1 and 2 are dynamin-like GTP hydrolase enzymes, critical to mitochondrial function, due to their ability to stabilise fission/fusion dynamics (Sidarala *et al*., 2022).

Fission creates new organelles and helps quality control within the mitochondria. It is facilitated by the formation of a multimeric complex comprising of dynamic-related protein 1, which exerts a mechanical force around the OMM, cutting it into two pieces (Supinski *et al.*, 2020). It is crucial that cytochrome c is not released during mitochondrial fission/fusion, as it assists with ATP synthesis. If apoptosis occurs, cytochrome c can disperse into the cytosol and trigger apoptosis of the cell (Halestrap *et al*., 1997; Ow *et al.,* 2008).

1.2.4 Mitochondrial biogenesis (MB)

MB is the creation of new mitochondria in cells. The main controller of MB is a transcriptional coactivator, peroxisome proliferator-initiated receptor gamma coactivator 1-α (PGC1α). Disease mechanisms hinder MB, damaging the upkeep of appropriate levels of proteins *e.g.*, a mutated TOM complex seen in patients with Alzheimer's disease (Goyal and Chaturvedi, 2021). Treatments for MTDS include therapies that initiate MB and mitochondrial restoration, such as administration of resveratrol (sirtuin 1 activator) or human recombinant transcription factor a (Supinski *et al.,* 2020).

2. Mitochondrial disorders (MTDS)

MTDS refer to clinical syndromes linked with abnormalities of the ETC and OXPHOS. Clinical manifestations include mtDNA/nDNA depletion, muscle weakness and pigmentary retinopathy. The most common MTDS can be seen below in Table 1 (Zeviani and Donato, 2004). Primary mitochondrial diseases **(**PMD) are a heterogenous clinical cluster of disorders that occur due to dysfunction of the mitochondrial respiratory chain (MRC) (Chinnery, 2000). Deficiencies of almost 400 genes across the two genomes have been associated with PMD (Pitceathly *et al*., 2020).

Table 1. Common MTDS (Zeviani and Donato, 2004).

Types of MTDS	Phenotype	mtDNA mutation
Kearns-Sayre syndrome (KSS)	Ataxia, neuropathy and short stature.	Singular deletions or copies (commonly sporadic).
Pearson's syndrome	Death in infancy is common and anaemia.	Singular deletions or copies (commonly sporadic).
Mitochondrial Encephalopathy, Lactic Acidosis, and Stroke-like episodes (MELAS)	Stroke like episodes and lactic acidosis.	Heteroplasmic point mutations (inherited maternally).
Myoclonic epilepsy with ragged red fibres (MERRF)	Epilepsy, myoclonus and deafness.	Heteroplasmic point mutations (inherited maternally).
Neuropathy, ataxia and retinitis pigmentosa (NARP)	Weakness, ataxia and pigmentary retinopathy.	Heteroplasmic point mutations (inherited maternally).
Leber's hereditary optic neuropathy (LHON)	Loss of vision.	Homoplasmic point mutations (Inherited maternally)
Sensorineural hearing loss (SNHL)	Hearing loss.	Homoplasmic point mutations (Inherited maternally)

2.1. Heteroplasmic mutations

Heteroplasmic mutations, where only some copies of mtDNA contain the mutation, progress to various clinical phenotypes *e.g.,* Leigh syndrome (LS), MERRF, MELAS and NARP. The heterogenous symptoms of MTDS range from organ specific to multisystemic dysfunction with varying clinical classes (Bottani *et al.,* 2020).

For example, heteroplasmic mutations in LS are attributable to mtDNA mutations, however some defects are X-linked or sporadic. LS symptoms usually occur in early childhood and include developmental delay, cardiomyopathy, hypotonia and movement disorder (Kanungo *et al.,* 2018).

2.2. Mitochondrial myopathies (MitM)

MitM are developing muscle disorders caused by damage to OXPHOS in the mitochondria. Myopathy is disease of muscle tissue. It is a common symptom of adult-onset MTDS due to the elevated energy required by the skeletal muscle. It can result in proximal myopathy and weakness of limbs. Mutations in either mtDNA or nDNA can cause MitM (Ahmed *et al.,* 2018).

3. Therapies for mitochondrial disorders (MTDS)

Current treatments for MTDS are mostly symptomatic. One of the main symptomatic treatments used is the 'one-size-fits-all' approach, which is designed to treat all types of MTDS. This 'one-size-fits-all' approach includes diet, exercise, increasing MB with resveratrol, antioxidants, and pharmacological therapy (Bottani *et al.,* 2020). Dietary approaches can include the use of vitamin B2, vitamin B3 and a ketogenic diet. In terms of exercise, a combination of aerobic and endurance training has proven to be a safe and beneficial treatment for patients with mtDNA mutations (Tarnopolsky, 2014; Voet *et al.,* 2019). Many potential treatments have been suggested for MTDS in the future. These are usually more specific

therapies that can be broadly categorised into precision medicine approaches, personalised therapies, cell replacement therapies and gene therapies (Bottani *et al.,* 2020).

Precision medicine strategies may be able to treat MTDS with specific mutations or unusual metabolic hallmarks (Bottani *et al.,* 2020). Personalised therapy will soon allow patients to obtain earlier diagnoses, risk assessments and better treatments with lower costs. Both have the potential to customize therapy for individual patients and to produce the best response with the highest safety ensured. Cell replacement therapies and gene therapy also have great potential for the future (Vogenberg *et al*., 2010).

3.1 Precision medicine approaches for treating patients with MTDS

3.1.1 Gene therapies

Gene therapy is a current strategy to combat MTDS, which introduces modified gene products into the mitochondria via protein import machinery and inhibition of mutant mtDNA (Friedmann and Roblin, 1972, cited by Zeviani and Donato, 2004, p. 2167). This is done via sequence antigenomic peptide-nucleic acids (Zeviani and Donato, 2004). It can treat diseases of a single recessive genetic defect (Bottani *et al*., 2020). Similarly, it can target many organs at the same time, rendering it a desirable treatment method (Pitceathly *et al.,* 2020). Two gene therapies for treating spinal muscular atrophy and retinal dystrophy have been approved by the FDA (Mendell *et al*., 2017; Russell *et al*., 2017).

Adeno-associated virus (AAV)

AAV's are non-enveloped viruses belonging to the parvovirus family that can be manipulated to transport DNA to target cells. It is one of the safest forms of gene therapy using recombinant AAV particles with the DNA sequence of interest, but without viral genes (Rose *et al.,* 1966, cited by Naso *et al.,* 2017; Pitceathly *et al.,* 2020). AAV-mediated gene therapies for MTDS have undergone clinical trials for neurometabolic disorders *e.g.,* Hunter syndrome (Naso *et al.,* 2017; Pitceathly *et al.,* 2020). At present, there is an AAV-vector-based gene replacement clinical trial running in patients with LHON (Hanaford *et al.,* 2022).

AAV's have been utilised to transport molecular scissors such as Zinc finger nucleases (ZFNs) and Transcription activator-like effector nucleases (TALENS) *in vivo,* to remove mutated mtDNA. ZFNs and TALENS are chimeric nucleases that have engineerable, sequence-specific DNA attaching modules, bound to a non-specific DNA cleavage domain. They can undergo genetic modification by triggering DNA-double-strand breaks, which initiate error-prone non-homologous end joining or homologous directed repair at genomic locations (Bottani *et al*., 2020). TALENS and mitochondrially targeted ZFNs (mtZFNs) lowered mtDNA heteroplasmy and restored molecular and biochemical phenotypes in a mouse model of heteroplasmic MTDS (Bacman *et al.,* 2018; Gammage *et al.,* 2018).

Clustered regularly interspaced palindromic repeats (CRISPR) /CRISPR-like protein 9 (Cas9) technology

CRISPR/Cas9 is another gene-editing tool that can correct errors in the genome, as well as switch on/off genes in cells and organisms. It is a cheaper/less time-consuming alternative to TALENS. Its mechanism can be seen below in **Figure 4** (Redman *et al.,* 2016).

Editing the mitochondrial genome has proven difficult due to sub-efficient delivery of a single guide ribonucleic acid (sgRNA) and Cas9 enzyme complexes into the mitochondria (Bottani *et al*., 2020). In 2015 Jo *et al*., effectively engineered the mtDNA with CRISPR/Cas9 technology

and showed that the flagged Cas9 localised inside the mitochondria, whilst the sgRNAs permitted depletion of directed elements of mtDNA (Bottani *et al*., 2020).

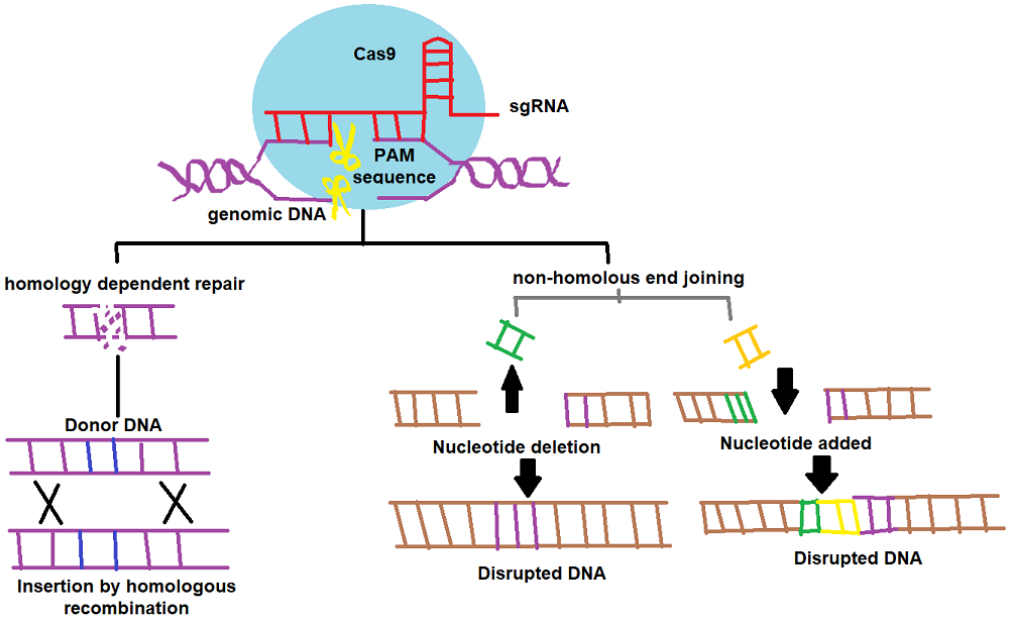

Figure 4. CRISPR/Cas9 mechanism. Cas9 cuts both strands of DNA and is directed to its target by a sgRNA, which attaches to genomic DNA, allowing for modification of the genome. For Cas9 to cut, the sequence of DNA must be at the 3' end of the sgRNA, this is called the protospacer adjacent motif (PAM). The DNA can be repaired via non-homologous end joining (resulting in random deletion/insertion of DNA) or homology dependent repair (where the homologous section of DNA is utilised as a repair template). Homology dependent repair allows for precise genome repair, as it can make alterations down to a single base pair (Redman *et al.*, 2016).

3.1.2 Cell replacement therapies

Cell replacement therapies utilise cell-based products to replace dead cells to re-establish the role of the impacted tissue (Lindvall *et al.*, 2004). It may be a possible cure to various MTDS, such as mitochondrial neurogastrointestinal encephalopathy (MNGIE). MNGIE is an autosomal recessive disorder caused by mutations in the thymidine phosphorylase (TP) gene (Hirano *et al.*, 2004). Autologous haematopoietic stem cell transplantation (AHSCT) and white blood cell-encapsulated thymidine phosphorylase are being investigated as a treatment. Following AHSCT, clinical and biochemical enhancements were effective in repairing TP activity and lowering thymidine concentrations to regular circulating levels in patients with MNGIE (Hirano *et al.*, 2006; Filosto *et al.*, 2012; Bottani *et al*., 2020; Pitceathly *et al.*, 2020).

3.1.3 Mitochondrial therapies

Mitochondria based gene editing tools

Hashimoto *et al.*, (2015) used a new molecular technique called mitochondrial-targeted TALENS (mitoTALENS), which show great promise for the future. mitoTALENS can be engineered to identify specific DNA sequences, to initiate double-stranded breaks to breakdown DNA (Pitceathly *et al.*, 2020). They can cause positive heteroplasmic shifts in cells lines with different pathogenic mtDNA mutations and have worked well in cultured mammalian cells (Ahmed *et al.*, 2018; Kazama *et al.*, 2019).

Mitochondrial transplantation (MT)

This is a current 'one-size-fits-all' method of restoring mitochondrial function, through immediate transplantation of good-quality mitochondria into target tissues *e.g.*, to repair function to diseased heart and liver (Cowan *et al*., 2017; Supinski *et al.*, 2020). Supinski *et al.*, (2020) described how MT into ischemic cardiac tissue increases cardiac function, myocardial contractility and may rescue other organs. Other MT transplants, such as into liver, may be a possible treatment for certain MTDS, particularly for disorders that impact this single organ (McCully *et al.*, 2017, cited by Parikh *et al.*, 2016, p. 181).

3.1.4 *In utero*

Molecular bypass (MBP) therapy for treating disorders with mtDNA instability

MBP therapy is an experimental treatment, which works by replenishing the regular number of deoxynucleotides (dNTPs) in the mitochondria of cells in patients with mtDNA degeneration syndromes (Marks, 2021). nDNA and mtDNA both rely on individual balanced pools of dNTPs to restore normal function of DNA replication and fix any DNA damage. By importing dNTPs from the cytosol to the mitochondria, it has the potential to restore mitochondrial dNTP discrepancies (Desler *et al*., 2006).

Diminished thymidine kinase 2 (TK2) activity has shown to cause imbalance of mitochondrial dNTPs and cause mtDNA mutations in non-dividing cells (Desler *et al*., 2006). MBP therapy with deoxypyrimidine monophosphates and deoxynucleoside were used in an open-labelled study, conducted on 16 early-onset TK2-patients. Results showed amelioration of motor abilities, discontinuation of feeding tubes and prolonged survival (Bottani *et al.*, 2020).

Pre-implantation therapies

Pre-implantation genetic diagnosis is a current preventative method, which facilitates families with a known history of mtDNA mutations (Rai *et al.*, 2018). It is an *in vitro* fertilization (IVF) technique, where the fertilized egg with the pathogenic mtDNA mutation is cultured until the blastocyst stage, then it is biopsied for genetic analysis, prior to implantation (Treff *et al.*, 2012; Sallevelt *et al*., 2017). However, it is limited, as it only helps women with small concentrations of mtDNA mutations in their oocytes (Bottani *et al*., 2020).

3.2 Personalised therapies for MTDS

3.2.1 Gene therapies

Allotropic gene expression is a technique used to override mtDNA mutations by re-expressing the absent mtDNA-encoded protein from the nucleus. In this instance, an engineered nuclear form of a mitochondrial gene encodes a protein that may be imported into the mitochondria. Nine patients with LHON syndrome, who had a G11778A mutation causing optic neuropathy, underwent a clinical trial with a single dose of a recombinant AAV2 carrying the normal ND4 gene, with the goal to improve their vision. 66% of the nine patients' visual acuity improved, resulting in amelioration of their visual field (Bottani *et al*., 2020).

3.2.2 Mitochondrial therapies

Mitochondrial donation (MD)

Mitochondrial spindle transfer (MST) is a new IVF treatment which includes transferring the mothers nDNA from an affected egg, into an unnucleated egg from an unaffected donor, leading

to an embryo bearing nDNA from both parents (Tachibana *et al*., 2009, cited by Bottani *et al*., 2020, p. 25).

The most utilised MD productive technologies include MST and Pronuclear Transfer (PNT), shown in **Figure 5**, which are both legally approved for use in the UK (Craven *et al*., 2010; Craven *et al*., 2017; Herbert and Turnbull, 2018).

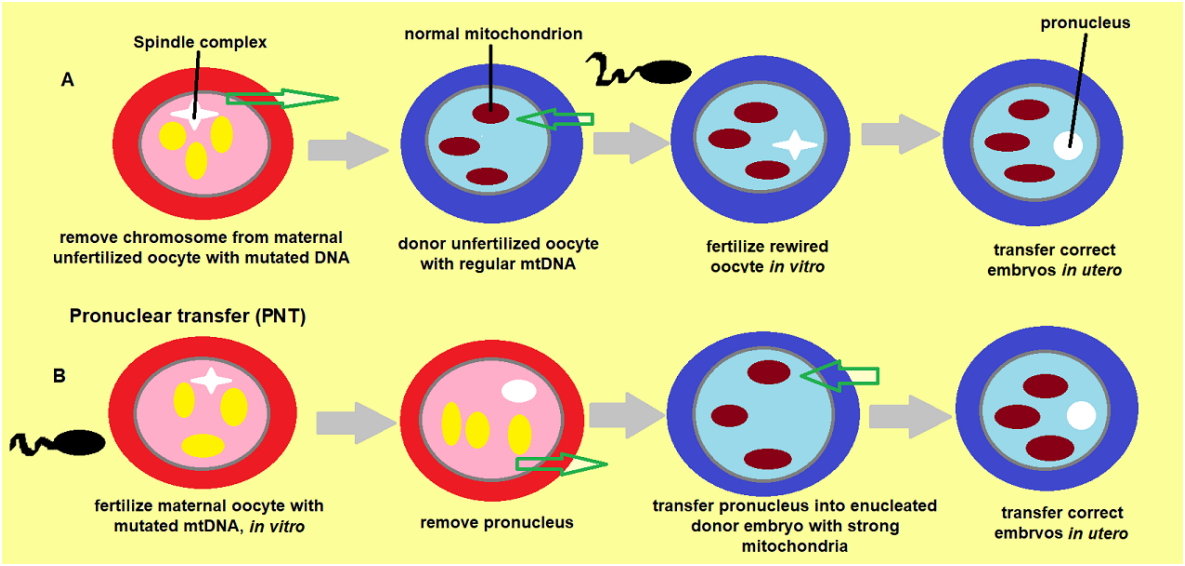

Figure 5. Mitochondrial donation. MST includes the transfer of nuclear genetic material from a patient's egg with mutated mtDNA, to an enucleated donor's unfertilized metaphase II oocyte, with healthy mitochondria. It creates an oocyte with a patient's nDNA, but without the mutated mtDNA (Bottani *et al*., 2020). PNT involves the transfer of a diploid nucleus into a donor zygote which has been enucleated. The zygote now contains healthy donor mtDNA, with both nDNA of the patient and the fertilising sperm (Craven *et al*., 2010).

At the meeting of The American Society for Reproductive Medicine, in 2016, Dr Jogn Zhang discussed the results of the utilisation of MST in a woman bearing a mtDNA mutation causing LS. The outcome was the birth of a healthy baby, with fewer than 10% mutated mtDNA in tissues, when analysed 2 days after birth (Hudson *et al.*, 2019). PNT was utilised in 2005 to avoid transmission of a mtDNA disorder in a mito-mouse, a mouse model that normally builds-up large-scale mtDNA deletions (Sato *et al.*, 2005).

Heteroplasmic shift

A method of correcting mtDNA mutations, focuses on the interference of mutant molecules, by utilising selective nucleases to move the heteroplasmy level under the maximum threshold (Bottani *et al*., 2020). Many techniques have been developed based on this concept *e.g.*, oligonucleotides. However, it still needs to be determined if shifts in heteroplasmy are maintained over time (Pitceathly *et al.*, 2020).

3.2.3 *In utero*

Foetal gene therapy uses intracellular delivery of genetic material for the treatment of disease, as described in sections 3.1.1 and 3.2.1. By introducing gene therapy for foetal treatment, scientists can access organs during developmental changes (Tsukamoto *et al.*, 1995, cited by David and Peebles, 2008, p. 203). This may also be used a therapeutic method for inherited disorders, *e.g.*, LS, which would normally result in early death or life-long irreparable destruction (Rashnonejad *et al*., 2019; Bottani *et al*., 2020). *In Utero* Gene Therapy (IUGT)

may overcome many of the disadvantages in postnatal gene therapy because of the tiny foetal size and the immature foetal immune system (Peranteau and Flake, 2020).

3.3 Genetic counselling (GC)

Most patients with MTDS are already symptomatic by the time they are diagnosed (Pitceathly *et al*., 2020). As there is huge clinical variability and genetic heterogeneity involved with the complexity of MTDS, patients and families usually experience a long and complicated diagnosis process. Therefore, GC is crucial for individuals suffering from MTDS and should be made available to all patients due to delayed diagnosis, no clear prognosis, issues of family planning and genetic testing (Vento and Pappa, 2013).

3.4 Clinical trials for MTDS

A growing number of clinical trials, which are normally double blind and placebo regulated, have investigated the therapeutic effect of vitamins, co-factors, nutritional supplements, and gene therapy for MTDS treatment. Ongoing clinical trials are shown in **Figure 6**. However, these ongoing trials have not yet demonstrated beneficial primary and secondary results (Pitceathly *et al.*, 2020).

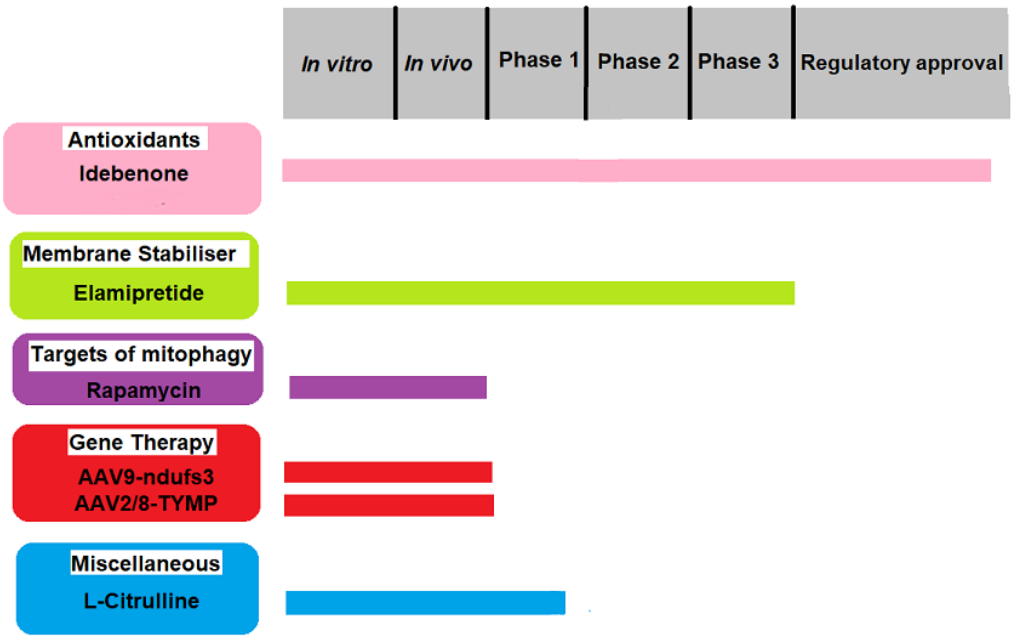

Figure 6. Progress in clinical trial development for MTDS. With idebenone (an antioxidant) reaching regulatory approval, elamipretide (a membrane stabiliser) reaching phase 3, rapamycin (a mitophagy target) and two gene therapies AAV9-ndufs3 and AAV2/8-TYMP reaching the *in vivo* stage, and L-citrulline (a miscellaneous compound) reaching phase 1 (Pitceathly *et al.*, 2020).

Failure of clinical trials in MTDS are mostly due to inadequacy of the compounds analysed. In addition, trial designs are hindered by the shortage of natural history data (due to their rarity), heterogeneity, validated biomarkers and result measures, to detect an effective treatment (Pitceathly *et al*., 2020).

4. Discussion

From this review it is evident that mitochondria are an essential organelle, critical for synthesizing ATP for eukaryotic life. The mitochondria have protein coding genes which are crucial for function and are derived from its own genome and nDNA. The mitochondrial genome is inherited from the mother, who transmits her oocyte mtDNA to all her female offspring, and subsequently transmit their mtDNA to their offspring, and so on. Being such an important structure with many functions, anything inhibiting the mitochondria's role in the body can lead to severe and often fatal results to the cell/body if not corrected.

These effects result in genetically heterogenous clinical syndromes linked with abnormalities of OXPHOS, and most MTDS occur due to dysfunction of the MRC. Clinical manifestations affect the body's organs, cells, and systems in various debilitating ways. Heteroplasmic and homoplasmic mutations have led to many clinical phenotypes such as LS, LHON and MERRF. High levels of mitochondrial ROS can trigger ETC irregularities and destroy mitochondrial elements, potentially causing muscle disorders such as MitM.

Due to the severity of MTDS, research must continue to explore the defects, origins, and clinical manifestations of these disorders, in order to find more precision/personalised therapies for patients. This information is also crucial in order to advise the patients, treat symptoms/manifestations, with the ultimate goal of preventing the disorders before they emerge.

Current treatments are limited to 'one-size-fits-all' approaches *e.g.*, dietary approaches. However, future perspectives for treating MTDS are exciting and promising, with many treatments currently undergoing clinical trials, including those completed as shown in Table 2 below.

Table 2. Completed clinical trials for MTDS.

Current trials	Location	Date	References
Dose escalating with KH176 to treat MELAS, LS and LHON.	Drug Research Unit in Ghent.	Completed October 15th, 2021.	(Clincosm, 2022).
EPI-743 for treating metabolism or MTDS.	Bethesda, Maryland, National Institutes of Health.	Completed March 19th, 2021.	(Clincosm, 2022).

Precision medicine approaches and personalised therapies are coming to the forefront, either of which may treat specific mutations at the genome level and prevent the disorders. Emerging precision medicine approaches include the use of gene therapy in AAV's and gene editing tools

such as CRISPR/Cas9. Personalised therapies include allotropic gene expression, MD with MST/PNT, and even foetal gene therapy for treating early-onset MTDS.

In summary, there is great hope for the future to treat MTDS, with these personalised and precision-based approaches. The more we learn about the rarity, complexity, and challenges of MTDS, the more precise methods of treatment we can develop. Current and future trials provide hope to overcome the extensive challenges of MTDS. We must not underestimate the importance of GC for patients suffering with MTDS. GC is crucial to explain to patients the complexities of the diseases and to support families/patients in their coping strategies, to discuss their options in terms of MTDS treatments, and to decide the best path to take so that MTDS can be better well managed in future generations.

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