Mitochondrial Medicine in the Omics Era

Joyeeta Rahman¹ and Shamima Rahman¹,²*

¹ Mitochondrial Research Group, UCL Great Ormond Street Institute of Child Health and
² Metabolic Unit, Great Ormond Street Hospital NHS Foundation Trust, London, UK

*Correspondence to:
Professor Shamima Rahman
Mitochondrial Research Group
Genetics and Genomic Medicine
UCL Great Ormond Street Institute of Child Health
London WC1N 1EH, UK.
Telephone: +44 (0)2079052608
shamima.rahman@ucl.ac.uk

Keywords:
Mitochondrial disease, OXPHOS, signalling, omics, genomics, transcriptomics, proteomics, metabolomics, mitochondrial stress response, treatment
Abstract

Mitochondria are dynamic bioenergetic organelles whose maintenance requires ~1500 proteins from two genomes. Mutations in either the mitochondrial or nuclear genome can disrupt a plethora of cellular metabolic and homeostatic functions. Mitochondrial diseases represent one the most common and severe groups of inherited genetic disorders, characterised by clinical, biochemical, and genetic heterogeneity, diagnostic odysseys, and lack of curative therapies. This review aims to discuss recent advances in mitochondrial biology and medicine arising from widespread utilisation of high-throughput omics technologies, and also includes a broad discussion of emerging therapies for mitochondrial disease. New insights into both bioenergetic and biosynthetic mitochondrial functionalities have expedited the genetic diagnosis of primary mitochondrial disorders, and identified novel mitochondrial pathomechanisms and new targets for therapeutic intervention. As we enter this new era of mitochondrial medicine, underpinned by global unbiased approaches and multi-faceted investigation of mitochondrial function, omics technologies will continue to shed light on unresolved mitochondrial questions, paving the way for improved outcomes for patients with mitochondrial diseases.

Search Strategy and Selection Criteria:

Since high-throughput omics technologies have in recent years been highly efficacious tools in mitochondrial research, we utilised the PubMed, Google Scholar and Web of Science databases to identify original research utilising omics technologies which have led to exciting developments in mitochondrial biology and disease. Search terms included the different subtypes of omics ("genomics", "transcriptomics", "proteomics", "metabolomics", etc.) combined with "mitochondrial". In addition, terms such as "global", "high-throughput", and "[genetic, metabolic, transcriptional, epigenetic] profiling" were valuable in identifying notable publications. Our omics research papers ranged from April 2008 to December 2017. We also consulted a collection of several hundred publications describing mitochondrial disease mutations (collated as part of our laboratory’s work in mitochondrial genetic diagnostics), as well as databases such as PanelApp and MitoCarta 2.0, to build and validate our updated list of causative mitochondrial disease genes. Reports ranged from February 1988 (identification of the first mtDNA deletion) to December 2017.
Mitochondrial Function and Dysfunction

Mitochondria are complex dynamic organelles that execute a myriad of functions pertaining to cellular metabolism and homeostasis. Cellular energy generation via oxidative phosphorylation (OXPHOS) is a hallmark feature of mitochondria, but mitochondria also play roles in calcium homeostasis, initiation of caspase-dependent apoptosis, cellular stress response, haem biosynthesis, sulphur metabolism, and cytosolic protein degradation. A unique feature of mitochondria is the mitochondrial DNA (mtDNA), a small circular genome (encoding only 37 genes) arising from the organelle’s endosymbiotic evolutionary origins. The mtDNA encodes 13 OXPHOS proteins; the remaining ∼1500 proteins of the mitochondrial proteome are encoded in the nuclear genome and transferred to the mitochondria via sophisticated import systems.

Mutations in >350 genes, of both mitochondrial and nuclear origin, have been reported to cause mitochondrial disease (Table 1). These genetic disorders may be defined as those in which mutations primarily or secondarily lead to OXPHOS dysfunction or other disturbances of mitochondrial structure and function including perturbed mitochondrial ultrastructure, aberrations in the production of cofactors and vitamins, or other impaired metabolic processes within the mitochondrion including the tricarboxylic acid (TCA) cycle and pyruvate metabolism. Mitochondrial diseases display heterogeneous phenotypic and biochemical presentations. This variation, together with an incomplete understanding of mitochondrial pathophysiology, besets mitochondrial diseases with diagnostic challenges and a lack of curative therapies. In recent years, however, high-throughput omics techniques, that is, high-throughput technologies capable of detecting differences in a multitude of molecular constituents in organisms (including genomics, transcriptomics, proteomics, metabolomics, and epigenomics) accompanied by sophisticated bioinformatics tools, have revealed new detail about mitochondrial functionalities and how they contribute to cellular health and disease. These novel techniques have been invaluable to several pillars of mitochondrial medicine, including elucidating basic aspects of mitochondrial structure and function, enhancing genetic diagnosis of mitochondrial disorders, and providing novel insights into therapeutic targets to ameliorate the effects of mitochondrial disease (Figure 1). This review aims to highlight the contributions of powerful omics technologies to mitochondrial medicine from the laboratory to the clinic.

Enhanced Understanding of Oxidative Phosphorylation

Perhaps the most well characterised function of the mitochondrion and the rationale behind its alternative name as the ‘powerhouse’ of the cell is cellular energy generation via OXPHOS. The
OXPHOS system, which is conserved from bacteria to higher eukaryotes, comprises five multimeric enzymes, named complexes I to V, and two mobile electron carriers (coenzyme Q$\text{_{10}}$, CoQ$\text{_{10}}$, and cytochrome c, cyt c). Complex I (NADH:ubiquinone oxidoreductase; CI) pumps four protons across the inner mitochondrial membrane (IMM) into the intermembrane space (IMS) through the oxidation of NADH and reduction of CoQ$\text{_{10}}$. Complex II (succinate-CoQ oxidoreductase; CII) transfers electrons from FAD-dependent sources through the reduction of CoQ$\text{_{10}}$; however, no protons are pumped across the IMM by this enzyme. Complex III (ubiquinol-cytochrome c oxidoreductase; CIII) oxidizes CoQ$\text{_{10}}$ and reduces cyt c, releasing a total of four protons into the IMS. The final electron acceptor is molecular oxygen which receives four electrons in a reaction catalysed by Complex IV (cytochrome c oxidase; CIV) to form water molecules. This reaction is coupled with proton transfer to the IMS. The proton gradient generated by the activity of these complexes creates a proton-motive force utilized by F$\text{_{1}}$F$\text{_{0}}$-ATP synthase (Complex V) to phosphorylate ADP to ATP, the major energy currency of the cell (Figure 2).

Although the structural and functional basis of OXPHOS has been agreed for decades, recent advances in omics technologies have divulged novel structural and functional insights. Quantitative proteomics has revealed the structural and functional importance of the 31 non-catalytically active supernumerary subunits of CI, including a novel cell-viability determining role for NDUFAB1. A combination of proteomics and patient based studies led to reassignment of NDUFA4 as a subunit of CIV rather than CI. The development of complexome profiling has provided valuable insight into the formation of OXPHOS complexes, notably the step-wise assembly of CI, whose structure at 4.2-Å resolution was recently determined by single-particle electron cryomicroscopy. Complexome analysis demonstrated that complex I was completely assembled, followed by the formation of quaternary molecular suprastructures known as supercomplexes (Figure 1), which challenged the assertion that prior supercomplex formation is necessary for assembly and stability of CI. Quantitative proteomics has also revealed supercomplex-associated proteins, such as COX7A2L, which stabilises the CIII$_2$/CIV supercomplex. The functional significance of supercomplexes is still unclear, but putative roles include limiting reactive oxygen species (ROS) production from CI, providing a kinetic and catalytic advantage for respiration, and preventing IMM protein aggregation.

Genome-wide CRISPR/Cas9 knock-out screens have also been a useful tool in elucidating novel mitochondrial functionalities. A CRISPR-based genetic screen revealed the essential role of OXPHOS in cytosolic aspartate production, which is vital for cell proliferation and nucleotide biosynthesis.
In addition, a genome-wide CRISPR ‘death screen’ led to the discovery of both mitochondrial and non-mitochondrial proteins essential for OXPHOS in vitro by deep sequencing cells dying in galactose medium because of CRISPR-induced OXPHOS inhibition. In future, genome-wide CRISPR/Cas9 screens will continue to uncover novel aspects of mitochondrial function and will be invaluable tools to identify novel therapeutic targets in vivo.

Mitochondria as Signalling Organelles and Gatekeepers of Cellular Homeostasis

An emerging concept in mitochondrial biology is the role of mitochondria as signalling organelles in constant communication with the nucleus and other subcellular compartments to titrate energy production and metabolites to the cell’s specific needs and nutrient availability. Omics studies have revealed details about retrograde signals from the mitochondrion to the cytosol, which include ROS, the mitochondrial membrane potential, key intermediary metabolites, mitochondrial bioenergetic (AMP/ATP) and redox (NAD+/NADH) ratios, calcium fluxes across the mitochondrial membrane, and gasotransmitters such as hydrogen sulphide. Mitochondrial ROS production, although initially postulated to be detrimental and to contribute to mitochondrial and cellular pathology, at physiological concentrations appears to be integral to cell signalling and the modulation of gene expression. Locally, H$_2$O$_2$ and other ROS fine-tune mitochondrial morphology, by shifting the balance of fission (fragmented mitochondria) and fusion (long tubular mitochondria). Elsewhere in the cell, mitochondrial H$_2$O$_2$ has been implicated in modulating cell survival, autophagy, and cell migration by regulating kinase-driven pathways. At an organismal level, it is important to note that ROS signalling differentially affects different cell types. The mitochondria house whole or partial components of several converging metabolic pathways including the TCA cycle, folate metabolism, and sulphur metabolism. Not only are the enzymes and metabolites in these pathways important for their respective metabolic functionalities, but these species also have pleiotropic roles as signalling molecules. In addition to generating reducing equivalents that feed into the OXPHOS system, the TCA cycle has numerous cataplerotic roles, including provision of precursors for the biosynthesis of complex lipids, proteins, carbohydrates and nucleotides. It is thus perhaps unsurprising that a number of TCA cycle intermediates have signalling roles impacting on critical cellular processes, including histone acetylation and demethylation by acetylCoA and α-ketoglutarate respectively (Figure 1). SuccinylCoA is required for protein succinylation, a post-translational modification that has been suggested to have wide-ranging cellular effects in health and disease, including immune function and cancer. Furthermore, the α-ketoglutarate/succinate ratio has recently been shown to influence human stem cell differentiation. However, it should be noted that evidence for physiological relevance of succinylation is still lacking. Impaired mitochondrial ATP synthesis is
associated with increased AMP/ATP ratio, leading to activation of AMP-activated kinase (AMPK), a principal cellular bioenergetic sensor regarded as the epicentre of nutrient-dependent signalling. High-throughput proteomic techniques have recently begun to identify targets of AMPK phosphorylation, including the mitochondrial fission factor MFF, thus linking nutrient sensing and mitochondrial dynamics. AMPK also alters the mitochondrial NAD⁺/NADH redox ratio, leading to sirtuin-mediated deacetylation of the master transcriptional coactivator PGC1α, enhancing its gene expression and promoting mitochondrial biogenesis.

Application of Omics Technologies to Mitochondrial Diagnostics

Genomics

The initial era of mitochondrial genomics began thirty years ago with the discovery of sporadic large-scale mtDNA rearrangements in patients with ragged-red fibre myopathies and of maternally inherited mtDNA point mutations in families with Leber hereditary optic neuropathy (LHON). The small size and known sequence of the mitochondrial genome led to a plethora of reports of novel disease-associated mutations, spanning almost every base of the mtDNA [www.mitomap.org]. During this period it became obvious that many paediatric cases of mitochondrial disease must be caused by nuclear gene defects, based on the circumstantial evidence of an increased incidence of consanguinity in affected families and the absence of mtDNA mutations in many patients. The search for nuclear gene mutations causing OXPHOS deficiency remained elusive until the identification of SDHA mutations in a patient with Leigh syndrome caused by deficiency of complex II, the only OXPHOS complex entirely encoded by nuclear genes. Despite this breakthrough, for many years most cases of mitochondrial disease remained resistant to genetic diagnosis because of the limitations of the available methods, which were restricted to candidate gene sequencing, with/without prior linkage analysis or (in the case of consanguineous families) homozygosity mapping.

In recent years mitochondrial disease genetics has been completely transformed by the emergence of next generation sequencing (NGS) technologies. Rapidly reducing costs have allowed wide-scale adoption of whole exome sequencing (WES) and whole genome sequencing (WGS) in large cohorts of patients. The increasing application of NGS of extended gene panels, WES and WGS to routine mitochondrial disease diagnostics has led to a dramatic increase in the genetic diagnostic rate of these disorders, from 10-20% in the pre-NGS era to >50% in some cohorts. Mitochondrial diseases are particularly tractable to NGS because WES typically reveals a relatively small number of biallelic possibly pathogenic variants. Review of these variants by an expert mitochondrial physician can expedite diagnosis. Resources such as Leigh Map and MSeqDR can enhance the interpretation of
The compendium of peer-reviewed validated phenotypic associations within the Leigh Map can help to prioritise causative genes by observed phenotype, particularly within the context of functional groups with notable phenotypic heterogeneity such as mitochondrial translation defects and mtDNA maintenance disorders. Furthermore, sharing of genetic variants through Genematcher and other resources can reveal novel disease genes by identifying other unrelated patients with variants in the same gene and similar (or even identical) clinical phenotypes. Together, these approaches have led to the identification of >350 genes responsible for human mitochondrial disease (Table 1), with >100 genes discovered in the last 5 years alone (Figure 1).

What do these advances in genomics mean for routine clinical practice? Translation of the new genetics to the clinic is being facilitated by rapidly decreasing costs and times for WES and WGS, so that many centres are increasingly taking an 'exome first' approach, followed by later muscle biopsy only if functional data are needed to prove pathogenicity of identified mutations. However, diagnosis of mitochondrial disease remains complex and the order of investigations needs to be contextual, depending on the precise clinical situation. Muscle biopsy in parallel with genetic testing is recommended in individuals with rapidly progressive disease and an unstable clinical condition. In adult patients sequencing of muscle DNA may be needed to identify heteroplasmic mtDNA mutations with low mutant load. In routine diagnostics it is not yet possible to match the high diagnostic rate achieved by some research groups, because of lack of resources and expertise to perform necessary investigations to analyse the functional effects of variants of unknown significance (VUS). Remaining challenges are largely around clinical interpretation of the functional significance of variants identified by WES and WGS. Initiatives such as the Clinical Genome Resource (ClinGen) programme are valuable because they aim to develop systematic methods for clinical annotation and interpretation of genomic variants by disease specialists. Finally, integration with phenotypic data, ideally in human phenotype ontology (HPO) format, will also be critical to improved genetic diagnostics going forwards.

**Transcriptomics**

WES and WGS are powerful techniques, but have yielded the genetic cause in only 25-50% of cases with mitochondrial disease. Reasons for failure to diagnose 100% of cases are complex, but include challenges in identifying de novo mutations, cryptic splice site defects, copy number variants, insertion/deletion events and mutations in deep intronic or regulatory regions or in refractory regions such as repeat sequences. Parent-child 'trio' sequencing allows identification of de novo mutations, and is the strategy employed effectively in the Deciphering Developmental
Disorders (DDD) and Genomics England 100,000 Genomes (100K) projects. Recent studies demonstrated the power of whole transcriptome sequencing using the technique of RNA sequencing (RNAseq) to prioritise candidate genes (e.g. those with reduced expression or even monoallelic expression) and to identify deep intronic variants affecting splicing. RNAseq is also helpful in the interpretation of variants identified by WGS. For example, RNAseq of primary muscle RNA samples successfully identified the genetic defect in 21% of 50 patients with suspected genetic myopathies whose diagnosis had been resistant to WES and WGS. This study emphasised the importance of investigating a clinically relevant tissue in unsolved cases. However, since many mitochondrial disease genes are housekeeping genes, they are widely expressed including in cultured skin fibroblasts. Accordingly, Kremer et al successfully employed RNAseq in cultured skin fibroblasts to diagnose 10% of a cohort of 48 patients with suspected mitochondrial disease remaining undiagnosed after WES. RNAseq does have challenges, being subject to batch effects and requiring robust methodological and filtering strategies. Since it relies on the gene of interest being expressed in the tissue being investigated, for genes not expressed in fibroblasts and where the affected tissue is inaccessible (brain, heart) RNAseq of reprogrammed iPS cells may be a promising approach.

Transcriptomics analysis may also reveal biomarkers of mitochondrial disease. For example, microarray based gene expression profiling of a mouse model with mutations in the Twinkle helicase and patient-derived transmitochondrial cybrids bearing the common m.3243A>G mtDNA mutation, associated with MELAS syndrome, identified fibroblast growth factor 21 (FGF21) and growth and differentiation factor 15 (GDF15), respectively, as mitochondrial disease biomarkers (Figure 1).

Proteomics
Another tool that has recently been adopted into the mitochondrial diagnostic armamentarium is quantitative proteomics. Progress is being made in cataloguing all ~1500 predicted human mitochondrial proteins, and the submitochondrial localisation of the mitochondrial proteome has recently been studied in Saccharomyces cerevisiae and human cells. Moreover, mass spectrometry based cross-linking interactome analysis has been used to investigate the many interactions of mitochondrial proteins, and to identify C17orf89 (NDUFAF8) as a novel complex I assembly factor that was mutated in a patient with Leigh syndrome. All of this information will be invaluable in identifying new disease genes for mitochondrial disease, and may help to resolve the 25-50% of cases undiagnosed after WES/WGS. As discussed above, complexome profiling has been used to study individual OXPHOS complexes. This technique revealed TMEM126 as a complex I
assembly factor, defects of which were subsequently linked to human disease. More recently, complexome profiling has been extended to analyse the entire mitochondrial ribosome. An elegant multinational study used mitoribosomal profiling to demonstrate reduced steady-state levels of small but not large mitoribosomal subunit proteins in fibroblasts from patients with mutations in the small ribosomal subunit MRPS34. It is anticipated that mitoribosome profiling will enable improved understanding of the pathogenesis of mitochondrial translation disorders, the most rapidly growing subgroup of genetic mitochondrial disease. Increasingly, quantitative proteomics is being used to interrogate the effects of metabolic reprogramming in various mitochondrial diseases. Increased glycolytic enzymes and protein acetylation, decreased oxidoreductases and altered heterogeneous ribonucleoprotein levels (needed for gene regulation) were observed in the liver of a mouse model of ethylmalonic encephalopathy. Proteomics will also be critical to understanding the importance of post-translational modifications of mitochondrial proteins, including lysine acetylation, malonylation and succinylation, in health and disease.

Metabolomics
The search for biomarkers and therapeutic targets for mitochondrial disease has been ongoing for decades. Initial biochemical markers of mitochondrial disease, such as lactate and pyruvate, have low sensitivity and specificity. Mass spectrometry based 'metabolomics' enables comprehensive and systematic profiling of thousands of small molecules in biological samples, and is the latest technology being used to search for the elusive perfect biomarker and/or drug target for mitochondrial disease. Metabolomics can be used to analyse the multitudinous downstream effects of mitochondrial dysfunction, including the consequences of oxidative stress, NAD+/NADH redox imbalance and energy deficiency, on a global scale.

Most metabolomics studies have investigated cell and animal models of mitochondrial disease and to date limited global metabolomics datasets are available for human mitochondrial disease. Caenorhabditis elegans models with mutations in subunits of OXPHOS complexes I-III displayed abnormalities of carbohydrate, amino acid and fatty acid metabolism, and of cellular defence pathways. Metabolic profiling of spent media from cultured muscle cells with pharmacologically induced OXPHOS defects revealed alterations of 32 metabolites, including elevations of lactate, several amino acids, TCA cycle intermediates and creatine. Gas chromatography/liquid chromatography-mass spectrometry (GC/LC-MS) profiling of blood and urine samples from a relatively small number of patients with a specific subgroup of Leigh syndrome caused by mutations in the LRPPRC gene (known as 'French Canadian' Leigh syndrome) revealed 45 'signature
metabolites'. These metabolites included ketones (β-hydroxybutyrate and acetoacetate), acylcarnitines (of multiple chain lengths but especially C14 and C16), amino acids (alanine, citrulline, asparagine), lipids (increased cholesterol/HDL ratio) and kynurenine (an intermediate of the de novo NAD\(^+\) synthesis pathway), as well as TCA cycle metabolites, lactate and pyruvate. So far, studies validating these results in other mitochondrial patients have not been reported, so it remains uncertain whether this metabolic profile is specific to this subgroup of Leigh syndrome, or common to all forms of Leigh syndrome or even to all mitochondrial diseases. It is difficult to make comparisons between studies in lower organisms and those in humans, because published studies have generally investigated different gene defects and different parameters in different organisms, with very few paired studies. However, a few patterns appear to be emerging. For example, increased levels of alanine, aspartate, glycine and branched chain amino acids have been observed in Cl, II and III knockouts of *C. elegans* and the ‘deletor’ mouse model of mitochondrial myopathy, which harbours a human mutation in the Twinkle DNA helicase, as well as in human plasma and urine samples.\(^74\) Conversely, discrepant observations between the aforementioned studies included decreased arginine in a Cl *C. elegans* knockout, the *Ndufs4*\(^{-/-}\) mouse model and human plasma and cell cultures, but elevated arginine levels in a CII *C. elegans* knockout and spent media from human cell cultures.\(^74\)

Systematic metabolomics studies of large cohorts of patients with mitochondrial disease are needed to determine the generalisability of these results and to identify the optimal robust biomarker set(s) for a) use as outcome measures in clinical trials or b) further investigation as possibly tractable therapeutic targets. Conducting such studies will be challenging because of multiple confounders, including clinical and genetic heterogeneity, batch effects and lack of standardised protocols. Few centres have large cohorts of living patients who can be clustered into specific genetically confirmed subgroups, thus international collaboration will be crucial to perform clinically meaningful metabolomics studies.

The LSFC study also suggested that methylation reactions might be perturbed in these patients,\(^77\) and intriguingly a recent study of mouse models with TWNK mutations affecting mtDNA maintenance has also implicated one-carbon metabolism in the pathogenesis of mitochondrial disease.\(^78\) Investigation of the ‘methylome’ may shed further light on the role of dysfunctional one-carbon metabolism in the pathogenesis of mitochondrial disease. Finally, a new area of interest is that of disorders of mitochondrial lipid metabolism.\(^79\) Lipidomics of mitochondrial and mitochondria-
associated membranes will enhance our understanding of cellular lipid trafficking and the pleiotropic mitochondrial defects caused by disturbances in membrane lipid homeostasis.80

**Integrated Omics**

A combined omics approach, integrating data from transcriptomics, proteomics and metabolomics, is still in its infancy. A recent study used ‘multi-omics’ analysis to identify the transcription factor ATF4 as a key regulator of the mitochondrial stress response in mammals.81 Previous studies combining two omics modalities include a microarray and quantitative SILAC (stable isotope labelling by amino acids in cell culture) proteomics study of a cell model overexpressing PGC1α that revealed a critical role for cellular iron in regulating mitochondrial biogenesis.82 Combined proteomics (mass spectrometry) and metabolomics (nuclear magnetic resonance) profiling of urine from patients with the m.3243A>G mutation revealed marked differences from controls, particularly of proteins involved in lysosomal pathways, calcium-binding and antioxidant defence.83 Going forwards, addition of phenotypic information from large cohorts of patients with mitochondrial disease sequenced in the 100K and other WGS projects will increase the power of multi-omics datasets.

**New Mitochondrial Disease Mechanisms**

Mechanisms underpinning mitochondrial disease pathology are poorly understood. However, in recent years, utilisation of omics technologies to elucidate novel pathophysiological mitochondrial mechanisms has led to exciting and often unexpected developments. Bioenergetic deficit due to ineffective OXPHOS is a starting point for explaining the pathophysiology of mitochondrial disease, and can be especially true for patients with mutations affecting OXPHOS components. However, failure to detect ATP deficiencies both in vitro and in vivo has demonstrated that energy deficits cannot solely explain mitochondrial disease pathophysiology. Other putative mitochondrial pathomechanisms include increased ROS production (or decreased antioxidant protection), loss of mitochondrial membrane potential, impaired mitochondrial calcium handling, and activation of apoptotic pathways.

Mitochondrial diseases may be caused by mutations of proteins with known roles, including OXPHOS structural subunits and assembly factors, mtDNA maintenance, mitochondrial translation, mitochondrial lipids and mitochondrial dynamics (Table 1). Conversely dozens of mitochondrial disease mutations affect proteins with undetermined functions. Elucidating the activities of these uncharacterised proteins provides valuable insight into mitochondrial pathophysiology and has been substantially facilitated by non-targeted global analyses. Investigation of mitochondrial protein-
protein interactions with a novel biotinylation proximity (BioID) assay enabled functional understanding of modified mitochondrial carrier protein SLC25A46, mutations in which are linked to Leigh syndrome and optic atrophy. These studies implicated SLC25A46 in several physiological processes including mitochondrial dynamics, since a hyperfused mitochondrial phenotype was observed in patient cells. Identified binding partners of SLC25A46 suggested additional roles, including being an integral component of the mitochondrial contact site and cristae organizing system (MICOS), a protein complex responsible for linking cristae junctions to the IMM, as well as mitochondrial lipid homeostasis by interacting with the ER membrane protein complex (EMC), which is responsible for phospholipid transfer from the ER, the site of cellular phospholipid synthesis, to the mitochondria (Figure 1). Defects in these processes contribute to perturbed mitochondrial ultrastructure, altered inheritance of mtDNA, and dysfunctional OXPHOS. Similar pathology and neurological phenotype have been observed in patients with mutations in the QIL1 MICOS subunit.

In other cases, proteins may have moonlighting functions that can contribute to disease pathology. AGK, deficiency of which causes Sengers syndrome (cataracts and cardiomyopathy), was originally annotated as acylglycerol kinase, and AGK mutations were hypothesised to perturb mitochondrial lipid homeostasis, although evidence was lacking in Sengers patients. However, quantitative proteomics studies recently revealed AGK to be a component of the TIM22 mitochondrial import machinery (Figure 2), a pathway involved in importing the ANT1 ADP/ATP translocase. This now explains previous observations of ANT1 deficiency in Sengers patients. In other instances WES has revealed novel roles for non-mitochondrial proteins in mitochondrial (patho)physiology. For example, the JAK-STAT cytokine STAT2, previously known for its role in innate immunity, was shown to be a novel regulator of mitochondrial fission; patients deficient in STAT2 exhibited a hyperfused mitochondrial and severe neurological phenotype similar to other mitochondrial dynamic defects.

Increasing evidence is accumulating that mitochondrial stress responses triggered by the molecular defect are the major contributor to mitochondrial disease, rather than the OXPHOS defect itself. While OXPHOS is upregulated in the fed state, mitochondria metabolically adapt in fasting or starvation conditions by upregulating biosynthetic pathways. Investigation of how mitochondrial disease affects this metabolic switch has led to significant developments in elucidating disease pathology, particularly within the context of mtDNA maintenance disorders. Metabolic and transcriptional remodelling elicited by mitochondrial dysfunction has been most comprehensively investigated in the deletor mouse model. Skeletal muscle of deletor mice mimics several starvation and stress mitochondrial phenotypes including transcriptional upregulation of metabolic cytokines
FGF21 and GDF15, altered one-carbon metabolism in the mitochondrial folate cycle leading to altered downstream nucleotide pools, enhanced glutathione biosynthesis, and induction of the mitochondrial unfolded protein response (UPR\textsuperscript{mt}) (Figures 1 and 2).\textsuperscript{58,78} Collectively, these metabolic and transcriptional changes are known as the integrated mitochondrial stress response (ISR\textsuperscript{mt}) and are present exclusively in affected tissues, which may at least partially explain tissue specificity of mtDNA maintenance disorders.\textsuperscript{1} Global metabolic, transcriptomic and proteomic analyses in other animal models, as well as human patients, will help to determine the generalisability of changes in ISR\textsuperscript{mt} in the pathophysiology of mitochondrial disorders.

Thus, omics methods have been extremely valuable in the study of mitochondrial disease pathophysiology and will continue to expedite the discovery of novel pathomechanisms. Remaining challenges include understanding genotype-phenotype relationships, which are complex for both mtDNA and nuclear-encoded mitochondrial disorders. The same gene defect, and sometimes even the same mutation, can lead to multiple phenotypes. This has been known for decades for mtDNA mutations, especially large-scale deletions and the m.3243A>G point mutation.\textsuperscript{91,92} This was postulated to arise from varying heteroplasmic mtDNA mutation loads in different tissues, but this explanation has been challenged by the observation of widely differing phenotypes with the same nuclear gene defect. For example, the same nuclear-encoded mitochondrial disease can range in severity from a fatal infantile cardiomyopathy to a late onset disorder with ovarian failure and psychiatric manifestations (impaired mitochondrial translation caused by recessive AARS2 mutations),\textsuperscript{92,94} or from fatal infantile encephalomyopathy to a late onset pure ocular myopathy (e.g. impaired mtDNA maintenance caused by recessive mutations in RRM2B).\textsuperscript{95,96} Genetic and/or environmental modifiers have been postulated but supportive evidence is scarce. Some clarity appears to be emerging for the mtDNA maintenance disorders. For example, in the case of homozygosity for POLG\textsuperscript{p.Ala467Thr}, there appears to be a correlation between the nature of the secondary mtDNA defect caused by defective polymerase gamma function (depletion, multiple deletions or point mutations) and the severity of the phenotype.\textsuperscript{97} Conversely, a single phenotype may be associated with extreme genetic heterogeneity, classically in the case of Leigh syndrome which has been linked to \textasciitilde100 different gene defects.\textsuperscript{47,98} Certain mitochondrial disease genes appear to have a predilection for particular organs, but mechanisms underpinning tissue specificity also remain largely unknown. It is possible that different elements of the phenotype may be caused by different mechanisms.\textsuperscript{99}
**Translational Medicine**

The promise of omics means that it is now more important than ever to become 'trial ready' for mitochondrial diseases. However, many challenges continue to impede the development of effective therapies for mitochondrial disease. A major hindrance has been the extreme clinical heterogeneity of affected patients, even within genetically homogeneous groups caused by the same recessive defect. It is very unlikely that a single therapy will reverse all the phenotypic manifestations of a mitochondrial disease, particularly in multisystem disorders which can affect as many as 7 organ systems. Additional historical obstacles to the development of rational therapies for these disorders have included the inaccessibility of the double-membraned mitochondrion, making it difficult for drugs and gene therapy to penetrate the correct mitochondrial subcompartment; a lack of viable candidate therapies; dearth of animal models for preclinical trials; lack of validated outcome measures to monitor therapeutic efficacy; inadequate natural history data; and insufficient numbers of genetically confirmed cases to conduct meaningful clinical trials. Important outstanding questions include determining which patients will benefit from which treatments, and defining what constitutes 'successful' treatment.

Several developments in the mitochondrial field in recent years have begun to address these challenges. The large gene sequencing programmes discussed above have transformed the genetic landscape of mitochondrial disease, so that many more patients are receiving a timely genetic diagnosis, sometimes within days of clinical presentation. The availability of numerous animal models has facilitated testing of novel therapies for mitochondrial disease. Several national mitochondrial cohorts have been established and international consortia have begun to perform natural history studies, set up Delphi panels to establish outcome measures for mitochondrial disease, and document current standard practices for monitoring and management. International collaboration will likely pave the way for relatively large clinical trials, even for the rarest genetic disorders, although innovative trial designs will be needed for ultra-rare disorders. Another caveat is that the introduction of treatments via 'back-door' compassionate use programmes may mean that all known patients are already receiving a candidate treatment, even before a clinical trial can be rolled out, making it impossible to ever perform a proper clinical trial of that compound. The thorny route to licensing for lysosomal storage disorders (LSDs), another group of inborn errors of metabolism with complex multisystem presentations, will provide important lessons for the mitochondrial field. Of 70 orphan drug designations for LSDs, only 14 have received FDA approval over a 30 year period. Novel routes to drug licensing for rare diseases will need to be explored.
Here we will focus on recently emerging novel therapies for mitochondrial disease, including targets/pathways revealed by high throughput omics studies (Figure 3). Therapeutic strategies currently in development can be divided into two main groups – pharmacological and gene therapy based approaches.

**Pharmacological approaches**

**Vitamins and cofactors**

Very few ‘treatable’ mitochondrial disorders that respond to specific pharmacological interventions are recognised. These include disorders of CoQ\textsubscript{10} biosynthesis, disorders of riboflavin transport and metabolism, thiamine and ketogenic diet responsive forms of pyruvate dehydrogenase complex deficiency, and biotin-thiamine responsive basal ganglia disease caused by mutations in *SLC19A3* encoding a thiamine transporter.\textsuperscript{112}

**Antioxidants**

Antioxidants proposed as mitochondrial therapeutics include vitamins C and E, N-acetylcysteine (to replenish glutathione stores), alpha-lipoic acid, and CoQ\textsubscript{10} and its analogues including ubiquinol and idebenone,\textsuperscript{100} the latter having been licensed recently for the treatment of LHON. Modified redox modulating agents under investigation include EPI-743 and KH176,\textsuperscript{113,114} currently in phase 2 trials (Table 2). It should be noted that off-target effects of antioxidants are currently incompletely understood. By remodelling ROS signalling, high dose antioxidants could potentially cause unwanted side effects in treated patients. For example, antioxidants attenuated health-promoting effects of physical exercise in healthy human volunteers in one study.\textsuperscript{115} Furthermore, antioxidants may have differential effects in different tissues; identical doses of a mitochondrially targeted antioxidant MitoQ improved hematopoietic stem cell dysfunction but were toxic to neural stem cells derived from the same mouse model.\textsuperscript{27}

**Replenishing reducing equivalents to restore redox balance**

Mitochondrial dysfunction leads to depletion of reducing equivalents, especially NAD\textsuperscript{+}. Supplementation with nicotinamide riboside (NR) was associated with clinical improvement in two mouse models of mitochondrial disease.\textsuperscript{116,117} Two phase 1 trials recently demonstrated increased steady-state levels of NAD\textsuperscript{+} following oral administration of NR to healthy volunteers,\textsuperscript{118,119} and clinical trials in patients with primary mitochondrial diseases are likely to follow.

**Stabilising mitochondrial membrane lipids**

Elamipretide (MTP-131) is a novel tetrapeptide (H\textsubscript{3}Arg-Tyr(2,6-diMe)-Lys-Phe-NH\textsubscript{2}) that has been suggested to stabilise cardiolipin, a complex IMM-specific lipid critical for integrity of the OXPHOS
complexes. A phase 1/2 clinical trial of elamipretide in genetically confirmed mitochondrial myopathy has been completed.\textsuperscript{120} Results have not yet been reported, but an open label extension is ongoing (clinicaltrials.gov).

**Stimulating mitochondrial biogenesis**

Agents with potential to correct mitochondrial dysfunction by manipulating pathways that impact mitochondrial biogenesis include bezafibrate, resveratrol and decanoic acid (C10),\textsuperscript{100,121} some of which are undergoing clinical trials (Table 2).

**Nucleoside supplementation**

Another approach that has shown encouraging results in cell and animal models is enhancing nucleoside supply as a treatment approach for mtDNA depletion caused by impaired nucleoside salvage, particularly thymidine kinase 2 (TK2) deficiency.\textsuperscript{122,123} Selected TK2-deficient patients have been treated on a compassionate basis, with so far unreported results (http://enmc.org/publications/workshop-reports/recommendations-treatment-mitochondrial-dna-maintenance-disorders).

**Metabolic remodelling**

Rapamycin has recently emerged as a potential therapy for mitochondrial disease, with improved disease outcomes in three mouse models.\textsuperscript{124-126} In deletor mice, rapamycin successfully ameliorated several aspects of the ISR\textsuperscript{mt} stress response in addition to directly inhibiting mTOR targets.\textsuperscript{125} Furthermore, low-dose rapamycin was reported to enhance longevity of TK2-deficient mice,\textsuperscript{126} a result resonating with the longstanding observations of lifespan-extending properties of rapamycin in several model organisms of ageing. Similar effects of increased lifespan and reversal of neuropathology have been observed after maintaining ndufs4\textsuperscript{-/-} mice with Leigh syndrome in a hypoxic chamber.\textsuperscript{127} However, amelioration of disease was only observed in 11% hypoxia, not in 17% oxygen or intermittent hypoxia, so it is difficult to imagine how this approach might be translated into clinical practice.

**Gene therapy**

Genetic approaches to treating mitochondrial disease can also be divided into two groups – those targeting the mtDNA, and those aimed at correcting nuclear gene defects. Different strategies are needed because of the peculiar characteristics of the mtDNA, namely the high copy number leading to the phenomenon of heteroplasmy (coexistence of mutant and wild type mtDNA in varying proportions in different tissues), unique genetic code and exclusive maternal inheritance.

**Mitochondrial genome editing**
For heteroplasmic mtDNA mutations, genome editing to selectively destroy the mutant sequence is an attractive option and has been pursued using increasingly sophisticated tools for >15 years. Initial proof-of-principle studies demonstrated that restriction endonucleases could selectively destroy mutant mtDNA leaving wild type genomes intact. Subsequent studies used zinc finger nucleases and mitochondrial TALENs to target restriction endonucleases to the mitochondrion, initially in cell models of mitochondrial disease and more recently in whole animals. Currently CRISPR/Cas9 gene editing is not technically possible against mtDNA in situ, because of difficulties of importing RNA into the mitochondrion.

Harnessing the nuclear genome to correct mtDNA defects

Another approach to correcting a mtDNA defect is to insert a vector containing the relevant mitochondrial gene recoded using the universal nuclear genetic code, so that the recoded gene can be inserted into and expressed from the nucleus, a process known as allotopic expression. Including a mitochondrial targeting sequence in the recoded gene ensures that the encoded protein can be directed to the mitochondrion. This approach has been used to treat a rat LHON model and human clinical trials are ongoing (Table 2). A recent preliminary report demonstrated safety and suggested efficacy of rAAV2-ND4 therapy.

Gene therapy for nuclear-encoded mitochondrial disorders

Gene therapy involves the correction of a genetic abnormality by delivering a ‘correct’ copy of the affected gene to cells using a vector. The most promising gene therapy vectors at present are adeno-associated virus (AAV) vectors which belong to the parvovirus family but are non-pathogenic in humans. The availability of multiple naturally occurring AAV subtypes with different tissue tropism is attractive for multisystem disorders such as mitochondrial disease. AAV-mediated gene therapy has been administered in knockout mouse models of ethylmalonic encephalopathy, mitochondrial neurogastrointestinal encephalomyopathy and Leigh syndrome (ndufs4) with varying degrees of success. However, the huge number of nuclear gene defects now linked to mitochondrial disease poses a significant challenge to the use of gene therapy as a universal panacea for mitochondrial disease; it is difficult to conceive how gene therapies for more than 350 different gene defects can be brought to clinic in a realistic time frame. Furthermore, whether public health systems can cope with the enormous costs of these therapies and whether single-dose therapies will attract commercial investment are logistical issues that need to be addressed. Another gene therapy approach is CRISPR/Cas9 gene editing to correct nuclear-encoded mitochondrial disease. This has recently been demonstrated in a proof-of-principle study in an induced pluripotent stem cell model of primary CoQ10 deficiency. However, homology directed repair is an inefficient repair mechanism, and this will need to be addressed going forwards.
**Reproductive technologies**

An area of remarkable progress has been the development of the mitochondrial replacement techniques metaphase spindle cell transfer (MST) and pronuclear transfer (PNT) to avoid transmission of pathogenic mtDNA mutations.\(^{139-141}\) These techniques involve the transfer of nuclear genetic material from the biological mother’s egg, before (MST) or after (PNT) fertilisation, to a donor egg or zygote, with minimal carryover of the biological mother’s mtDNA. These therapies are intended to reduce risk of transmitting mitochondrial disease for women with a high proportion of mutated mtDNA, in whom pre-implantation genetic diagnosis is unlikely to be an effective reproductive option. After an extensive public debate about the related ethical and societal challenges, the UK Parliament passed a bill legalising mitochondrial donation therapy in 2015. In March 2017 the first UK clinic was licensed to perform PNT by the Human Fertilisation and Embryology Authority, which is now considering applications for mitochondrial donation therapy. The first baby born after MST was delivered in Mexico in 2016, to a woman carrying the m.8993T>G Leigh syndrome mutation, and was reported to be well at 7 months of age.\(^{142}\) A remaining issue is that studies of embryonic stem cells have led to concerns about the possibility of later reversion to the mutant mtDNA haplotype.\(^{143}\) Long-term neurodevelopmental follow-up of children born after mitochondrial donation therapies will be needed, to determine whether these concerns are justified.

**Conclusions**

The mitochondrion is a complex organelle where many pathways and cell functions overlap. Furthermore, critical crosstalk with other subcellular organelles contributes to the remarkable complexity and heterogeneity of mitochondrial disorders. Recent advances in multi-faceted omics technologies have, and will continue to, disentangle enigmatic features of mitochondrial disease revolutionise the field of mitochondrial medicine and hopefully pave the way for the development of effective therapies at long last.

**Acknowledgements**

This work was supported by a Great Ormond Street Hospital Children’s Charity Research Leadership Award (V1260) to SR and PhD studentship to JR, and by the NIHR Great Ormond Street Hospital Biomedical Research Centre. We also acknowledge research grant funding from the Lily Foundation. The views expressed are those of the author(s) and not necessarily those of the NHS, the NIHR or the Department of Health.
Competing interest statement

SR has previously received research funding from Vitaflo International Ltd.

References


Figure Legends

Figure 1. The Contributions of Omics Technologies to Mitochondrial Medicine. In recent years, high-throughput omics technologies coupled with sophisticated bioinformatic analyses have made significant contributions to the mitochondrial field. The genomic revolution (1) has increased diagnostic yield for mitochondrial disease patients and has also expedited the discovery of dozens of novel disease genes. Metabolomics has been a valuable tool in disentangling novel mitochondrial disease pathomechanisms, such as changes to one-carbon cycle metabolism in certain mitochondrial disorders (2). The structure and function of OXPHOS complexes within multimeric supercomplexes has greatly benefited from the use of quantitative proteomics (3). In addition to producing reducing equivalents for OXPHOS, the metabolites and enzymes in the TCA cycle also affect redox ratios in the mitochondrion, thereby modulating downstream epigenetic regulation through the actions of NAD⁺-dependent sirtuins (4). Transcriptomics has been a valuable tool in analysing gene expression and has been responsible for the identification of novel mitochondrial disease biomarkers (5). A global analysis of the mitochondrial interactome has revealed novel links between mitochondrial dynamics, mitochondrial cristae organization, and ER-mitochondrial communication (6).

Figure 2. Mitochondrial Maintenance and Function. The mitochondria serve a multitude of biosynthetic and bioenergetic functions. The process of OXPHOS requires the coordinated effort of numerous metabolic reactions and structural components including: the maintenance of mtDNA so that it can be effectively replicated, transcribed, and translated to produce structural components of OXPHOS enzymes; the production of iron-sulphur cofactors with vital redox properties; and the production of reducing equivalents and other metabolites from the TCA cycle. Mitochondria also serve several biosynthetic functions necessary for a variety of cellular processes. These include one-carbon metabolism which feeds into pathways responsible for antioxidant defence, DNA methylation, and purine biosynthesis.

Figure 3. Current Mitochondrial Therapies in Development. Despite the lack of approved curative therapies for mitochondrial disease, a number of pharmacological and genetic approaches to ameliorating the pathology of mitochondrial disease are under investigation.
Table 1. Current Mitochondrial Gene Defects and Pathomechanisms

<table>
<thead>
<tr>
<th>Disease Mechanism</th>
<th>Gene Function</th>
<th>Gene(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Oxidative Phosphorylation Deficiency</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Complex I Subunits and Assembly Factors</td>
<td>NDUFA1, NDUFA2, NDUFA6, NDUFA9, NDUFA10, NDUFA11, NDUFA12, NDUFA13, NDUFB3,</td>
<td>NDUFA1, NDUFA2, NDUFA6, NDUFA9, NDUFA10, NDUFA12, NDUFA13, NDUFB3,</td>
</tr>
<tr>
<td></td>
<td>NDUFB8, NDUFB9, NDUFB10, NDUFB11, NDUFS1, NDUFS2, NDUFS3, NDUFS4, NDUFS6,</td>
<td>NDUFB8, NDUFB9, NDUFB10, NDUFB11, NDUFS1, NDUFS2, NDUFS3, NDUFS4,</td>
</tr>
<tr>
<td></td>
<td>NDUFS7, NDUFS8, NDUFS11, NDUV2, NDUFA1, NDUFA2, NDUFA3, NDUFA4, NDUFA5,</td>
<td>NDUFS7, NDUFS8, NDUFS11, NDUV2, NDUFA1, NDUFA2, NDUFA3, NDUFA4,</td>
</tr>
<tr>
<td></td>
<td>NDUFA8, ACAD9, ECSIT, FOXRED1, NUBPL, TIMMDC1, TMEM126B, MT-ND1, MT-ND2, BT-</td>
<td>NDUFA8, ACAD9, ECSIT, FOXRED1, NUBPL, TIMMDC1, TMEM126B, MT-ND1,</td>
</tr>
<tr>
<td></td>
<td>ND-ND3, MT-ND4, MT-ND5, MT-ND6</td>
<td>MT-ND4, MT-ND5, MT-ND6</td>
</tr>
<tr>
<td>Complex II Subunits and Assembly Factors</td>
<td>SDHA, SDHB, SDHC, SDHD, SDHAF1, SDHAF2</td>
<td></td>
</tr>
<tr>
<td>Complex III Subunits and Assembly Factors</td>
<td>UQCRB, UQRC2, UQCRFS1, UQCRQ, CYC1, BCS1L, HCCS, TIC19, LYRM7, UQCC2, UQCC3,</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MTCYB</td>
<td></td>
</tr>
<tr>
<td>Complex IV Subunits and Assembly Factors</td>
<td>COX4I1, COX4I2, COX5A, COX6A1, COX6B1, COX7B, COX8A, NDUFA4, SURF1, SCO1,</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SCO2, COX10, COX15, COA3, COA5, COA6, COA7, COX14, COX20, FASTKD2, PET100,</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PET117, CEP89, MT-CO1, MT-CO2, MT-CO3</td>
<td></td>
</tr>
<tr>
<td>Complex V Subunits and Assembly Factors</td>
<td>ATP5A1, ATP5D, ATP5E, ATPAF2, TMEM70, USMG5, MT-ATP6, MT-ATP8</td>
<td></td>
</tr>
<tr>
<td>Disorders of Mitochondrial DNA Maintenance</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nucleotide Pool Maintenance</td>
<td>ABAT, AK2, DGU0K, RRM2B, SAMHD1, SUCLA2, SUCLG1, TK2, TYPMP</td>
<td></td>
</tr>
<tr>
<td>Replication, Maintenance, and Transcription of</td>
<td>DNA2, FBXL4A, MGME1, MPV17, POLG, POLG2, SSBP1, SLC25A4, TINKW</td>
<td></td>
</tr>
<tr>
<td>mtDNA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mitochondrial Translation Defects</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mitochondrial tRNAs</td>
<td>MT-PA, MT-TC, MT-TD, MT-ND, MT-TE, MT-TF, MT-TG, MT-TH, MT-TI, MT-TK, MT-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TL1, MT-TL2, MT-TM, MT-TN, MT-TP, MT-TQ, MT-TR, MT-TS, MT-TV, MT-TW, MT-TY</td>
<td></td>
</tr>
<tr>
<td>Mitochondrial Aminoacyl-tRNA Synthetases</td>
<td>AARS2, CAR2, DARS, DARS2, EARS2, FARS2, GARS, HARS2, IARS, IARS2, KARS, LARS,</td>
<td></td>
</tr>
<tr>
<td></td>
<td>LARS2, MARS2, NARS, QARS, RARS, RARS2, SARS2, TARS2, VARS2, WAR2, YARS2</td>
<td></td>
</tr>
<tr>
<td>tRNA Modification</td>
<td>ELAC2, MTFT, NSUN3, PDE12, QRS1, QRT1, TMT5, TRMT10C, TRNT1</td>
<td></td>
</tr>
<tr>
<td>Mitochondrial rRNA</td>
<td>MT-RNR1, MT-RNR2</td>
<td></td>
</tr>
<tr>
<td>RNA Processing</td>
<td>PNPT1</td>
<td></td>
</tr>
<tr>
<td>Mitoribosome Subunits and Assembly</td>
<td>ERAl1, MRPL3, MRPL12, MRPL44, MRPS2, MRPS7, MRPS16, MRPS22, MRPS23, MRPS34,</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MRMR2, RMND1</td>
<td></td>
</tr>
<tr>
<td>Protein Synthesis</td>
<td>C12orf65, GFM1, GFM2, GTPBP3, GU1, LRPBP6, MTO1, MTPAP, PUS1, TACO1, TRMU,</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TSFM, TUFM</td>
<td></td>
</tr>
<tr>
<td>Mitochondrial Membrane Phospholipid and Import</td>
<td>AGK, CHKB, DNAJC19, GFER, MIPEP, PAM16, PLA2G6, PMPCA, SERAC1, SLC25A3, SLC25A5,</td>
<td></td>
</tr>
<tr>
<td>Machinery</td>
<td>SLC25A10, SLC25A12, SLC25A22, TAZ, TIMM58, TIMM50, XPNPSE3</td>
<td></td>
</tr>
<tr>
<td>Mitochondrial Dynamics</td>
<td>DNM1L, GDP1, MFL, MFN2, MTO1, OPA1, STAT2, TRAK1, YME1LI</td>
<td></td>
</tr>
<tr>
<td>MICOS Complex</td>
<td>CHCHD10, QHL1, SLC25A46</td>
<td></td>
</tr>
<tr>
<td>ER-Mitochondrial Tethering</td>
<td>EMC1</td>
<td></td>
</tr>
<tr>
<td>Mitochondrial Protein Quality Control</td>
<td>AFG3L2, ATAD3A, CLPB, CLPP, CLPX, HSPA9, HSPD1, HSPE1, LONP1, PITRM1, SACS,</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SPG7, TRAP1</td>
<td></td>
</tr>
<tr>
<td>Toxicity</td>
<td>ECHS1, ETHE1, HIBCH</td>
<td></td>
</tr>
<tr>
<td>Antioxidant Defence</td>
<td>NNT, TXN2</td>
<td></td>
</tr>
<tr>
<td>Mitochondrial Quality Control Defects</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tricarboxylic Acid Cycle Enzymes</td>
<td>ACO2, DHTKD1, FH, IDH3A, IDH3B, MDH2, OGDH</td>
<td></td>
</tr>
<tr>
<td>Pynvate Metabolism</td>
<td>DLAT, DLD, MPC1, PC, PDHA1, PDHB, PDHX, PDK3, PDP1, PDP2</td>
<td></td>
</tr>
<tr>
<td>Fatty Acid Metabolism</td>
<td>CRAT, ETF4, ETFB, ETDFH, FA2H, HSD1B10, PYCR1, SLC25A1</td>
<td></td>
</tr>
<tr>
<td>CoA Metabolism and Transport</td>
<td>COASY, PANK2, SLC25A4</td>
<td></td>
</tr>
</tbody>
</table>
### Vitamin and Cofactor Metabolism Defects

- **Coenzyme Q<sub>10</sub> Biosynthesis**: COQ2, COQ4, COQ5, COQ6, COQ7, COQ8A, COQ8B, COQ9, PDSS1, PDSS2
- **Iron-Sulphur Cluster Protein Biosynthesis**: ABCB7, FDXR, FDX1L, FXN, ISCA1, ISCA2, ISCU, LYRM4, NFS1, NFU1
- **Lipoic Acid Biosynthesis**: BOLA3, GLRX5, IBA57, LIAS, LIPT1, LIPT2, MECR
- **Cytochrome c**: CYCS
- **Biotin Metabolism**: BTD, HLCS
- **Thiamine Metabolism and Transport**: SLC19A2, SLC19A3, SLC25A19, TPK1
- **Mitochondrial One-Carbon Metabolism**: SLC25A26, SLC25A32
- **Heavy Metal Metabolism**: SLC25A24, SLC33A1, SLC39A8
- **Selenoprotein Biosynthesis**: SECISBP2, SEPSECS
- **NADPH Metabolism**: NADK2, NAXD, NAXE
- **Riboflavin Metabolism and Transport**: FLAD1, SLC52A2, SLC52A3

### Other Cellular Defects Associated with Mitochondrial Dysfunction

- **Ca<sup>2+</sup> Homeostasis**: ANO10, C19orf70, CISD2, CYP24A1, MICU1, MICU2, WFS1
- **Heme Biosynthesis**: ABCB6, ALAS2, SFXN4, SLC25A38
- **Apoptosis Defects**: AIFM1, APOPT1, DIABLO, HTRA2, PTRH2
- **DNA Repair**: APTX, XRCC4
- **Miscellaneous or Unknown Function**: ALDH1B1, ALDH18A1, BDH1, CA5A, CTBP1, C1QBP, C19orf12, DCC, DIAPH1, FHF1, IF5A, OPA3, PNP4A, PNP4B, POP1, PP2A, ROB1, RTN4P1, SLC44A1, STXBP1, TANGO2, TMEM65, TMEM126A

### Table 2. Current Mitochondrial Disease Therapies in Clinical Trials

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Phase</th>
<th>Mechanism of Action</th>
<th>Disorder</th>
<th>Age Range (Years)</th>
<th>Clinical Trial Identifier</th>
<th>Primary Outcome Measure(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPI-743 (Vatiquinone®)</td>
<td>2</td>
<td>Mitochondrial Redox Modulator</td>
<td>PMD</td>
<td>1+</td>
<td>NCT01370447</td>
<td>Change in neuromuscular function, IAE, NPMDS</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>LS</td>
<td>1-18</td>
<td>NCT02352896</td>
<td>NPMDS</td>
</tr>
<tr>
<td>Idebenone (Raxone®)</td>
<td>2</td>
<td>Mitochondrial Redox Modulator</td>
<td>MELAS</td>
<td>8-65</td>
<td>NCT00887562</td>
<td>Mean Change in Cerebral Lactate Concentration</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>Mitochondrial Redox Modulator</td>
<td></td>
<td></td>
<td>NCT02274005</td>
<td>BCVA</td>
</tr>
<tr>
<td>KH176</td>
<td>2</td>
<td>Mitochondrial Redox Modulator</td>
<td>PMD, MM, MELAS, MIDD</td>
<td>18+</td>
<td>NCT02909400</td>
<td>Movement Disorders</td>
</tr>
<tr>
<td>RTA408 (Omavexalone)</td>
<td>2</td>
<td>Anti-oxidant and Anti-inflammatory</td>
<td>MM</td>
<td>18-75</td>
<td>NCT02255422</td>
<td>Change in Peak Workload</td>
</tr>
<tr>
<td>Bezafibrate</td>
<td>2</td>
<td>PPARα Activator</td>
<td>MM</td>
<td>16-74</td>
<td>NCT02398201</td>
<td>Change in Respiratory Chain Enzyme Activity</td>
</tr>
<tr>
<td>Cyclosporine</td>
<td>2</td>
<td>Immunomodulator</td>
<td>LHON</td>
<td>18+</td>
<td>NCT02176733</td>
<td>BCVA</td>
</tr>
<tr>
<td>KL1333</td>
<td>1</td>
<td>NAD+ Modulator</td>
<td>MRCD, MELAS</td>
<td>19-45</td>
<td>NCT03056209</td>
<td>IAE</td>
</tr>
<tr>
<td>MTP-131 (Elamipretide; Bendavia®)</td>
<td>2</td>
<td>Cardiolipin Stabiliser</td>
<td>PMD</td>
<td>16+</td>
<td>NCT02805790</td>
<td>IAE</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>MM</td>
<td>16-65</td>
<td>NCT02367014</td>
<td></td>
</tr>
<tr>
<td>Arginine and Citrulline</td>
<td>2</td>
<td>Nitric Oxide Precursors</td>
<td>PMD</td>
<td>3-18</td>
<td>NCT02809170</td>
<td>Reactive hyperemic index</td>
</tr>
<tr>
<td>Allogeneic Hematopoietic Stem Cell Transplant</td>
<td>1</td>
<td>Cellular Therapy</td>
<td>MNGIE</td>
<td>5-55</td>
<td>NCT02427178</td>
<td>Neutrophil Count</td>
</tr>
<tr>
<td>rAAV2-ND4 (GS010)</td>
<td>3</td>
<td>Gene Therapy</td>
<td>LHON</td>
<td>15+</td>
<td>NCT03293524</td>
<td>BCVA</td>
</tr>
</tbody>
</table>

Key: BCVA - Best Corrected Visual Acuity; IAE - Incidence of Adverse Events; LHON - Leber Hereditary Optic Neuropathy; LS - Leigh Syndrome; MELAS - Mitochondrial Encephalopathy Lactic Acidosis and Stroke-like Episodes; MIDD - Maternally Inherited Deafness and Diabetes; MM - Mitochondrial Myopathy; MNGIE - Mitochondrial Neurogastrointestinal Encephalopathy; NPMDS - Newcastle Paediatric Mitochondrial Disease Score; PMD - Primary Mitochondrial Disorder