

# Mitochondrial energy generation disorders: genes, mechanisms, and clues to pathology

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Inherited disorders of oxidative phosphorylation cause the clinically and genetically heterogeneous diseases known as mitochondrial energy generation disorders, or mitochondrial diseases. Over the last three decades, mutations causing these disorders have been identified in almost 290 genes, but many patients still remain without a molecular diagnosis. Moreover, while our knowledge of the genetic causes is continually expanding, our understanding into how these defects lead to cellular dysfunction and organ pathology is still incomplete. Here, we review recent developments in disease gene discovery, functional characterization, and shared pathogenic parameters influencing disease pathology that offer promising avenues toward the development of effective therapies.

Mitochondrial energy generation disorders (hereafter termed mitochondrial diseases) are a heterogeneous group of rare disorders involving defective oxidative phosphorylation (OXPHOS).<sup>2</sup> The OXPHOS system comprises five enzyme complexes, situated in the mitochondrial inner membrane. The first four complexes and two electron carriers form the respiratory chain, which generates a proton gradient used by complex V (F<sub>1</sub>F<sub>0</sub>-ATP synthase) to generate the majority of cellular ATP. For the purpose of this review, we have focused on genes and mechanisms that have a demonstrated defect in primary energy generation (e.g. components of the OXPHOS system) or a clear expected role in mitochondrial homeostasis and the ability to generate energy (e.g. components of the TCA cycle and pyruvate dehydrogenase complex (PDC) that feed into OXPHOS, or those affecting mitochondrial membranes and structure).

Mitochondrial diseases are clinically diverse and can present at any age (1). They can manifest in a tissue-specific or a multisystemic manner, but most often they affect organs with the highest energy demands such as brain, skeletal muscle, eyes, and heart (2). Mitochondrial diseases can show any mode of

inheritance: maternal; X-linked; autosomal recessive; autosomal dominant; and *de novo*. This is due to the dual involvement of both the mitochondrial and nuclear genomes in maintaining the mitochondrial proteome. Mitochondrial DNA (mtDNA) is present in thousands of copies in most cells, which means the heteroplasmic load of a mutation can vary from 0 to 100% and may also differ between tissues in an individual. The threshold level above which the proportion of mutant mtDNA causes cellular dysfunction varies between mutations and tissues, further complicating the pathogenesis of mtDNA disease, as described in detail elsewhere (1, 2).

Disorders of energy generation have a minimum birth prevalence of 1 in 5000 live births (3, 4). Mutations in mtDNA appear to account for ~75% of adult-onset disease (4) but only ~25% of childhood-onset disease (5, 6).

## Gene discoveries

In 1988, the first genetic causes of human mitochondrial disease were published when large deletions in mtDNA and the m.11778G→A point mutation in *MT-ND4* were identified as causing mitochondrial myopathy (MIM 251900) and Leber's hereditary optic neuropathy (LHON, MIM 535000), respectively (7, 8). The following year, the first nuclear cause of mitochondrial disease was identified by the finding of pathogenic mutations in the X-chromosome-encoded PDC subunit gene, *PDHA1* (9).

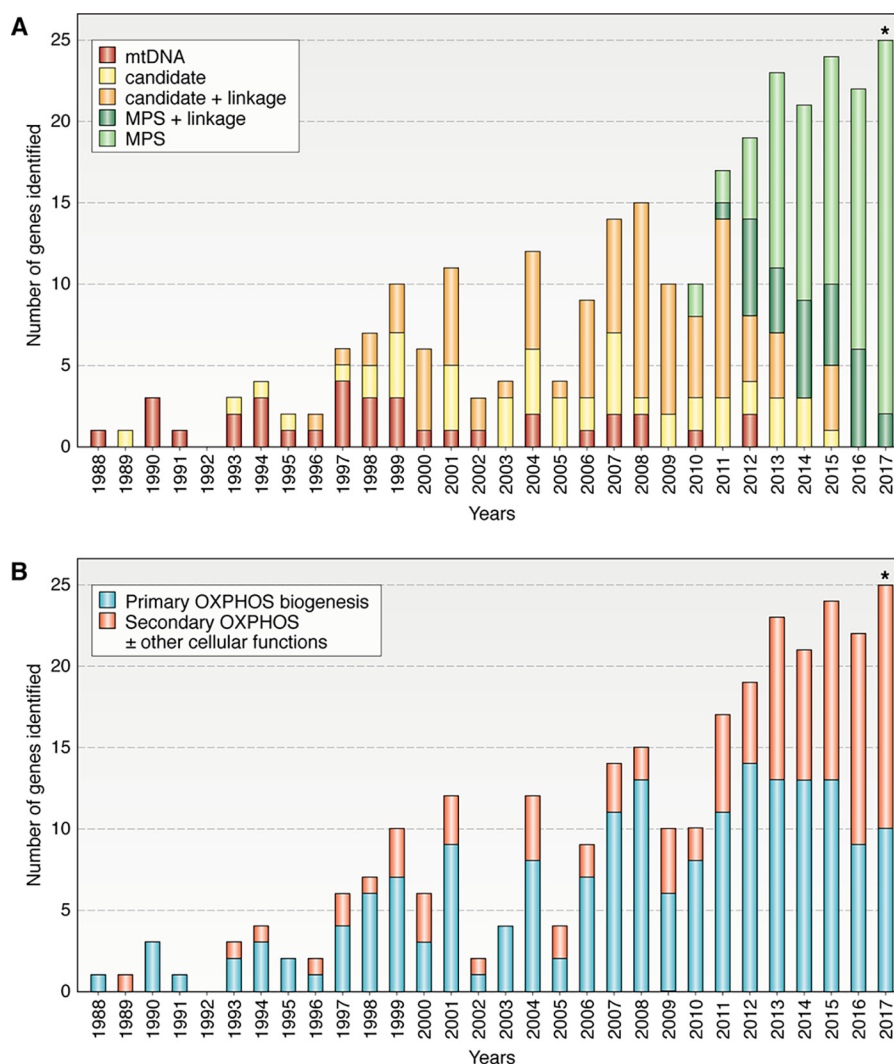
In the following 4 years, mutations in another six mtDNA genes and a second nuclear encoded PDC subunit gene (*DLD*) were shown to cause mitochondrial disorders (Fig. 1 and Table S1) (10–16). This signaled the beginning of a 16-year period where 94 additional nuclear encoded gene defects were identified by candidate gene sequencing (often in conjunction with linkage studies or homozygosity mapping from 1996) (Fig. 1A). The advent of massively parallel (or next generation) sequencing (MPS) led to the identification of two genes encoding the mitochondrial complex I assembly factors, *NUBPL* and *FOXRED1*, as disease genes in 2010 (17). This technology has now enabled the discovery of another 116 novel mitochondrial disease genes. Candidate gene sequencing and linkage studies continue to be used, leading to the discovery of an additional 42 nuclear disease genes. The utility of linkage studies and homozygosity mapping is now successfully being combined with MPS for mutation identification, currently accounting for about 20–30% of yearly gene discoveries.

It has been suggested that the rate of novel gene discoveries in Mendelian disorders has been declining in recent years (post-2013) (18). However, this does not yet seem to hold true

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<sup>2</sup> The abbreviations used are: OXPHOS, oxidative phosphorylation; PDC, pyruvate dehydrogenase complex; TCA, tricarboxylic acid; ROS, reactive oxygen species; MPS, massively parallel sequencing; MIM, Online Mendelian Inheritance in Man (<https://omim.org/>).



**Figure 1. Discovery of genes linked to mitochondrial energy generation disorders: mode of identification and link to OXPPOS biogenesis by year (1988–2017\*).** *A*, because mtDNA was first linked to human disease in 1988, all 37 genes encoded by the mitochondrial genome have had mutations reported; however, only 35 of these have had adequate levels of evidence reported to confirm pathogenicity (32). The first nuclear gene to be linked to mitochondrial energy generation disorders was identified in 1989 by candidate gene sequencing; an additional 138 genes have been identified by this technique to date (with or without the assistance of linkage or homozygosity studies). Since 2010, MPS technology (including targeted sequencing panels and whole-exome or whole-genome sequencing and RNAseq) has identified 116 additional nuclear genes. Linkage or homozygosity studies have aided in the identification of 119 (or 47%) of the 254 nuclear genes associated with mitochondrial disease. *B*, in recent years, the number of genes linked to secondary defects in OXPPOS as well as to additional cellular functions has been steadily increasing, most likely due to the relatively unbiased nature of MPS in identifying gene defects. \*, numbers are complete up to Nov. 23, 2017.

for mitochondrial diseases, which have averaged 22 novel gene discoveries per year since 2012 (Fig. 1A), including 25 for 2017 thus far (listed in Table S1). As of November 23 2017, 289 mitochondrial disease genes have been identified (35 mtDNA encoded genes and 254 nuclear encoded disease genes); numbers refined and updated from Refs. 19, 20. Recent studies of mitochondrial disease cohorts undergoing mtDNA and whole-exome sequencing typically report finding a molecular diagnosis in 30–60% of patients (21–24). Although some patients will have had mutations in known disease genes missed for technical reasons, the general consensus in the field is that known disease genes account for only ~60% of patients with suspected mitochondrial diseases.

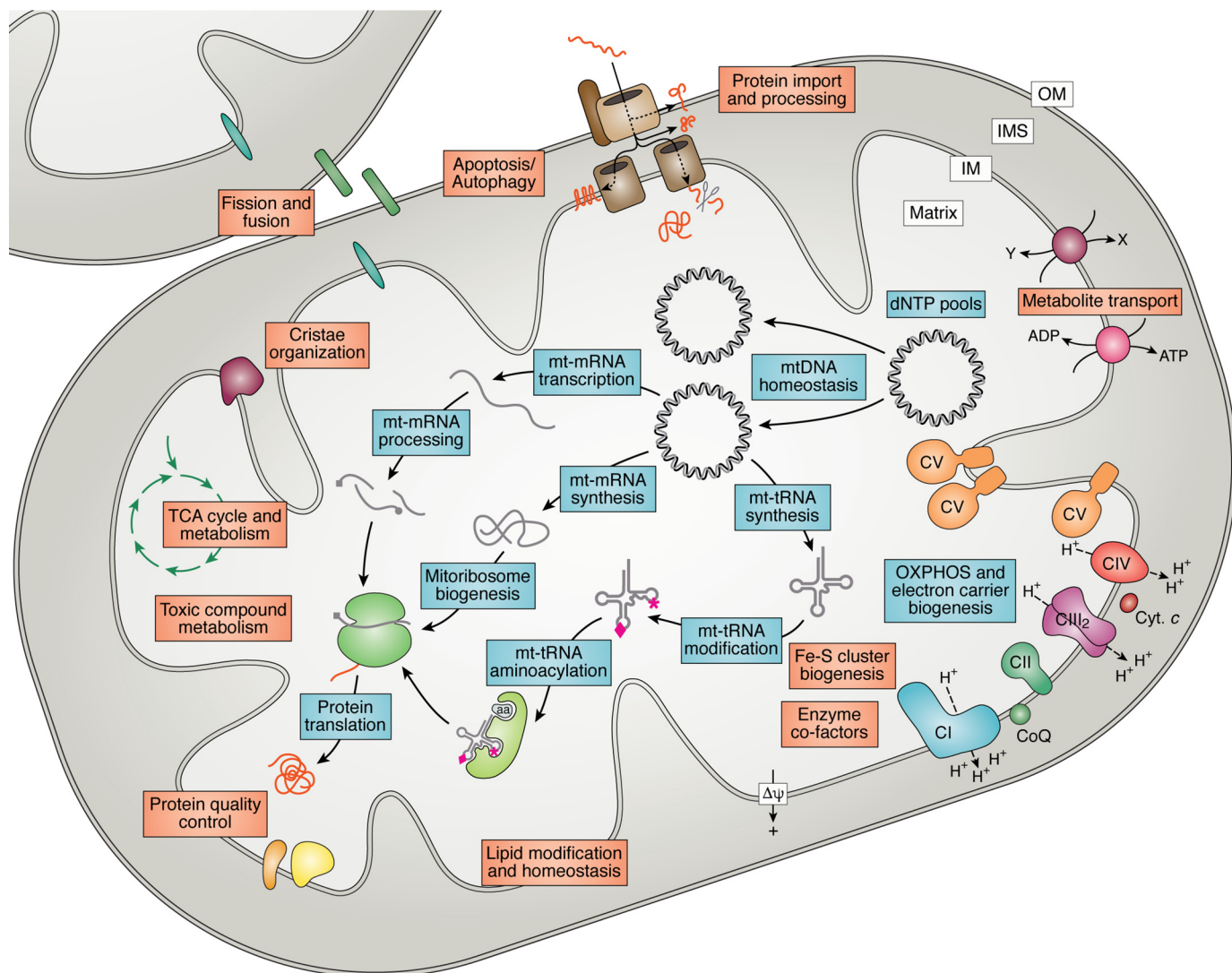
### Categorizing OXPPOS disease genes: direct versus wider cellular impact

The 289 genes we have categorized as causing mitochondrial disease can be divided into those that have a primary role

specific to OXPPOS biogenesis and those whose impacts on OXPPOS are indirect or involve other cellular functions. Interestingly, as disease gene discovery has become more reliant on MPS, the number of genes with secondary or more indirect roles in OXPPOS has increased to around half of all new findings (Fig. 1B). Based on their function, the encoded proteins can be roughly grouped into the following categories: 1) OXPPOS subunits, assembly factors, and electron carriers; 2) mtDNA maintenance; 3) mtDNA expression; 4) enzyme cofactors; 5) mitochondrial homeostasis and quality control; and 6) general metabolism (Fig. 2). Individual references for genes are listed in Table S1 or detailed in Refs. 19, 20, 25–29.

### Genes with a primary role specific to OXPPOS biogenesis

The larger portion (185 of 289) of the mitochondrial disease genes falls into one of the first three categories, with a primary



**Figure 2. Functional categories of genes impacting mitochondrial energy generation.** Mutations in genes functioning in a wide variety of pathways have been linked to mitochondrial diseases and can be separated into those with a primary role specific to OXPHOS biogenesis (blue boxes) or those with a secondary impact on OXPHOS that may also involve other cellular functions (orange boxes). These pathways include the following: 1) OXPHOS subunits, assembly factors, and electron carriers; 2) mtDNA maintenance (including dNTP homeostasis); 3) mtDNA expression (including synthesis, processing, and modification of mt-rRNAs, mt-tRNAs, mt-mRNAs, mitoribosome biogenesis, and translation); 4) enzyme cofactors (including Fe-S cluster biogenesis); 5) mitochondrial homeostasis and quality control (including mitochondrial protein import, lipid modification and homeostasis, mitochondrial morphology comprising fission/fusion and cristae organization factors, protein quality control, and apoptosis/autophagy); and 6) general metabolism (e.g. TCA cycle and metabolite transport). CI–CV, OXPHOS complexes I–V; Cyt. c, cytochrome c; CoQ, ubiquinone/coenzyme Q;  $\Delta\psi$ , mitochondrial membrane potential; aa, amino acid; OM, outer membrane; IMS, intermembrane space; IM, inner membrane.

role specifically related to OXPHOS biogenesis (Fig. 3). Mitochondrial DNA itself encodes 13 subunits of OXPHOS complexes I and III–V, along with 22 tRNAs and 2 rRNAs that are essential for expression of the 13 subunits. The dual genetic nature of mitochondria means that many additional nuclear encoded proteins are required, not just for the biogenesis of the OXPHOS complexes themselves but for the maintenance and expression of mtDNA (30, 31). Pathogenic mutations have been reported in all 37 mtDNA genes (26, 32); however, for two genes (*MT-CO3* and *MT-RNR2*), the evidence for pathogenicity has yet to be sufficiently validated (32).

Disorders of mtDNA maintenance can cause mtDNA depletion or multiple deletions of mtDNA (27). Many of these genes are involved in the regulation of mitochondrial or cytosolic nucleotide pools, as mitochondria rely on cytosolic *de novo* syn-

thesis or salvage pathways to maintain a balanced supply of dNTPs (27). Other disease genes impacting on mtDNA homeostasis encode proteins involved in replication and repair pathways, as well as mtDNA nucleoid packaging (27, 31). This includes the mitochondrial polymerase- $\gamma$ , encoded by *POLG*, in which almost 200 mutations have been reported, making it the most common nuclear cause of mitochondrial disease (33).

Numerous nuclear encoded and mtDNA encoded factors are required for transcription and translation of the 13 mtDNA-encoded OXPHOS polypeptides. Hence, genes linked to OXPHOS disorders include those vital for expression and stability of the long polycistronic mtDNA transcripts, which require post-transcriptional processing and modification to produce stable functional mt-mRNAs, mt-rRNAs, and mt-tRNAs (29, 31). Likewise, mitochondria



Some of the genes we have categorized as having a primary effect on OXPHOS have dual roles or subcellular localizations that may contribute to their impact on OXPHOS and disease pathology. For example, mutations in *SUCLA2* and *SUCLG1* leading to mtDNA depletion syndromes are thought to result from a nucleotide imbalance due to their role in generating ATP and GTP, rather than their function as subunits of the TCA cycle enzyme succinate-CoA ligase (42). Additionally, some of the genes implicated in mitochondrial RNA processing and modification (*ELAC2*, encoding RNase Z; *PUS1*, encoding pseudouridylylase synthase 1; *TRIT1*, encoding tRNA isopentenyltransferase; *TRNT1*, encoding CCA-adding tRNA nucleotidyltransferase) have been found to also function in the nucleus (43–46).

### **Genes with a secondary impact on OXPHOS biogenesis as well as other cellular functions**

Mutations in genes in this category often impact other cellular functions, including enzyme cofactors, metabolism, or mitochondrial homeostasis and quality control (Fig. 3). A significant proportion of these genes are those involved in Fe-S cluster biogenesis. Fe-S clusters are found in OXPHOS complexes I–III, thereby directly affecting OXPHOS biogenesis, but also in other mitochondrial and cellular enzymes, such as aconitase (TCA cycle), electron transfer flavoprotein-ubiquinone oxidoreductase (electron transfer to ubiquinone, e.g. fatty acid oxidation), and isopropylmalate isomerase (amino acid synthesis) (47). These defects can thus have a wider impact on diverse cellular pathways, along with OXPHOS function.

Other genes in this category may influence general mitochondrial homeostasis or biogenesis, thereby impacting OXPHOS function via a number of mechanisms. In general, these genes can be categorized into mitochondrial protein import, mitochondrial lipid or membrane homeostasis, mitochondrial fission/fusion and cristae organization, and/or mitochondrial protein quality control (19, 48, 49). For some, a mechanism for their effect on OXPHOS function has been elucidated. An example is *TAZ*, encoding a phospholipid transacylase involved in remodeling the mitochondrial inner membrane phospholipid cardiolipin, in which mutations cause Barth syndrome (MIM 302060) (48). In patient cell lines, supercomplexes consisting of OXPHOS complexes I, III, and IV in various stoichiometries are destabilized, resulting in impaired OXPHOS efficiency (48, 50). In contrast, the *ATAD3* locus, recently identified to underlie diverse clinical presentations often involving cerebellar defects (51–53), is linked to mitochondrial functions that include regulation of mtDNA maintenance and translation. *ATAD3* patient cell lines show defects in cellular cholesterol and mtDNA homeostasis, providing a basis for the disease pathology, yet a clear link between *ATAD3* and OXPHOS impairment is still lacking (52).

Additional genes responsible for mitochondrial disorders affect mitochondrial and cellular metabolism, including components of the PDC and TCA cycle, enzymes involved in toxic compound metabolism, and carrier proteins involved in metabolite and cofactor transport across cellular and mitochondrial membranes (19, 54). We have elected not to include genes involved in other metabolic pathways, such as mitochondrial fatty acid oxidation, in which secondary inhibition of OXPHOS

function may result from altered metabolite concentrations or cellular signaling pathways (55).

Assigning many of these secondary genes to a distinct category is difficult as they can have dual or unresolved roles. An example is *AGK*, encoding a mitochondrial inner membrane lipid-modifying acylglycerol kinase, in which defects lead to Sengers syndrome (MIM 212350) (48). Recent studies show that *AGK* also functions in the carrier protein import pathway of mitochondria, independent of its kinase activity (56, 57). Likewise, mutations in the mitochondrial ATP/ADP transporter (*ANT1*), encoded by *SLC25A4*, can impact not only on the ADP/ATP ratio but also on mtDNA homeostasis by altering mitochondrial dNTP equilibrium (58).

Other genes that could be classified into several categories include *DNAJC19* (protein import and cardiolipin homeostasis) (59) and *MECR* (mitochondrial fatty acid synthesis and synthesis of the cofactor lipoic acid) (54, 60). Similarly, although considered a member of the carrier family, *SLC25A46* localizes to the mitochondrial outer membrane where it functions in mitochondrial cristae architecture and endoplasmic reticulum/mitochondrial contacts implicated in lipid transfer (61). It is interesting to note that complex V and the associated mitochondrial contact site and cristae-organizing system (MICOS) play roles in inner membrane cristae structure and curvature (62, 63), and some patients with mutations affecting complex V show altered mitochondrial morphology (64).

Generally, an isolated OXPHOS enzyme deficiency (e.g. complex I deficiency) is suggestive of a mutation in either a structural subunit or assembly factor of the affected OXPHOS complex. However, there are many exceptions to this, with mutations in genes encoding OXPHOS subunits and assembly factors sometimes resulting in a combined OXPHOS deficiency. These pleiotropic effects may arise due to secondary alterations in mitochondrial membranes or mtDNA, changes in cofactor or metabolic regulation, or secondary impact due to changes in OXPHOS supercomplexes. In contrast, mutations in the other mitochondrial disease genes would be assumed to affect multiple OXPHOS complexes, and this is observed in 30–60% of patients (3, 19). Yet, isolated OXPHOS deficiency has been found for genes that would be expected to have pleiotropic effects, such as mutations in *ETHE1*, encoding a mitochondrial sulfur dioxygenase, which results in complex IV deficiency and ethylmalonic encephalopathy (MIM 602473) due to a toxic build up of hydrogen sulfide (65).

### **From genotypes to phenotypes: underlying mechanisms**

In recent years, our knowledge of the genetic architecture of mitochondrial disorders has increased rapidly and will continue to do so as additional genes are identified by genomic analyses in patients, in parallel with studies in model systems that identify or validate novel candidate genes based on proteomic analyses, genetic screens, or other approaches (41, 66–68). However, our understanding of how these basic defects lead to specific types of cellular dysfunction, organ involvement, and disease severity remains fragmentary.

The pathogenesis of mitochondrial disorders shows both genetic heterogeneity and pleiotropy. The former is demonstrated by Leigh syndrome (MIM 256000), an infantile neuro-

**Table 1**  
**Mechanisms linking genotype to phenotype**

Abnormalities triggering cellular dysfunction	Cellular consequences
ATP synthesis and adenine nucleotide pools	↓ ATP levels Altered AMP/ATP and ADP/ATP ratios → changes in cellular signaling/function
Mitochondrial membrane potential	Changes to ATP levels, Fe-S cluster biogenesis, and mitochondrial protein import Altered Ca <sup>2+</sup> homeostasis → aberrant cell signaling and/or cell death Changes in mitochondrial morphology and turnover
ROS	Changes to ROS signaling pathways Damage to protein, lipids, nucleic acids → cellular stress responses, and/or cell death
NAD <sup>+</sup> /NADH and redox balance	Altered cellular signaling pathways and effects on mitochondrial biogenesis
TCA cycle metabolites	Altered cellular signaling pathways and epigenetic changes
Amino acids and one-carbon metabolism	Changes to nutrient sensing signaling networks and transcriptional responses

degenerative disorder with characteristic lesions in the basal ganglia and brainstem caused by mutations in >75 genes to date (69). These genes are split across most of the categories listed in Fig. 3, suggesting that defects in many different processes can trigger a common tissue-specific pathology. In contrast, a number of the most common genes underlying mitochondrial disease show pleiotropy. For example, autosomal recessive mutations in *POLG* can cause a range of syndromes such as Leigh syndrome, mitochondrial DNA depletion syndrome 4A (Alpers type, MIM 203700), mitochondrial recessive ataxia syndrome (including *MIRAS* and *SANDO*, MIM 607459) and others that differ in age of onset, severity, and in the combination of diverse neurological symptoms plus muscle, gut, liver, pancreas, or renal involvement (33). Autosomal dominant *POLG* mutations can cause many of the same features plus Parkinsonism, hypogonadism, and cataracts, but there is only weak correlation between the specific *POLG* mutations and phenotype (33). Similarly, the m.3243A→G mutation in the mtDNA *MT-TL1* gene is best known for causing MELAS. However, many patients lack stroke-like episodes, instead suffering from one or more symptoms such as seizures, short stature, myopathy, cardiomyopathy, ophthalmoplegia, deafness, diabetes, gastrointestinal or renal symptoms (70).

Many factors contribute to the tissue specificity of mitochondrial disorders. Mitochondrial quantity varies by up to 30-fold in different tissues, based on cytochrome *c* levels, whereas large-scale proteomic analyses suggest that ~25% of mitochondrial proteins are not shared between any given pair of tissues (71). This variation means that the control of mitochondrial metabolic flux can be determined by different components of the OXPHOS system in different tissues (72).

Inadequate ATP synthesis is an obvious issue for cells with constant or intermittently high energy demands such as neurons, cardiomyocytes, and renal tubular cells, which need to propagate action potentials or reabsorb metabolites (73). Similarly, pancreatic beta cells must increase the ATP/ADP ratio above a threshold level to trigger insulin release (74), and astrocytes must recycle neurotransmitters released by neurons, a process that likely accounts for a large proportion of the brain's energy budget (75). Apart from an ATP deficit, inadequate ATP synthesis can also increase the AMP/ATP ratio, leading to activation of AMP-activated protein kinase and multiple signaling cascades (76). Some organs show marked increases in energy demand during growth and development, e.g. brain glucose consumption increases 3–4-fold per cm<sup>3</sup> during the first few

years of life before declining to adult levels (77). Thresholds for ATP requirement, and how they respond to changes in demand, undoubtedly contribute to different susceptibility to OXPHOS dysfunction between and within tissues, and to why conditions like Leigh syndrome can show substantial presymptomatic periods of good health in early life.

The mitochondrial membrane potential is the second obvious parameter impacted in bioenergy disorders and might be expected to decrease (*i.e.* become more electroneutral) if electron transfer and proton pumping are restricted. Apart from driving ATP production, the mitochondrial membrane potential is also required for biogenesis of Fe-S clusters, to drive mitochondrial import of many nuclear encoded proteins (78), and for import of calcium from the cytosol and endoplasmic reticulum to sequester transient increases and prevent aberrant calcium signaling or induction of cell death by opening of the mitochondrial permeability pore (73, 79). Mitochondria with decreased membrane potential are prone to cleavage of OPA1, triggering mitochondrial fission, and to accumulation of PINK1 on the outer membrane, which induces degradation by mitophagy following recruitment of Parkin and LC3 (49, 80). It is important to note that mitochondrial membrane potential can also be increased by mitochondrial dysfunction. This has been reported for the *MT-ATP6* m.8993T→G mutation, which leads to a leucine to arginine change that appears to block the proton channel of complex V, resulting in increases in membrane potential, reactive oxygen species (ROS) production, lipid peroxidation, and susceptibility to apoptosis (81, 82).

Although ATP and mitochondrial membrane potential could be considered primary targets of OXPHOS defects, mitochondria are also key components of metabolic signaling pathways, and a range of metabolites play key roles in influencing cellular function. A detailed review of mitochondrial signaling pathways is beyond the scope of this review, but excellent reviews of pathways involved in nutrient sensing and stress responses to energy deficit, oxidative stress, and mitochondrial unfolded proteins are available (49, 76, 80). Mitochondrial metabolism is also now recognized to play key roles in regulating inflammation and immune responses (49, 83). We will therefore focus on some of the key metabolites that are dysregulated by mitochondrial dysfunction and may jointly contribute to triggering a range of signaling pathways mediating cellular homeostasis and pathology, as summarized in Table 1 and discussed below.

OXPHOS complexes I and III are regarded as the major sites of mitochondrial ROS generation under normal conditions,

and ROS are key mediators of homeostatic mechanisms regulating cell proliferation, maintenance of stemness, differentiation, and stress responses (49, 78). Modest increases in ROS levels can trigger a protective reaction via activation of antioxidant response elements (76), but if this is exceeded then oxidation of proteins, lipids, and nucleic acids can trigger other stress responses or cell death (73). Damaging increases in ROS production can occur in defects affecting not just complexes I and III (49, 84) but complex V, as described above, and other processes leading to increased mitochondrial membrane potential. Mutations in proteins that are key components of ROS homeostasis, such as thioredoxin 2, can also prevent the cell from adequately buffering increased ROS production (85).

Primary or secondary defects of complex I inhibit NADH oxidation, and cell lines and tissues from complex I-deficient mouse models show a decreased ratio of  $\text{NAD}^+/\text{NADH}$ , which can sometimes be reflected in plasma by an increase in the ratio of hydroxyacylcarnitines to acylcarnitines (84, 86, 87). Decreased  $\text{NAD}^+$  levels inhibit SIRT1-PGC1 $\alpha$  signaling, potentially down-regulating mitochondrial biogenesis.

A number of TCA cycle intermediates influence cellular signaling, and primary defects in TCA cycle enzymes or secondary alteration caused by OXPHOS disorders can impact a range of processes. Citrate exported from the mitochondria to the cytosol is converted by ATP citrate lyase to acetyl-CoA, which can be used for protein lysine acetylation, and a decrease of citrate levels can decrease acetylation of specific histone H3 marks (78). Increased 2-ketoglutarate levels result in increased generation of the “oncometabolite” 2-hydroxyglutarate, which can inhibit enzymes such as hypoxia-inducible factor prolyl hydroxylases and Jumonji-domain family histone lysine demethylases, leading to activation of hypoxic signaling and marked epigenetic changes (79). Increased levels of succinate or fumarate can also result in protein succinylation and transcriptional changes, contributing to a number of TCA cycle defects that result in predisposition to tumors such as paragangliomas, pheochromocytomas, myomas, and gliomas (49).

Amino acid metabolism can also be dysregulated in mitochondrial disease. Although the specific responses vary, muscle of “deletor” mice shows an amino acid starvation-like response, with activation of Akt signaling and release of FGF21, a hormone-like cytokine into the blood, leading to mobilization of lipids from storage fat (49). Serine levels are particularly elevated in “deletor” mouse muscle and in a HEK-293 cell model of mtDNA depletion (88, 89). In the cell model, this involved an ATF4-mediated increase in serine biosynthesis and a decrease in serine consumption reflecting decreased formate production (89). This defect in cellular one-carbon metabolism was predicted to impact purine and thymidylate synthesis as well as cellular methylation reactions. More broadly, amino acid abnormalities influence the nutrient-sensing signaling network and can lead to global transcriptional responses in a highly tissue-specific manner (90).

### Conclusions and future directions

Investigations over the last 30 years have led to the identification of almost 290 genetic causes for disorders of mitochondrial energy generation, making these among the most geneti-

cally diverse group of inherited diseases. Since the widespread uptake of MPS technologies from 2012, there has been an average of 22 new disease genes identified each year. Challenges remain in detecting and interpreting pathogenic DNA variants, particularly in non-coding regions. Analytical approaches continue to evolve and are complemented by improvements in data sharing and comprehensive databases cataloguing population-based genetic variation (91), together with transcriptomic and proteomic analyses (37, 92, 93). It therefore seems likely that the current rate of gene discovery will continue for some years.

Whether they affect OXPHOS directly due to their primary function, or in a secondary manner that may also affect other cellular functions, categorizing these genes according to their functional roles emphasizes the wide array of cellular pathways that converge at OXPHOS biogenesis. In many cases, the roles can be ambiguous, as the proteins may have dual functions, alternative cellular localizations, or less clear roles in mitochondrial pathways. However, for some, their identification as a disease gene has aided in their functional characterization, resulting in new insights into mitochondrial and cellular pathways and disease pathology (e.g. *ATAD3A* and *SLC25A46*). In others, it has led to a treatment, as in the case of biotin and thiamine supplementation in *SLC19A3* mutations (94).

Although our understanding of the heterogeneity and pleiotropy of mitochondrial disorders is still incomplete, a number of parameters influencing disease pathology have been identified that span many of the gene classification categories and affect different facets of cell signaling. The last Cochrane review of mitochondrial therapy in 2012 concluded: “there is currently no clear evidence supporting the use of any intervention in mitochondrial disorders” (95). However, a series of preclinical studies have provided encouraging evidence supporting the use of small molecule therapies targeting cellular signaling pathways related to NAD bioavailability and mitochondrial biogenesis, quality control, and stress responses (96, 97). Although for the vast majority of mitochondrial diseases there are currently no effective treatments or cures, the continued identification of disease genes, their mitochondrial and cellular functions, and their shared pathogenic parameters are leading to a greater understanding and an increasing number of clinical trials (2).

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