Review Article

Blackout in the powerhouse: clinical phenotypes associated with defects in the assembly of OXPHOS complexes and the mitoribosome

Daniella H. Hock, David R. L. Robinson and David A. Stroud

Department of Biochemistry and Molecular Biology, Bio21 Molecular Science and Biotechnology Institute, University of Melbourne, Parkville 3052 Victoria, Australia

Correspondence: David A. Stroud (david.stroud@unimelb.edu.au)

Mitochondria produce the bulk of the energy used by almost all eukaryotic cells through oxidative phosphorylation (OXPHOS) which occurs on the four complexes of the respiratory chain and the F₁–F₀ ATPase. Mitochondrial diseases are a heterogenous group of conditions affecting OXPHOS, either directly through mutation of genes encoding subunits of OXPHOS complexes, or indirectly through mutations in genes encoding proteins supporting this process. These include proteins that promote assembly of the OXPHOS complexes, the post-translational modification of subunits, insertion of cofactors or indeed subunit synthesis. The latter is important for all 13 of the proteins encoded by human mitochondrial DNA, which are synthesised on mitochondrial ribosomes. Together the five OXPHOS complexes and the mitochondrial ribosome are comprised of more than 160 subunits and many more proteins support their biogenesis. Mutations in both nuclear and mitochondrial genes encoding these proteins have been reported to cause mitochondrial disease, many leading to defective complex assembly with the severity of the assembly defect reflecting the severity of the disease. This review aims to act as an interface between the clinical and basic research underpinning our knowledge of OXPHOS complex and ribosome assembly, and the dysfunction of this process in mitochondrial disease.

Introduction

Mitochondria are ubiquitous organelles that have their own DNA and translation machinery. The human mitochondrial (mt) DNA is a circular double-stranded molecule containing 16,569 base pairs and 37 genes [1]. Two of these genes encode mitochondrial ribosomal RNAs (mt-rRNA) which are required for the assembly of the mitochondrial ribosome (mitoribosome). Another 22 genes encode mitochondrial transfer RNAs (mt-tRNAs) which are responsible for deciphering RNA sequences during protein translation. The mt-tRNA for valine (tRNAval) is also present as a structural component of the mitoribosome [2–4] (Figure 1, lower inset). The remaining 13 mitochondrial genes encode highly hydrophobic transmembrane proteins that are translated on mitoribosomes and assembled into complexes I, III, IV and V of the oxidative phosphorylation (OXPHOS) system [5–8] (Figure 1, upper inset). Besides the 37 genes encoded on mtDNA, over a thousand other proteins encoded on nuclear DNA (nDNA) are translated in the cytosol and imported into the mitochondria via dedicated protein import machinery [9,10]. Mitochondria are responsible for generating the majority of cellular ATP via OXPHOS, which occurs on the mitochondrial respiratory chain (MRC) and the F₁F₀-ATPase. The MRC consists of four multiprotein complexes (complexes I–IV) embedded in the inner mitochondrial membrane (IMM) and two electron carriers called coenzyme Q (CoQ, Q) and cytochrome c (Cyt C, C). Complexes I, III and IV are also found together in higher order structures known as supercomplexes or respirasomes [11], though the precise function this coalescence is not fully clear [12].
MRC as a whole, as well as the two electron carriers are involved in a series of redox reactions that creates a proton gradient across the IMM, which is in turn used by the fifth complex, complex V or the F$_1$F$_0$-ATPase, to synthesise ATP.

The MRC, complex V and the mitoribosome are built through a series of orchestrated steps that require the help of other proteins not part of the mature complex, often called assembly factors. Not all assembly factors directly promote assembly, the controlled coalescence of subunits into complexes through stabilisation of assembly intermediates, but many instead provide critical services in the form of maturation and delivery of cofactors such as heme, copper and iron–sulfur clusters (Fe–S), post-translational modification of subunits and regulation of translation. Furthermore, transcription of mtDNA, mitochondrial mRNA processing, mitochondrial tRNA maturation, mitoribosome assembly and translation all directly influence OXPHOS complex
biogenesis function [5–8]. Dysfunction in any of the aforementioned processes can lead to mitochondrial disease. Given the central importance of mitochondria to cellular function, disease can affect any tissue in the body, though tissues and organs of high energy demand such as heart, brain and muscle are frequently affected. Furthermore, some patients present with tissue or organ specific phenotypes (e.g. cardiomyopathy) while others exhibit multi-system disorders or delayed onset, reflecting the different requirements for mitochondrial energy production in different cell types, the function of affected protein, their tissue-specific expression or their being encoded on nuclear or mitochondrial DNA [5–8]. There are over 300 known disease genes, located in either nuclear DNA (nDNA) or mitochondrial DNA (mtDNA), implicated in mitochondrial diseases ([5,13], this review). Broadly speaking, these can be separated into two categories: genes that are involved in primary energy generation (i.e. subunits or assembly factors of the OXPHOS system, mitoribosome, involved in transcription, mtDNA homeostasis and related systems) and genes that have a secondary function (i.e. protein quality control, membrane structure and metabolite transport) [5,14,15]. Of the over 190 known primary disease genes, more than half lead to defective assembly of the OXPHOS complexes or the mitoribosome ([5], this review).

The current diagnostic paradigm for mitochondrial disease involves a combination of whole exome (WES) or whole genome (WGS) sequencing, with linkage studies and homozygosity mapping [5,13]. In the case of mtDNA mutations, diagnosis can be even more challenging considering that the mtDNA is present in multiple copies per cell and might contain a heterogenous population of mutated and non-mutated mtDNA, a phenomenon termed heteroplasmy. In the case of mitochondrial disease, the ratio of mutated to non-mutated mtDNA is termed mutation load, which is particularly important in the onset and tissue-specific manifestation of mitochondrial diseases [16]. Diagnostic yield when using strategies combined with WES or WGS is only 30–68% [17–20], suggesting that other novel disease genes and variants remain to be discovered. The principal challenge in the current diagnosis paradigm is validation of variants of uncertain significance (VUS) detected in patients with suspected mitochondrial disease [21]. To address this, investigators often turn to functional studies. Studies such as these, sometimes continuing long after confirmed diagnosis, have been incredibly informative to our understanding of mitochondrial complex assembly. The results of these studies can later provide information invaluable to those attempting to solve new undiagnosed cases. For example, we now know from cultured cell line knockout studies that loss of proteins required for some steps of complex I assembly does not greatly impact assembly and function of the enzyme, whereas loss of proteins required for other steps leads to catastrophic failure in complex biogenesis [22]. Perhaps unsurprisingly, in patients null mutations have been described in genes required for the former step, but not the second (see the below sections for detail). Armed with this information, an investigator may be able to prioritise a novel VUS in a gene associated with one of these steps for follow-up studies. It is, therefore, the intention of this review to present a common ground to both basic researchers interested in the assembly of mitochondrial complexes, as well as more clinically focused audiences, in the hope that both will benefit from each other’s ongoing efforts to understand the function and impacts of dysfunction in these complex molecular machines.

Due to the large number of steps in the biogenesis of these complexes, most of the following sections have been separated into sub-sections describing distinct stages of assembly and the impacts of disease on this process. Subunits (i.e. structural proteins found in the fully assembled complex) in the below sections are indicated in bold typeface, whereas assembly factors and other proteins are listed in regular typeface. The nomenclature for some subunits and assembly factors is complicated, different groups may use different symbols and for most complexes the subunit naming convention differs between humans and other organisms. For the purposes of this review, we have chosen to refer to the subunits and assembly factors by their Human Genome Organisation (HUGO) approved gene symbol [23], though have aimed to include the commonly used protein symbol in parenthesis at first mention. Finally, due to the large number of case reports for some disease genes we have chosen to cite the first example for each commonly observed phenotype. Further case reports can be found by referring to the relevant entry in the Online Mendelian Inheritance in Man database (https://www.omim.org), with the OMIM entry number cited for each gene in the relevant table.

**Complex I**

Mitochondrial complex I (NADH, Ubiquinone reductase) is the first complex in the MRC. The fully assembled complex I is L-shaped with a hydrophobic arm embedded in the IMM and a hydrophilic arm extending into the mitochondrial matrix [24,25]. The latter contains the catalytic N-module, which is involved in oxidation of NADH to NAD+, and the Q-module which transfers electrons via Fe–S clusters to Coenzyme Q [26,27]. This
in turn triggers conformational changes leading to pumping of protons across the membrane arm (ND-modules) and creation of the proton-motive force used by complex V [28]. Complex I consists of 45 subunits (44 distinct subunits as one subunit, NDUFAB1 is present in two locations within the complex) [29]. Of the 44 distinct subunits, 14 are classified as core subunits since they possess catalytic function and homologues are present in all organisms with complex I, including bacteria [30,31]. Of the 14 core mammalian subunits, 7 are encoded on mtDNA: ND1, ND2, ND3, ND4, ND4L, ND5 and ND6, all of which are present in the membrane arm, while the remainder are encoded on nuclear DNA. Four are located in the Q-module: NDUFS2, NDUFS3, NDUFS7, NDUFS8; and three in the N-module: NDUFS1, NDUFSV1, NDUFSV2. The remaining 30 subunits (prefixed NDUFA, NDUFB, NDUFC and NDUFAB1) are known as accessory or supernumerary subunits, with 25 of them being characterised as essential for the assembly and stability of the complex [22]. The assembly of complex I occurs through the sequential addition of modules seeded by core subunits to which accessory subunits are added. Although the order of module coalescence is still not fully understood, the assembly pathway for complex I is one of the most well studied of all mitochondrial complexes and there is an increasingly well accepted model (Figure 2) [22,24,32].

Assembly of the Q and ND1 modules

The Q-module subassembly contains nuclear-encoded subunits NDUFA5, NDUFS2, NDUFS3, NDUFS7 and NDUFS8 and appears to be one of the earlier modules to assemble (Figure 2) [22,24,32]. With the exception of NDUFA5, all of the above subunit genes have been associated with Leigh syndrome, leading to early childhood death (Table 1). In line with this, their gene-editing based ablation in the commonly used human embryonic kidney cell (HEK293T) model system leads to complete loss of complex assembly and activity [22]. The assembly factors NDUFAF5 and NDUFAF7 are required for maturation of the Q-module [33–35] and they contain the S-adenosylmethionine (SAM)-dependent methyltransferase domains [33,36] that provide post-translational modifications to subunits within the Q-module. NDUFAF5 is responsible for hydroxylation of an arginine residue in NDUFS7 [34] and requires the assembly factor NDUFAF8 for its stabilisation, though its molecular function in this capacity is not yet clear [37,38]. On the other hand, NDUFAF7 is required for dimethylation of an arginine residue in NDUFS2 [33,35]. While the function of these modifications is unknown, their loss results in complex I dysfunction [39,40]. There have been no patients reported for NDUFAF7, however, a

Figure 2. Schematic depicting the complex I assembly pathway showing known mitochondrial disease genes in red, mtDNA-encoded subunits underlined and assembly factors in italics.

Complex I assembly is characterised by coalescence of distinct modules assembled individually prior to final assembly into large intermediates and eventually the functional complex. The Q-module joins the ND1 module and coalesces with ND2, ND4 and ND5 modules. The N-module (harbouring the site of NADH oxidation) is the last module to join the complex in the assembly pathway. Fe–S, iron–sulfur, IMS, intermembrane space; IMM, inner mitochondrial membrane; Matrix, mitochondrial matrix.
Table 1. Defects affecting biogenesis of the Q and ND1 modules

| Gene   | S. F. | Types of genetic variants and protein impact | Clinical presentations and relevant information | Ref. | OMIM |
|--------|-------|---------------------------------------------|-----------------------------------------------|------|------|
| Q-module | | | | | | |
| NDUF2  | X     | Various missense leading to uncertain impact or near absence of protein. | Leigh syndrome, optic atrophy, hypertrophic cardiomyopathy, elevated blood lactate. | [380,381] | 602985 |
| NDUF3  | X     | Missense leading to uncertain protein impact. | Leigh syndrome, optic atrophy. | [382] | 603846 |
| NDUF7  | X     | Predominantly missense but also intronic (nonsense) leading to truncated protein or uncertain impact on protein. | Leigh syndrome, hypotonia, may present normal blood lactate and pyruvate. | [383,384] | 601825 |
| NDUF8  | X     | Missense leading to uncertain impact on protein or reduced levels. | Ranging from severe Leigh syndrome, elevated blood lactate and pyruvate, hypotonia, early death to slowly progressive neurological disease at the end of first decade of life. | [385–387] | 602141 |
| NDUFAF3 | X     | Missense leading to uncertain protein impact. | Severe lactic acidosis, optic atrophy, respiratory failure and variable brain involvement including Leigh syndrome, myoclonic seizures, macrocephaly and cavitating leukoencephalopathy. | [41,388,389] | 612911 |
| NDUFAF4 | X     | Missense leading to decreased protein levels or uncertain protein impact. | Leigh syndrome, hypotonia, elevated blood and cerebrospinal fluid lactate, may present severe infantile cardiomyopathy. | [42,390] | 611776 |
| NDUFAF5 | X     | Predominantly missense but also intronic (nonsense) leading to uncertain protein impact. | Most common presentation of Leigh syndrome, but also fatal lactic acidosis, hyponatremia, hypotonia and bilateral optic neuropathy. Variable survival depending on variant. Decreased CI activity. | [36,391–393] | 612360 |
| NDUFAF8 | X     | Nonsense (frameshift and intronic) and missense leading to uncertain protein impact. | Leigh syndrome, may present optic atrophy and elevated blood lactate. | [38] | 618461 |
| ND1 module | | | | | | |
| MT-ND1 | X     | Multiple, predominantly missense variants with protein levels dependant on variant, and mutant mtDNA load. | LHON, MELAS. | [43–45] | 516000 |
| NDUFA1 | X     | Missense leading to decreased protein levels. | X-linked. Variable presentation from severe Leigh syndrome to developmental delay, hypotonia, elevated blood lactate and myoclonic epilepsy and survival to childhood. | [48] | 300078 |
| NDUFA8 | X     | Missense leading to reduced proteins levels. | Psychomotor retardation, severe quadriplegia, elevated blood lactate, cerebral atrophy, hypotonia, epilepsy with survival to adulthood. | [394] | 603359 |
| NDUFA13 | X     | Missense or nonsense (frameshift) leading to reduced protein levels. | Hypotonia, dyskinesia, sensorial impairments or Leigh syndrome and mild hypertrophic cardiomyopathy. | [395,396] | 609435 |
| NDUFA6 | X     | Predominantly missense but also frameshift and intronic leading to decreased protein levels or uncertain protein impact. | Leigh syndrome is the most common presentation, but also Fanconi syndrome, lactic acidosis, bilateral striatal necrosis and progressive dystonia in childhood. | [47,66,397,398] | 612392 |

Continued
Table 1. Defects affecting biogenesis of the Q and ND1 modules

| Gene | A. S. F. | Types of genetic variants and protein impact | Clinical presentations and relevant information | Ref. | OMIM |
|------|----------|--------------------------------------------|-----------------------------------------------|------|------|
| TIMMDC1 | X | Intronic variant causing frameshift and early termination leading to decreased protein levels. | Infantile muscular hypotonia, developmental delay, neurological deterioration. | [46] | 615534 |

S., subunit; A.F., assembly factor; LHON, Leber’s hereditary optic neuropathy; MELAS, mitochondrial encephalopathy, lactic acidosis and stroke-like episodes.

number have been reported for NDUFAF5 and NDUFAF8 (Table 1). For NDUFAF5, the majority of patients harbour missense mutations leading to impaired complex assembly. The phenotype is predominantly Leigh syndrome, however, many patients have survived until adolescence or adulthood (Table 1). NDUFAF8 was only recently reported to be a disease gene, with the patients described having presumably null mutations leading to a classic Leigh syndrome phenotype [38]. The assembly factors NDUFAF3 and NDUFAF4 are required for maturation of the Q-module and have been found in association with both NDUF53 and NDUF5 subunits [41] as well as the Q-module intermediate [32]. While the precise molecular function of NDUFAF3 and NDUFAF4 are not known, they appear to be essential for progression to the next assembly stage [22,32,42]. Consistent with this, patients for both have presented with severe infantile disease due to predominantly missense, presumably null variants (Table 1).

Following association with NDUFAF3 and NDUFAF4, the assembled Q-module joins with the mtDNA-encoded ND1 subunit and the assembly factor TIMMDC1 [32]. Numerous mutations in ND1 have been reported leading to Leber hereditary optic neuropathy (LHON) and mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS) (Table 1). Generally, these patients have high mutant loads or are in some cases homoplasmic (100%) for the variants [43–45]. Like most mutations in mtDNA, variants in ND1 generally lead to adult onset disease. TIMMDC1 is only a recently identified disease gene with a single homozygous intronic variant detected, leading to a splicing defect and no detectable protein [46] (Table 1). The assembly factor NDUF6 is also required for ND1 biogenesis. While many missense variants in the NDUF6 gene lead to Leigh syndrome, it does not appear to be stably associated with an intermediate and its precise function is not yet known [47]. Once the nascent Q–ND1 module is formed, it subsequently incorporates the subunits NDUF3, NDUF8 and NDUF13 [32] to produce the final Q–ND1 intermediate. Both NDUF8 and NDUF13 are disease genes with missense variants leading to complex I deficiency, however, the few patients identified have survived into young adulthood (Table 1). The reason for only missense variants leading to reduced protein levels being reported for NDUF8 and NDUF13 might be explained by the fact that loss of either protein products in HEK293T cells leads to complete loss of complex assembly and activity [22], suggesting that complete absence of these proteins might be not tolerated. Finally, while NDUF1 is a subunit of the ND1 module, it is not added to the complex I until late during assembly [32]. Many missense variants in NDUF1, encoded on the X chromosome, have been linked to complex I deficiency with phenotypes ranging from mild (survival into childhood) to severe (Leigh syndrome and death in early childhood) [48] (Table 1).

Assembly of the ND2 module

In the early steps of the assembly of the ND2 module, the subunits ND2, NDUC1 and NDUC2 associate with assembly factors ACAD9, ECSIT, NDUF1 and COA1 (Figure 2). To this subcomplex, ND3 and the assembly factors TMEM126B and TMEM186, and subsequently ND4L and ND6 are added [32,49]. The aforementioned assembly factors are also known to form the mitochondrial complex I intermediate assembly (MCIA) complex which is essential for the assembly of the ND2 module [49]. COA1, which was originally suggested to be a complex IV assembly factor [50], has recently been shown to be involved in the early stages of ND2 translation [51]. Patients have been described for all mtDNA-encoded subunits present in this intermediate (ND2, ND3, ND4L and ND6). The phenotype spectrum is broad and depends on both the variant itself and mutant load, however, Leigh syndrome, LHON and associated phenotypes are predominant (Table 2). Although ND4L is transcribed as a bicistronic mRNA with ND4 (of the ND4 module) [52] no variants have been described that affect the both proteins. All assembly factors present in the MCIA complex, with the
exception of ECSIT and newly identified members COA1 and TMEM186 [49], are known disease genes (Table 2). The function of NDUFAF1 (formerly CIA30) was first linked to complex I assembly through the use of fungal [53] and then mammalian [54] model systems, and eventually led to the identification of patients presenting with cardiomyopathies [55–57]. Fibroblasts from NDUFAF1 patients have reduced levels of complex I and defective assembly of the ND2 module [55]. ACAD9 was originally thought to be involved in β-oxidation...
based on sequence homology with other members of the acyl-CoA family as well as its ability to bind acetyl-CoA substrates in vitro [58]. The association with cardiomyopathy [59], demonstration of its interaction with other MCIA members and requirement for complex I assembly [60] solidified its role as an assembly factor. ACAD9 is one of the most common causes of complex I deficiency and while it typically presents as cardiomyopathy, the phenotype can be quite varied with milder presentations such as mild growth retardation and exercise intolerance also noted (Table 2) [61]. Interestingly, while it is now clear that ACAD9 deficient cells lack β-oxidation defects, riboflavin supplementation has been shown to lessen symptoms in patients and improve complex I activity [61]. ACAD9 is a flavoprotein (riboflavin is a precursor to flavin adenine dinucleotide; FAD), however, a catalytically dead mutant retains the ability to rescue complex I assembly, suggesting that ACAD9 has a secondary role in mitochondrial function [62]. TMEM126B was identified as an assembly factor through complexome profiling studies [63] with patients later being identified. The role of TMEM126B in complex I assembly is still not completely clear, although we know this protein has multiple transmembrane domains and may act as an anchor for the other MCIA subunits, despite their levels remaining unaffected by loss of TMEM126B [49]. Patients harbouring mutations in TMEM126B also present milder phenotypes compared with those with mutations in NDUFAF1 and ACAD9 (Table 2).

At this stage of complex I biogenesis there appears to be multiple parallel routes to the final complex, either the ND2 module joins with the Q–ND1 intermediate or the ND4 intermediate [32] (Figure 2). Once either the Q–ND1–ND2 subcomplex or ND2–ND4 subcomplex is assembled the ND2 module subunits NDUFA9, or NDUFA10 and NDUFS5 are respectively incorporated. NDUFA11 appears to be added later during assembly once the membrane arm is fully built [32]. Despite the timing of these subunit additions, all are critical for complex I assembly in gene-edited HEK293T cells [22]. Patients have been identified for NDUFA9, NDUFA10 and NDUFA11 (Table 2) and generally present with severe infantile Leigh syndrome or encephalocardiomyopathy and isolated complex I deficiency [64–68], though there have been reports of milder NDUFA9 and NDUFA11 cases presenting with childhood-onset progressive dystonia [69] and late-onset myopathy respectively [68].

Assembly of the ND4 and ND5 modules
The assembly steps of the ND4 and ND5 modules are less well characterised than the other modules, with assembly factors only identified in recent years. In the case of the ND4 module, a subcomplex containing four accessory subunits NDUFB5, NDUFB6, NDUFB10 and NDUFB11 assemble early and are followed by the addition of NDUFB1 and the mtDNA-encoded subunit ND4. Like for NDUFA10 and NDUFS5 (discussed in the ND2 module section), NDUFB4 seems to be incorporated into the ND4 module once the intermediate ND2–ND4 subcomplex is formed [32]. In our HEK293T model system, loss of all nuclear subunits present in the ND4 module leads to turnover of almost all complex I subunits, leaving only an intermediate containing Q/ND1 subunits module intact [22]. As such, defects in genes associated with the ND4 module lead to severe disease (Table 3). ND4, NDUFB10, NDUFB11 are known disease genes and patients with variants in ND4 largely present with similar clinical features to those with mutations in other mtDNA-encoded complex I subunits, with LHON the dominant phenotype but also Leigh syndrome and encephalopathy have been reported. Indeed, one ND4 variant (11778A), accounts for more than half of the reported primary cause of LHON in Caucasian families [70] and over 90% in the Chinese families [71]. Although ND4 and ND4L (found in the ND2 module) are transcribed as a bicistronic mRNA [52], no cases have been described where both proteins are affected. For NDUFB10 there is a single known case that presented with severe neonatal cardiomyopathy leading to infantile death. The patient harboured a compound frame shift variant leading to early termination and a missense (p.C107S) variant leading to a mutation in the conserved CXnC motif important for import of the protein into mitochondria [72]. Consistent with an import defect, tissues had reduced but not absent levels of NDUFB10 and accumulation of complex I intermediates. Similarly, variants in NDUFB11 also typically lead to infantile cardiomyopathy, though the presentation is complicated by the gene being present on the X chromosome (Table 3) [66,73,74]. Interestingly, female patients appear to suffer strong skewing of X-chromosome inactivation toward the mutant allele [73].

The assembly factors TMEM70, FOXRED1 and ATP5SL are found associated with a near complete ND4 module [22,32,75]. While their function in assembly of this module is not clear yet, all three interact with subunits of the ND4 module and their loss, either in model systems or patient cell lines [22,76] leads to accumulation of membrane assembly intermediates. While no patients have been identified for ATP5SL, both TMEM70 and FOXRED1 are known mitochondrial disease genes (Table 3). FOXRED1 was identified through high-
| Gene       | S. | A. F. | Types of genetic variants and protein impact                                                                 | Clinical presentations and relevant information                                                                                                                                                                                                                                                                                                                                 | Ref.   | OMIM   |
|------------|----|-------|-------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------|--------|
| ND4 module |     |       |                                                                                                             |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |        |        |
| MT-ND4     | X  |       | Missense with unclear protein impact.                                                                        | LHON, early onset Leigh syndrome, late-onset encephalopathy, may present lactic acidosis. Phenotype severity may correlate with mutation load.                                                                                                                                                                                                                                                                                               | [418–421] | 516003 |
| NDUFB10    | X  |       | Missense and nonsense leading to impaired protein import.                                                    | Fatal lactic acidosis and cardiomyopathy.                                                                                                                                                                                                                                                                                                                                                                                                                                             | [72]   | 603843 |
| NDUFB11    | X  |       | Nonsense but also missense and deletion leading to reduced protein levels.                                    | X-linked gene. Encephalopathy, cardiomyopathy, MIDAS, LIMD, sideroblastic anaemia                                                                                                                                                                                                                                                                                                                                         | [66,73,74] | 300403 |
| FOXRED1    | X  |       | Missense, frameshift and nonsense leading to unclear protein impact                                         | Variable phenotype from severe neonatal lactic acidosis with early death to Leigh syndrome, hypotonia, lactic acidosis, hypertrophic cardiomyopathy and survival into adolescence and adulthood. Decreased CI activity                                                                                                                                                                                                                       | [77,78,422] | 613622 |
| TMEM70     | X  |       | Clinical cases are presented at the complex V defects section                                                |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |        |        |
| ND5 module |     |       |                                                                                                             |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |        |        |
| MT-ND5     | X  |       | Predominantly missense but also deletion leading to frameshift with protein impact dependant on variant and mutant mtDNA load | Variable onset MELAS, Leigh syndrome, LHON. May present as combination of the previous phenotypes with hypotonia, failure to thrive, cardiomyopathy, renal failure, myopathy. Phenotypes may vary according to age of onset and mutation load                                                                                                                                                                                                                     | [86,87,423–427] | 516005 |
| NDUFB3     | X  |       | Missense but also nonsense leading to unclear protein impact                                               | Variable phenotype from mild short stature, distinctive facial appearance surviving into childhood and decreased CI assembly to severe encephalopathy, myopathy, hypotonia, lactic acidosis, failure to thrive                                                                                                                                                                                                                                           | [88–90] | 603839 |
| NDUFB8     | X  |       | Predominantly missense but also frameshift                                                                   | Leigh syndrome, fatal infantile lactic acidosis,                                                                                                                                                                                                                                                                                                                                                                                                                                          | [91]   | 602140 |

Continued
complex I associated pool where it associates with many different proteins containing the LYR motif [85] and a
NDUFB8 function, Rendon and co-workers [79] identi- FOXRED is an FAD-dependent oxidoreductase, and although it is not clear if FAD binding is important in its
ND5 module assembly as it has been shown to interact with newly synthesised
fi
having been identi-
ND5 modules appears to rely on the presence of a properly built ND4 module but not vice versa [22]. Defects
module [22], however, its molecular function is not yet known. Interestingly the stability of both the ND2 and
translation of assembly intermediates for both complexes [75,80]. In line with this dual role, patients commonly
have cardiac and brain involvement and defects in both OXPHOS complexes [81] or isolated complex I or V
cated in the assembly of both complexes I and V, with functional studies showing that its loss leads to accumu-
lion of assembly intermediates for both complexes [75,80]. In line with this dual role, patients commonly
have cardiac and brain involvement and defects in both OXPHOS complexes [81] or isolated complex I or V
deficiencies, varying according to tissue type [81–84] (Tables 3 and 14).

The ND5 module contains the core subunit ND5 and accessory subunits NDUFB2, NDUFB3, NDUFB7, NDUFB8, NDUFB9 and NDUFA1. NDUFA1 is unique in that it is found in two pools, a soluble non-
complex I associated pool where it associates with many different proteins containing the LYR motif [85] and a
complex I associated pool [24]. In the case of the latter it is found twice, associating with the two LYR motif containing complex I subunits, NDUFB7 of this module and NDUFA6 of the N-module (discussed below) (Figure 2). Interestingly, loss of NDUFA1 in the gene-edited HEK293T model system leads to cell death
(wheras loss of the other accessory subunits leads to a presumed shift to glycolysis), and while there is a severe
complex I assembly defect the essential role of NDUFA1 appears to be related to its non-complex I associated
pool [22]. Little is known about assembly of the ND5 module with the only intermediate observed containing
all known subunits [32]. The recently identified assembly factor DMAC1 [22] is thought to be required for
ND5 module assembly as it has been shown to interact with newly synthesised ND5 and other subunits of the
module [22], however, its molecular function is not yet known. Interestingly the stability of both the ND2 and
ND5 modules appears to rely on a properly built ND4 module but not vice versa [22]. Defects in ND5 lead to the typical late onset (LHON, MELAS) phenotypes observed in patients with mutations in
mtDNA-encoded complex I subunits (Table 3) although there have been many reports of more severe infantile
presentations [86,87]. Variants have been identified in NDUFB3, NDUFB8 and NDUFB9. Patients present with
typical severe childhood phenotypes underpinned by complex I dysfunction (e.g. encephalopathy, myopathy,
hypotonia), dependent on the variants impact on protein function [88–92]. In line with the requirement of
these genes for complex I assembly in the HEK293T model system [22], in all patients, residual levels of the
presumably semi-functional proteins have been detected [88–92].

Assembly of the N-module
The N-module, which is the site of NADH oxidation, is probably pre-assembled into two subcomplexes containing
the NDUVF1 and NDUVF2 subunits and NDUFS1 and NDUFA2 subunits before incorporation with the Q–ND1 subcomplex (Figure 2) [32]. Defects have been identified in all four genes encoding these subunits (Table 4). In line with their central role in complex I enzyme function [24,25], patients present with severe disease, typically Leigh syndrome in infants, although some variants in NDUFA2 which is not thought to be
directly involved in NADH catalysis, lead to less severe disease and survival into childhood [93–102]. The

| Gene     | S. | A. | F. | Types of genetic variants and protein impact | Clinical presentations and relevant information | Ref. | OMIM     |
|----------|----|----|----|---------------------------------------------|-----------------------------------------------|------|---------|
| NDUFB9   | X  |    |    | Missense leading to decreased protein levels | Early onset progressive hypotonia, increased blood lactate | [92] | 601445 |

S., subunit; A.F., assembly factor; LHON, Leber’s hereditary optic neuropathy; MIDAS, mitochondrial dysfunction-associated senescence; LIMD, lethal infantile mitochondrial disease; MELAS, mitochondrial encephalopathy, lactic Acidosis and stroke-like episodes.
assembly factor NUBPL has a CXXC motif that binds iron–sulfur clusters and is thought to aid their delivery to NDUFS1 and NDUFS1. Depletion of NUBPL in model systems leads to turnover of some N-module subunits and accumulation of intermediates [103]. NUBPL was identified in the same high-throughput sequencing study as FOXRED1 [77] and since then a few other patients have been identified [72,77,104]. Patients have milder symptoms than those harbouring mutations in the two known NUBPL substrates, typically presenting with leukoencephalopathy and childhood ataxia followed by other variable but progressive symptoms suggesting that biogenesis of NDUFS1 and NDUFS1 remains partially functional.

The subunits NDUF6, NDUFA7, NDUFA11, NDUFA12, NDUFS4, NDUFS6, NDUFS1 and the other copy of NDUFA1, associated with LYR motif containing NDUF6, seems to be incorporated at later

Table 4. Defects affecting biogenesis of the N-module

| Gene   | A. S. F. | Types of genetic variants and protein impact                                      | Clinical presentations and relevant information                                                                 | Ref.     | OMIM    |
|--------|----------|----------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------|----------|---------|
| NDUFA2 | X        | Majority missense but also intronic and frameshift with unclear protein impact. | Variable phenotype from severe Leigh syndrome, hypertrophic cardiomyopathy, severe lactic acidosis to cystic leukoencephalopathy and survival into childhood. Impaired CI assembly/activity. | [93,94]  | 602137  |
| NDUFA6 | X        | Frameshift, nonsense and missense leading to unclear protein impact.             | Variable phenotype from optic atrophy, motor regression and survival into childhood to severe fatal lactic acidosis and brain abnormalities, hypotonia, seizures. Decreased CI assembly/activity. | [106]    | 602138  |
| NDUFA12| X        | Nonsense leading to complete absence of protein.                                | Leigh syndrome, dystonia, hypotonia, normal hearing and vision.                                               | [107]    | 614530  |
| NDUFS1 | X        | Predominantly missense but also nonsense with unclear protein impact.           | Variable severe phenotypes including Leigh syndrome, bilateral optic atrophy, hyperlactatemia, mental retardation, macrocytic anaemia, cavitating leukoencephalopathy, dystonia, hypotonia mostly leading to early death. Decreased CI assembly/activity. | [95–98]  | 157655  |
| NDUFS4 | X        | Variable. Nonsense, intronic, duplication and deletion causing frameshift. Unclear or undetected protein levels. | Variable presentations with predominant Leigh syndrome phenotype but also brain atrophy, cardiac hypertrophy, hypotonia, may present lactic acidosis and decreased CI activity. | [108–111]| 602694  |
| NDUFS6 | X        | Intron, missense and deletion leading to uncertain or decreased protein level. | Severe presentation of fatal lactic acidosis, Leigh syndrome.                                                  | [112–114]| 603848  |
| NDUFS1 | X        | Missense, nonsense and intronic leading to unclear protein impact.             | Leigh syndrome and may present progressive muscular hypotonia, myoclonic epilepsy, elevated plasma and cerebrospinal fluid lactate. CI deficiency. | [95]     | 161015  |
| NDUFS2 | X        | Intron deletion and insertion causing protein frameshift leading to decreased protein levels. | Hypertrophic cardiomyopathy and encephalopathy, Leigh syndrome, hypotonia.                                    | [100–102]| 600532  |
| NDUFA2 | X        | Predominantly nonsense but also deletion causing frameshift leading to undetectable protein levels. | Severe progressive brain abnormalities distinct from Leigh syndrome, hypotonia, may present apnoea, normal or mildly elevated plasma lactate. | [77,116,118,119]| 609653 |
| NUBPL  | X        | Predominantly missense but also frameshift leading to decreased protein levels. | Leukoencephalopathy, may present elevated serum and cerebrospinal fluid lactate.                              | [77,104] | 613621  |

S., subunit; A.F., assembly factor.
assembly stages of the complex I [32]. Some of these subunits appear to be able to be dynamically exchanged from the assembled complex, possibly to prevent accumulation of oxidatively damaged subunits [105]. Patients have been described for \textit{NDUFA6} [106], \textit{NDUFA12} [107], \textit{NDUFS4} [108–111], and \textit{NDUFS6} [112–114]. Patients with mutations in the latter three generally present with severe childhood Leigh syndrome (Table 4). Interestingly, loss of these proteins in the HEK293T model system leads to only mild defects in complex I assembly, turnover of only selected N-module subunits, and mild impacts on complex I function [22]. In line with this, mutations found in these patients generally lead to complete absence of the proteins (Table 4). In contrast, the known patients for \textit{NDUFA6}, which is required for N-module assembly in model systems [22], present with variable symptoms ranging from severe infantile disease to survival into childhood, presumably due to differing effects on protein stability and function [106]. These examples highlight the delicate balance between the role of the protein in complex assembly and the severity of the variant and presentation of phenotypes in patients.

Finally, the N-module requires the assembly factor NDUFAF2 for its biogenesis (Figure 2). NDUFAF2 is found associated with a complex I intermediate that lacks the N-module, but contains a near complete Q–ND1–ND2–ND4–ND5 subcomplex [22,32,115]. HEK293T cell lines lacking some N-module subunits as well as patients with mutations in \textit{NDUFAF2} contain this partially built assembly as a stable intermediate [22,105]. NDUFAF2 is thought to have evolved through duplication of the \textit{NDUFA12} gene [116], and the structure of the intermediate isolated from a fungal \textit{NDUFS6} knockout model (lacking the N-module) shows NDUFAF2 occupying the site usually taken by \textit{NDUFA12} in the mature complex [117], giving rise to the suggestion that the assembly factor caps the near final Q–ND1–ND2–ND4–ND5 intermediate priming it for addition of the N-module. NDUFAF2 patients present with complex I deficiency and severe progressive childhood disease. Most patients present with no detectable NDUFAF2 protein [77,116,118,119], which given the loss of associated N-module in patients is reflected in the similar complex I defect upon loss of NDUFAF2, \textit{NDUFA12}, \textit{NDUFS4} and \textit{NDUFS6} in gene-edited model systems [22], as well as the similarity of phenotypes observed in patients for these genes (Table 4).

**Complex II**

Mitochondrial complex II (or succinate dehydrogenase) is an important enzyme that participates in both central metabolic processes relevant to mitochondrial energy generation: the MRC and the tricarboxylic acid cycle (TCA). This enzyme is responsible for the oxidation of succinate to fumarate with the extracted electrons used to reduce Coenzyme Q [120]. Complex II is the smallest complex in the OXPHOS system and the only one where all subunits are of nuclear origin (SDHA, SDHB, SDHC, SDHD). While all except for \textit{SDHC} have been linked to mitochondrial disease (Table 5), genes associated with complex II are more commonly known for their association with tumorigenesis, particularly heritable paragangliomas (discussed below). \textit{SDHA} is the most frequently associated with mitochondrial disease, with multiple variants and presentations leading to complex II deficiency having been described [121–129]. In general, patients carrying mutations in the \textit{SDHA} locus predicted or shown to lead to reduced protein levels present with classical mitochondrial disease phenotypes including Leigh syndrome (Table 5). There are few examples of \textit{SDHB} and \textit{SDHD} patients with phenotypes in line with classical mitochondrial dysfunction. For the former, there are only three known patients [130,131] all harbouring the same homozygous transversion leading to an aspartate to valine substitution, reduced levels of \textit{SDHB} protein and assembled complex II. Two of these patients presented neurologic impairment, developmental regression and leukoencephalopathy [130,131], but the third, a sibling of one affected individual, was asymptomatic [130] suggesting incomplete penetrance. As such this variant is still classified as a VUS (see OMIM 185470.0020). For \textit{SDHD} there are only two known patients, both variants leading to reduced levels of protein and impaired complex II assembly, complex II deficiency, however, both presenting with fatal hypertrophic cardiomyopathy or encephalomyopathy [131,132] suggesting this is a genuine mitochondrial disease gene.

The assembly of this complex is not well characterised, with the known steps dominated by the insertion of two cofactors, thought to be catalysed by the assembly factors SDHAF1–4 (Figure 3). The first step is likely the flavination of the SDHA subunit by the assembly factor SDHAF2 [133,134]. Although the requirement for SDHAF2 in the flavination of SDHA has been disputed in some cancer cell lines [135,136], evidence exists to the contrary, including both the absence of SDHA flavination in tumours harbouring heterozygous SDHAF2 mutations [133] and a structure of the bacterial homologue of SDHAF2 bound to SDHA [137]. SDHA also interacts with the assembly factor SDHAF4, which is proposed to prevent the generation of reactive oxygen species (ROS) from the oxidation of succinate by unassembled SDHA but has also been suggested to facilitate
the interaction of SDHA with SDHB [138]. The other notable step is the insertion of an Fe–S prosthetic group into SDHB, which is thought to be incorporated prior to the formation of an SDHA–SDHB intermediate and be promoted by SDHAF1 and SDHAF3 [139–142]. SDHAF1 is the only assembly factor known to be a mitochondrial disease gene, the two known homozygous missense variants lead to reduced complex II activity [143]. Patients present with infantile leukodystrophy and developmental regression, therefore having similarities

![Figure 3. Schematic depicting the complex II assembly pathway showing known mitochondrial disease genes in red and assembly factors in italics.](image)

Heme b binds SDHC and SDHD. SDHA is flavinated (FAD) in the presence of SDHAF2. SDHB receives iron–sulfur (Fe–S) clusters through the assistance of SDHAF1 and SDHAF3 and binds SDHA to finalise the assembly of the complex. IMS, intermembrane space; IMM, inner mitochondrial membrane; Matrix, mitochondrial matrix.
to those harbouring the VUS in the associated subunit SDHB, discussed above (Table 5) [131,143]. Little is known about the assembly of the membrane anchored CII subunits, SDHC and SDHD. The heme b group situated at their interface is incorporated is required for their stability [144], but appears to play no catalytic role in the enzymatic function of the complex [145]. Interestingly the presence of both matrix exposed subunits, SDHA and SDHB is required for the stability of the SDHC/SDHD [134,141].

Unlike for the other OXPHOS complexes, the genes encoding succinate dehydrogenase subunits and assembly factors are better known as being implicated in tumorigenesis over mitochondrial disease. Mutations in SDHA [146], SDHB [147], SDHC [148], SDHD [147] and SDHAF2 [133,149] are linked to heritable paragangliomas, gastrointestinal stromal tumours, renal carcinomas and pituitary adenomas [150]. This is likely due to metabolic and epigenetic alterations [151] as unlike the mutations leading to complex II deficiency discussed above, those leading to tumorigenesis are typically heterozygous with modest effects on complex function. The impact of these mutations on complex II assembly and function are discussed in excellent recent reviews from Dalla Pozza et al. [151] and Bezawork-Geleta et al. [152].

**Complex III assembly**

Complex III or the cytochrome bc<sub>1</sub> complex sits at the centre of the MRC, using electrons from complexes I and II via Coenzyme Q to reduce cytochrome c, while also pumping protons into the intermembrane space from the matrix [153]. Complex III forms an obligate homodimer (CIII<sub>2</sub>), with each monomer being composed of one mtDNA-encoded subunit (CYB) and nine nuclear-encoded subunits, CYC1, UQRC1, UQRC2, UQCRFS1, UQCRH, UQCRB, UQCRQ, UQCR10, UQCR11 [154,155]. CYB and the nuclear-encoded cytochrome c1 (CYC1) and UQCRFS1 are the three subunits with electron transfer capabilities [153,156]. Much of the human complex III assembly pathway has been extrapolated from studies of the yeast complex, which presents a similar structure and subunit composition [157–159]. Assembly can be broken down into many discreet steps (Figure 4), which are summarised below.

**Translation of CYB and initial steps of complex III assembly**

The assembly of complex III begins with the translation of mtDNA-encoded cytochrome B (CYB) on mitribosomes and its co-translational insertion into the IMM (Figure 4) [158]. For efficient translation, the

![Figure 4. Schematic depicting the complex III assembly pathway showing known mitochondrial disease genes in red, mtDNA-encoded subunits underlined and assembly factors in italics. CYB is joined by UQCRB and UQCRQ. The subcomplex joins with a tetramer of UQRC1 and UQRC2, CYC1, UQCRH and UQCR10, resulting in the first dimeric intermediate, pre-CIII<sub>2</sub>. UQCRFS1 receives the iron–sulfur cluster from the assembly factor LYRM7 and is assembled into the pre-CIII<sub>2</sub> structure with the aid of the translocase BCS1L. The incorporation of UQCR11 subunit occurs at later stages of complex III assembly. The assembly factor TTC19 clears proteolytic fragments arising from UQCRFS1 maturation. IMS, intermembrane space; IMM, inner mitochondrial membrane; Matrix, mitochondrial matrix.](https://doi.org/10.1042/BCJ20190767)
mitoribosome must be bound by a dimer of the assembly factors UQCC1 and UQCC2 [160,161]. This dimer interacts with the newly translated polypeptide at the mitoribosome exit tunnel and remains bound to CYB after its incorporation into the IMM [161], which is believed to be aided by the insertase OXA1L [162,163]. Like all other mtDNA protein coding genes, the phenotype and disease onset due to mutations in CYB depend on the variant and mutant load. Though many patients present with relatively mild symptoms (myopathy expressed as exercise intolerance; [164–169]), the classic LHON phenotype is also a common presentation [170–174] (Table 6). Interestingly, for many of these variants, complex III deficiency as well as high levels of mutant load appear to be restricted to muscle tissue. There are two known patients for the assembly factor UQCC2, both with mutations leading to near absence of the protein [160,175] (Table 6). In contrast with those with CYB mutations, the one UQCC2 patient harbouring complete loss of the protein due to defective mRNA splicing showed complex III deficiency in both the expected muscle tissue but also skin fibroblasts [160]. Another noteworthy point is that defects in UQCC2 also lead to a combined complex I defect [160,175], which is likely due to the reliance of complex I on the presence of complex III for its assembly [176].

Table 6. Defects affecting translation of CYB and initial steps of complex III assembly

| Gene   | S. | A. F. | Types of genetic variants and protein impact                                      | Clinical presentations and relevant information                                                                 | Ref.       | OMIM    |
|--------|----|-------|----------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------|-----------|---------|
| MT-CYB | X  |       | Predominantly missense but also nonsense leading to reduced levels of protein. Effect is often restricted to muscle tissue. | Variable phenotypes and onset depending on variant, spanning from mild exercise intolerance and lactic acidosis, may develop encephalopathy and seizures in adulthood, may include multisystemic involvement (growth retardation, deafness, cognitive dysfunction) to LHON. A single severe case of fatal infantile cardiomyopathy has also been described. | [164–167,286,428] | 516020  |
| UQCC2  | X  |       | Missense or intronic splicing defect, leading to undetectable or very low protein levels. | Lactic acidosis, growth retardation, neurological impairment.                                                   | [160,175] | 614461  |
| UQCC3  | X  |       | Single patient with missense leading to undetectable protein levels.              | Lactic acidosis, hypoglycaemia, hypotonia, delayed development.                                               | [178]     | 616097  |
| UQCRB  | X  |       | Single patient with deletion leading to C-terminal extension, but with unclear protein impact. | Episodic infantile lactic acidosis, hypoglycaemia, transient liver dysfunction, followed by normal psychomotor development in early childhood. Decreased CIII activity. | [179]     | 191330  |
| UQCRQ  | X  |       | Missense leading to unclear protein impact.                                       | Severe non-lethal psychomotor retardation dystonia, ataxia, ataxia, mildly elevated blood lactate. Decreased CIII activity and variable CI deficiency. | [180]     | 612080  |

S., subunit; A.F., assembly factor; LHON, Leber’s hereditary optic neuropathy.
Following membrane integration of CYB, the first of two heme b molecules is incorporated at the b₁ site [158,177], triggering the binding of the assembly factor UQCC3 which based on work on its fungal homologue is thought to promote the incorporation of a second heme b molecule at the b₁₁ site [177]. This leads to dissociation of the UQCC1–UQCC2 dimer, which is free to initiate another round of CYB translation [161,177]. There is a single known patient for UQCC3, harbouring a missense variant leading to undetectable protein [178] (Table 6). The patient presented with isolated complex III deficiency (complex I was at the lower end of the control range in muscle), displayed lactic acidosis, hypotonia and delayed development, and fibroblasts exhibited defects in CYB and complex III assembly [178]. Fully hemylated CYB is then stabilised by the binding of the first nuclear-encoded subunits UQCRB and UQCRQ (Figure 4) [177]. Both of these subunits are mitochondrial disease genes, although there are only single variants known for each with limited cases (Table 6). For UQCRB, the single known case harbours a homozygous mutation leading to deletion of the last seven amino acids of the protein and inclusion of a new stretch of 14 amino acids derived from non-coding exons [179]. The protein impact of this mutation is not clear, although the patient, an infant who presented with episodic lactic acidosis, hypoglycaemia and liver dysfunction, had impaired complex III activity in liver, lymphocytes and fibroblasts. Interestingly, by the age of four the patient showed normal growth and psychomotor development. Without further cases or related functional studies, the mechanism underpinning the mild impact of this variant is unknown. For UQCRQ there is a single homozygous missense variant with multiple affected cases, however, these present with a more severe psychomotor retardation phenotype (Table 6). The impact of the mutation on protein function is unknown, although muscle from the patients has only a moderate complex III activity defect [180]. As for most other patients with defects affecting complex III, complex I activity was also moderately impaired in some, but not all of the reported cases.

### Assembly of the pre-complex III dimer

Parallel to the coalescence of hemylated CYB with UQCRB and UQCRQ, matrix facing subunits UQCRCA and UQCRCD form a separate tetrameric module (i.e. a dimer of each), which is likely incorporated into the CYB-containing module simultaneously with CYC1 and UQCRH to yield the first dimeric intermediate of complex III, the pre-CIII₁₆ (Figure 4) [181]. This step coincides with the dissociation of UQCC3, which may prevent the dimerisation of earlier intermediates [181]. Following dimerisation, the subunit UQCR10 is added to pre-CIII₁₆ [158]. UQCRCA and CYC1 are mitochondrial disease genes (Table 7). Although there exist only a few patients for both, there is a consistent and similar phenotype and clinical progression to what is seen in the UQCRB patient [179]. Patients present with recurring episodes of metabolic acidosis that largely resolves in childhood or early adulthood [182–184]. Analysis of fibroblasts from the patients have combined complexes I and III defects in assembly, though the complex I enzyme defect is not significant for the CYC1 patients [184]. The mechanism underpinning the similarity in phenotypes between these patients and those with mutations in UQCRB is not yet clear, though suggests a similar impact on complex III assembly and function.

### Table 7. Defects affecting assembly of the pre-complex III dimer

| Gene | S. | A.F. | Types of genetic variants and protein impact | Clinical presentations and relevant information | Ref. | OMIM |
|------|----|-----|---------------------------------------------|-----------------------------------------------|------|------|
| CYC1 | X  |     | Missense leading to near absence of protein | Episodic severe infantile ketoacidosis, insulin-responsive hyperglycaemia, hyperammonemia followed by normal development in childhood or early adulthood. Isolated CIII deficiency | [184] | 123980 |
| UQCRCA | X |     | Missense leading to uncertain protein impact | Episodic infantile hypoglycaemia, lactic acidosis, ketonuria, variable liver failure followed by normal development in childhood. CIII deficiency and may present combined CI deficiency | [182,183] | 191329 |

S., subunit; A.F., assembly factor.
Biogenesis of UQCRFS1 and final steps of assembly

The Rieske iron–sulfur protein, UQCRFS1, is first imported into the matrix where it is bound by the chaperone LYRM7 which stabilises it and mediates the insertion of a 2Fe–2S cluster (Figure 4) [185,186]. Now-folded and containing an iron–sulfur cluster, UQCRFS1 is thought to be translocated into the IMM by the AAA+ ATPase BCS1L [187]. BCS1L is an inner membrane protein that forms a matrix-sided heptameric ring structure [188], allowing for the translocation of the hydrophilic folded C-terminus of UQCRFS1 across the inner membrane [187–189]. The incorporation of UQCRFS1 marks the important transition to a catalytically active complex III [158]. Both assembly factors and the UQCRFS1 gene itself are linked to mitochondrial disease, with multiple variants described, most of which lead to severely reduced protein levels of the corresponding protein (Table 8). Functional studies in fungal models have indicated that absence of the LYRM7 homologue leads to destabilisation and degradation of UQCRFS1 [185,190,191], although a small amount of UQCRFS1 is still assembled into complex III, which is not seen in cells lacking the homologue of BCS1L [187]. In line with this, patients for all three genes have some similarity in clinical presentation, particularly LYRM7 and UQCRFS1, both of which present severe infantile conditions consistent with complex III deficiency (Table 8) [192–195]. While BCS1L patients can also present with these symptoms, there is considerable symptomatic variability depending on the variant, including hepatic iron overload and the absence of a complex III defect entirely (Table 8) [196–202]. This had led to suggestions that BCS1L may have another yet to be characterised role in mitochondrial function [196].

Table 8. Defects affecting biogenesis of UQCRFS1 and final steps of CIII assembly

| Gene     | S. | A.F. | Types of genetic variants and protein impact                                      | Clinical presentations and relevant information                                                                 | Ref. | OMIM   |
|----------|----|-----|----------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------|------|--------|
| UQCRFS1  | X  |     | Missense, intronic, nonsense leading to total absence or decreased protein levels. | Severe hypertrophic cardiomyopathy, lactic acidosis, alopecia. Isolated CIII deficiency. Depending on severity of variant survival into childhood with slightly lightly impaired gross and fine motor skills. | [195] | 191327 |
| BCS1L    | X  |     | Predominantly missense but also nonsense leading to decreased protein levels or unclear protein impact. | Variable phenotype including i) mild Björnstad syndrome (pili torti, neurosensory deafness), ii) severe Leigh syndrome, tubulopathy, hepatic dysfunction, metabolic acidosis, iii) GRACILE syndrome. May not present CIII deficiency. | [196–201] | 603647 |
| LYRM7    | X  |     | Multiple. Nonsense, intronic, deletion, duplication, missense leading to decreased protein levels. | Lactic acidosis, early onset multifocal cavitating leukoencephalopathy, fatal neurologic decompensation. Isolated CIII deficiency. | [192–194] | 615831 |
| TTC19    | X  |     | Predominantly nonsense but also deletions likely leading to absence of protein. | Variable onset and neurological phenotypes including psychiatric symptoms, progressive neurodegenerative disorder, developmental delay, ataxia, as well as Leigh syndrome. Isolated CIII deficiency. Onset tends to late childhood with some cases presenting in adulthood. | [203,205–208] | 613814 |

S., subunit; A.F., assembly factor; GRACILE, growth retardation, amino aciduria, cholestasis, iron overload, lactic acidosis and early death.
The final steps of complex III assembly include the incorporation of the last subunit, UQCR11 [158], followed by transient association of the assembly factor, TTC19. Although with unknown function at the time, TTC19 was identified in patients with progressive neurological phenotypes and isolated complex III deficiency [203]. Functional studies using a mouse knockout model showed that TTC19 is involved in proteolytic clearance of protein fragments derived from the N-terminus of UQCRFS1 that likely inhibit the enzyme [204]. Many other patients and variants have since been reported, generally leading to loss of detectable protein and similar late-onset phenotypes (Table 8) [192–201,205–208]).

**Complex IV assembly**

Complex IV or cytochrome c oxidase (COX) is the last proton-pumping enzyme in the ETC and displays many interesting features compared with other OXPHOS complexes. For instance, complex IV has the highest ratio of known assembly factors per subunit as well as tissue and developmental specific isoforms which together adds complexity for the diagnosis of mitochondrial diseases [6,209,210]. Mammalian complex IV consists of 14 subunits of which three are core subunits encoded on mtDNA, MT-CO1 (frequently referred to as COX1), MT-CO2 (COX2) and MT-CO3 (COX3) with the remainder encoded by nDNA, COX4 (with 2 possible isoforms encoded on separate genes, COX4I1 and COX4I2), COX5A, COX5B, COX6A (2 possible isoforms, COX6A1-2), COX6B (2 isoforms, COX6B1-2), COX6C, COX7A (3 isoforms, COX7A1-3), COX7B, COX7C, COX8A and COX8C [211–213]. It is generally thought that assembly of complex IV occurs in a modular fashion (Figure 5) [210,214,215], with the three mt-DNA encoded acting as platforms for assembly of nDNA encoded subunits into modules.

**Assembly of the COX1 module**

The assembly of complex IV starts with translation of COX1, which requires a specific translational activator (TACO1) [216,217], and COA3 and COX14, which appear to interact with COX1 [218–220] and suggested to prevent its degradation [218,220]. Another assembly factor, CMC1, has also been implicated in stabilising COX1 at this stage of assembly [221]. At this early step, mutations in TACO1 have been linked to a range of phenotypes, commonly presenting as Leigh syndrome, optic atrophy and muscle involvement with variable

![Figure 5. Schematic depicting the complex IV assembly pathway showing known mitochondrial disease genes in red, mtDNA-encoded proteins underlined and assembly factors in italics.](https://example.com/figure5.png)

Complex IV assembly is driven by the coalescence of three distinct modules named after the mtDNA-encoded subunits they contain, COX1, COX2 and COX3. COX1 receives heme and copper (Cu) cofactors and joins COX4 and COX5A to form the COX1 module. COX2 receives copper and is assembled with COX5B, COX6C, COX7B, COX7C and COX8A to form the COX2 module. COX1 and COX2 module are integrated prior to addition of the COX3 module, which is composed of COX6A, COX6B and COX7A. NDUFA4 is the last subunit to be added to form the mature complex IV. IMM, inner mitochondrial membrane; Matrix, mitochondrial matrix.
The copper biosynthesis and insertion in mammalian cells has mostly been extrapolated from yeast studies. In this pathway, the CuB group is moved by COX17 to COX11, which is a metallochaperone [244,245], containing a conserved copper-binding motif [246]. COX11 also requires another assembly factor, COX19, to maintain its redox state [247] after copper binding. Finally, MITRAC7 is a COX1-specific chaperone that prevents COX1 degradation prior to the fusion of the COX1 and COX2 modules (Figure 5) [248]. Another protein thought to be involved in assembly of the COX1 module is HIGD1A. Based mainly on studies primarily performed in yeast, HIGD1A is thought to bind and stabilise COX4 and COX5A subunits prior to incorporation to COX1 [215] and potentially stay bound to regulate complex IV activity [249]. However, while mammalian HIGD1A associates with COX4 and COX5A [214,215], knockout of HIGD1A in mammalian cells had only a very minor effect on the stability of COX4 and COX5A subunits, or complex IV assembly more broadly [214,250].
| Gene     | A. | F. | Types of genetic variants and protein impact                                      | Clinical presentations and relevant information                                                                                                                                                                                                 | Ref.         | OMIM    |
|----------|----|----|----------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------|---------|
| MT-CO1   | X  |    | Missense, nonsense leading to unclear protein impact.                            | Majority of cases present survival into adulthood and phenotypes of late-onset Leigh syndrome, recurrent myoglobinuria, lactic acidosis, cerebellar ataxia, optic atrophy, hearing loss, myopathy.                                                                 | [224–226]   | 516030  |
| COX4I1   | X  |    | Missense or deletion/insertion leading to unclear or decreased protein levels.   | Short stature, increased chromosomal breaks, resembling Fanconi anaemia or severe cases of developmental delay, short stature and seizures, resembling Leigh syndrome.                                                                                                                                       | [232,233]   | 123864  |
| COX4I2   | X  |    | Missense leading to unclear protein impact.                                      | Exocrine pancreatic insufficiency, dyserythropoietic anaemia, calvarial hyperostosis, failure to thrive.                                                                                                                                                                                                   | [236]       | 607976  |
| COX5A    | X  |    | Missense leading to decreased protein levels.                                    | Early-onset pulmonary hypertension, lactic acidosis, heart abnormalities, failure to thrive.                                                                                                                                                                                                             | [237]       | 603773  |
| COA3     | X  |    | Missense and duplication causing frameshift leading to decreased protein levels. | Peripheral neuropathy, exercise intolerance, short stature. Survival to adulthood.                                                                                                                                                                                                                           | [227]       | 614775  |
| COX10    | X  |    | Missense leading to unclear protein impact.                                      | Variable severe phenotypes mostly presenting metabolic acidosis and anaemia combined with Leigh syndrome or hypertrophic cardiomyopathy but also tubulopathy, ataxia, hypotonia and early death.                                                                                                                | [238,239]   | 602125  |
| COX14    | X  |    | Missense leading to undetectable protein levels.                                | Severe lactic acidosis, microphthalmia, ketoacidosis, hypertrophic cardiomyopathy, respiratory distress, brain hypertrophy.                                                                                                                                                                                 | [218]       | 614478  |
| COX15    | X  |    | Missense, intronic and nonsense leading to unclear protein impact.              | Variable severe phenotypes. Fatal infantile hypertrophic cardiomyopathy, lactic acidosis, seizures, hypotonia or Leigh syndrome, failure to thrive, psychomotor delay, hypotonia, elevated plasma lactate and pyruvate.                                                                                                                | [240,241]   | 603646  |
| SURF1    | X  |    | Over 80 mutations from variable genetic nature.                                 | Most common cause of Leigh syndrome associated with CIV deficiency but variable severe phenotypes including Charcot-Marie-Tooth disease, (Reviewed in [242])                                                                                                                                            | (Reviewed in [242]) | 185620  |

Continued
Assembly of the COX2 module

The assembly of the COX2 module starts with the translation and membrane insertion of the first transmembrane domain of COX2, which is stabilised by COX20 (Figure 5) [251]. The insertion of the second transmembrane domain also leads to translocation of the globular copper-binding domain which occurs with the assistance of assembly factor COX18 [251]. Patients harbouring mutations in the mtDNA-encoded COX2 (MT-CO2) have been reported to present with phenotypes ranging from mild exercise intolerance and recurrent myoglobinuria or late-onset cerebellar ataxia to severe fatal lactic acidosis, depending on variant and mutant load [252–255]. There are some overlap in phenotypes seen in cases caused by mutations in the assembly factor COX20 [256–259], particularly ataxia and hypotonia with survival into adulthood reported (Table 10).

The insertion of the copper centre (CuA) in COX2 requires five known assembly factors: COX17, SCO1, SCO2, COA6 and COX16 (Figure 5). COX17 donates copper to SCO1 [260], a metallochaperone that delivers the copper to COX2. Copper delivery to COX2 also requires SCO2, another metallochaperone that reduces the disulphide bonds in COX2 to allow the copper insertion [261]. COA6 and COX16 also interact with COX2, probably at the time of copper insertion, and promote the function of SCO1 and SCO2 [262–264]. COX16 has also been shown to act in the recruitment of the COX1 module to the COX2 module in the next step of assembly, in a COX2-dependent manner [264]. Another putative assembly factor, TMEM177, was shown to associate with the COX2/COX20/SCO1/SCO2 intermediate [265], and while TMEM177 is thought to regulate COX20 levels, loss of TMEM177 does not impair complex IV assembly in mammalian HEK293T cells [265]. Of the proteins involved in copper assembly, mutations have been identified in SCO1, SCO2 and COA6, all leading to severe phenotypes and commonly linked to fatal infantile cardiomyopathy, with shared clinical presentations of encephalopathy, liver failure, respiratory distress and metabolic acidosis [88,266–272] (Table 10). COX7B is an X-linked gene, and while many variants have been described, all known cases are in females who present with facial dysmorphism, linear skin defects, short stature with variable presentation of tetralogy of Fallot and ventricular hypertrophy [276,277]. For COX8A, a homozygous intronic mutation has been shown to cause a more severe phenotype of Leigh-like syndrome, developmental delay, pulmonary hypertension, epilepsy and elevated lactate in blood and cerebrospinal fluid [278]. For PET100 and PET117 it is useful to consider the fungal studies where much more is known about their function. The yeast homologue pPet100 was found in an assembly intermediate containing pCox7, pCox7a and pCox8 (human COX7A, COX6C and COX7C) and loss of pPet100 leads to its

| Gene   | S. A. F. | Types of genetic variants and protein impact | Clinical presentations and relevant information | Ref. | OMIM |
|--------|----------|---------------------------------------------|-----------------------------------------------|------|------|
| TACO1  | X        | Duplication causing frameshift or missense leading to unclear protein impact. | Variable phenotype from mild mental retardation and survival to early adulthood to severe slowly progressive childhood-onset of Leigh syndrome, may present optic atrophy, dystonia, spastic tetraparesis, renal tubulopathy. | [216,222,223] | 612958 |

S., subunit; A.F., assembly factor.

© 2020 The Author(s). This is an open access article published by Portland Press Limited on behalf of the Biochemical Society and distributed under the Creative Commons Attribution License 4.0 (CC BY-NC-ND).
### Table 10. Defects affecting assembly of COX2 module

| Gene       | S. | A. F. | Types of genetic variants and protein impact | Clinical presentations and relevant information | Ref. | OMIM   |
|------------|----|-------|---------------------------------------------|-----------------------------------------------|------|--------|
| MT-CO2 (COX2) | X  |       | Missense, nonsense, deletion leading to unclear or decreased protein levels. | Variable phenotypes from mild exercise intolerance, recurrent myoglobinuria, late-onset cerebellar ataxia and survival into adulthood to severe cases of lactic acidosis and early death. | [252–255] | 516040 |
| COA6       | X  |       | Mostly missense but also nonsense leading to decreased protein levels. | Hypertrophic cardiomyopathy may present lactic acidosis. Decreased CI and CIV in heart but not effect in fibroblasts. | [88,271,272] | 614772 |
| COX7B      | X  |       | Intrinsic and deletion causing protein frameshift or nonsense leading to unclear protein impact. | X-linked gene. Facial dysmorphism, linear skin lesions with survival into childhood, may present tetralogy of Fallot, ventricular hypertrophy. | [276,277] | 300885 |
| COX8A      | X  |       | Intrinsic leading to unclear protein impact. | Leigh-like syndrome, developmental delay, pulmonary hypertension, epilepsy, elevated blood and cerebrospinal fluid lactate. Decreased CIV assembly. | [278] | 123870 |
| COX20      | X  |       | Predominantly missense but also intronic leading to decreased protein levels. | Most presentations include ataxia, hypotonia but can also present mild elevation of blood lactate, sensory neuropathy and static encephalopathy with reported survival into adulthood. | [256–259] | 614698 |
| PET100     | X  |       | Nonsense or missense abolishing first methionine leading to unclear protein impact. | Variable severe phenotypes. Fatal infantile lactic acidosis, brain abnormalities, severe coagulopathy or Leigh syndrome, elevated blood lactate, seizures, hypotonia. but Decreased CIV assembly/activity. | [274,279] | 614770 |
| PET117     | X  |       | Nonsense leading to unclear protein impact. | Brain and motor development regression with survival into adulthood. Decreased CIV activity/assembly. | [280] | 614771 |
| SCO1       | X  |       | Predominantly missense or frameshift may lead to decreased protein levels. | Liver failure, encephalopathy, hypotonia, metabolic acidosis, may present cardiac hypertrophy or respiratory distress. | [266,267] | 603644 |
accumulation together with another subcomplex composed of Cox5a and Cox6 (human COX4I1 and COX5A), thus preventing the assembly of the mature complex IV [273]. Consistently, the patients reported to harbour PET100 mutations show undetectable [279] or residual mature complex IV [274], with the former displaying a more severe phenotype and neonatal death [279]. In the case of pPet117, yeast studies have shown that it stabilises Cox15 oligomers and might function in heme A synthesis and/or transfer to pCox1, and is also required for complex IV assembly [275]. In contrast with this, mammalian studies have shown that PET117 interacts with PET100, MR-1S and other subunits of the COX1 and COX2 modules [215], suggesting that mammalian PET117 could have a distinct function to its yeast counterpart. Despite unknown effect of PET117 mutation on protein levels, the patients reported with a homozygous nonsense mutation display decreased complex IV assembly and a milder phenotype [280] when compared with PET100 patients [274,279].

Assembly of the COX3 module

The COX3 module is the last module to be added to the now complete COX1 and COX2 modules in the nascent complex IV assembly (Figure 5). This module consists of the core subunit COX3 and the nuclear-encoded subunits COX6A, COX6B and COX7A. Even though complex IV displays the highest assembly factor to subunit ratio amongst other OXPHOS complexes, it was surprising that no assembly factors have been found to be required for building the COX3 module. Although COX3 is not directly involved in electron transport, it is thought to play a regulatory role in enzyme function [211,281]. HIGD2A was previously thought to act as an assembly factor for the complexes I, III, IV supercomplex [282] but recently shown to be required for the assembly of the COX3 module [214]. While HIGD2A appears to be needed for the early steps of COX3 biogenesis, it is unclear if it is involved in COX3 translation, membrane integration or its integration with the nascent complex IV assembly. Following coalescence of the COX1, COX2 and COX3 modules, NDUFA4, formerly thought to be a complex I subunit [212], is likely the last subunit to be incorporated to form the mature complex IV [210].

Similar to other mitochondrially encoded genes, mutations in MT-CO3 (COX3) cause a variety of phenotypes and onsets including Leigh-like syndrome, myopathy, lactic acidosis, MELAS, LHON and recurrent myoglobinuria with survival into adulthood reported [172,283–286] (Table 11). Interestingly, the phenotype of recurrent myoglobinuria is also found in patients harbouring mutations in COX1 (MT-CO1) [225] and COX2 (MT-CO2) [252]. In the case of COX6A1 and COX6A2, mutations have been reported to cause different clinical presentations. While a homozygous deletion in COX6A1 was shown to cause a neuromuscular disease called Charcot–Marie–Tooth disease [287], mutations in COX6A2 lead to a muscle-specific presentation of myopathy and cardiomyopathy [288]. As COX6A2 is exclusively expressed in heart and skeletal muscle [289], the mutations reported in COX6A2 were shown to only affect complex IV activity in differentiated muscle and not in undifferentiated myoblasts [288]. For the only hydrophilic extramembrane subunit of complex IV facing the IMS, COX6B1 [290], pathogenic variants have been shown to cause severe infantile encephalomyopathy, cardiomyopathy and lactic acidosis. The last subunit to be assembled into complex IV, NDUFA4, has been reported to cause Leigh syndrome, congenital lactic acidosis and variable presentation of dystonia due to a homozygous intronic variant. The intronic variant leads to undetectable NDUFA4 protein levels via western blot analysis and was shown to not impair the assembly of the other 13 complex IV subunits [291].

Table 10. Defects affecting assembly of COX2 module  Part 2 of 2

| Gene | S. | A. | Types of genetic variants and protein impact | Clinical presentations and relevant information | Ref. | OMIM |
|------|----|----|---------------------------------------------|-----------------------------------------------|------|------|
| SCO2 | X  |    | Mostly missense but also nonsense and duplication leading to unclear or reduced protein levels. | Fatal infantile hypertrophic cardiomyopathy, encephalopathy, elevated blood lactate, respiratory distress. | [268–270] | 604272 |

S., subunit; A.F., assembly factor.
Although the exact mechanism in which COA7 is involved in complex IV assembly remains unclear, mutations in COA7 have been shown to cause leukoencephalopathy or spinocerebellar ataxia and axonal neuropathy with variable onset and survival into late adulthood is reported (Table 12). A patient reported with undetectable COA7 levels via western blot analysis also showed decreased levels of COX2 and COX3 subunits, and decreased assembly of complex IV [292], providing insights into the stage of assembly of which COA7 may be involved.

Complex V assembly
Complex V, also known as F_oF_1-ATPase, is the last enzyme in the OXPHOS system, utilising the proton gradient generated by complexes I, III and IV to power ATP synthesis. Complex V is composed of a membrane-embedded F_o section connected by an external stalk to a matrix soluble F_1 section containing the ATPase domains [293]. Like complexes I, III and IV, complex V is under dual genetic control, with two of the membrane subunits encoded on mtDNA (ATP6, MT-ATP6; ATP8, MT-ATP8) and the remaining 16 encoded on nDNA [294]. Much of what is known for complex V is extrapolated from studies in fungal models, though in recent years there is an increasing amount of literature documenting the assembly in mammals. The nomenclature for nuclear complex V genes has also undergone a recent overhaul [23], now all being prefixed ATP5 (e.g. ATP5F1A). Typically, the corresponding proteins are represented by single Greek and Latin letters (e.g. α or α-subunit for ATP synthase subunit alpha, which is the recommended name for the protein product of ATP5F1A) though there are some inconsistencies. As for other complexes, we have chosen to refer to the subunits and assembly factors by their gene name using the aforementioned formatting (bold typeface for subunit, regular typeface for assembly factor) though have included the commonly used protein symbol on the first instance.

Table 11. Defects affecting assembly of COX3 module

| Gene       | S. | A. F. | Types of genetic variants and protein impact | Clinical presentations and relevant information                                                                 | Ref.  | OMIM  |
|------------|----|-------|---------------------------------------------|---------------------------------------------------------------------------------------------------------------|-------|-------|
| MT-CO3 (COX3) | X  |       | Missense, nonsense, insertion, deletion leading to unclear or decreased protein levels depending on variant and mutation load. | Variable phenotype, including Leigh-like syndrome, myopathy, lactic acidosis, MELAS, LHON, recurrent myoglobinuria with cases of survival into adulthood. | [172,283–286] | 516050 |
| COX6A1     | X  |       | Deletion leading to decreased protein levels. | Recessive axonal type of Charcot-Marie-Tooth with survival into adulthood. | [287] | 602072 |
| COX6A2     | X  |       | Missense leading to decreased protein levels. | Congenital myopathy, cardiomypathy, isolated CIV deficiency in muscle and absent from undifferentiated myoblast. | [288] | 602009 |
| COX6B1     | X  |       | Missense leading to decreased protein levels. | Severe infantile encephalomyopathy, cardiomypathy, lactic acidosis. CIV deficiency detected in muscle and mildly fibroblasts. | [429,430] | 124089 |
| NDUFA4     | X  |       | Intronic leading to undetectable protein levels. | Leigh syndrome, congenital lactic acidosis, may present dystonia with survival into adulthood. | [291] | 603833 |

S., subunit; A.F., assembly factor; LHON, Leber’s hereditary optic neuropathy; MELAS, mitochondrial encephalopathy, lactic acidosis and stroke-like episodes.
Assembly of the F₁ catalytic module and central stalk

The assembly of complex V starts with the oligomerisation of three ATP5F1A (α-subunit) and three ATP5F1B (β-subunit) subunits into an alternating hexamer, in a series of assembly steps mediated by the assembly factors ATPAF1 and ATPAF2 respectively (Figure 6) [295]. These proteins likely act as placeholders to prevent the formation of homomeric complexes [296,297] and their loss in fungal models leads to aggregation of the subunits into large insoluble complexes [298]. In general, there are few reported cases for patients harbouring mutations affecting assembly or function of this module (Table 13). For ATP5F1A there are two known variants [299,300] with cases for both presenting with severe encephalopathy or microcephaly, followed by early infantile death. Tissue and fibroblast material from affected patients had reduced but not absent levels of ATP5F1A protein as well as lower levels of other subunits, suggesting stability of the F₁ module is

Table 12. Defects affecting unknown steps of complex IV assembly or function

| Gene (alias) | S. | A. | Types of genetic variants and protein impact | Clinical presentations and relevant information | Ref. | OMIM |
|-------------|----|----|--------------------------------------------|-----------------------------------------------|------|------|
| COA7        | X  |    | Predominantly missense but also intrinsic and deletion causing protein frameshift and undetectable protein levels | Variable phenotypes and onset with survival into late adulthood presenting leukoencephalopathy or spinocerebellar ataxia with axonal neuropathy | [292,431] | 615623 |

S., subunit; A.F., assembly factor.

Assembly of the F₁ catalytic module and central stalk

The assembly of complex V starts with the oligomerisation of three ATP5F1A (α-subunit) and three ATP5F1B (β-subunit) subunits into an alternating hexamer, in a series of assembly steps mediated by the assembly factors ATPAF1 and ATPAF2 respectively (Figure 6) [295]. These proteins likely act as placeholders to prevent the formation of homomeric complexes [296,297] and their loss in fungal models leads to aggregation of the subunits into large insoluble complexes [298]. In general, there are few reported cases for patients harbouring mutations affecting assembly or function of this module (Table 13). For ATP5F1A there are two known variants [299,300] with cases for both presenting with severe encephalopathy or microcephaly, followed by early infantile death. Tissue and fibroblast material from affected patients had reduced but not absent levels of ATP5F1A protein as well as lower levels of other subunits, suggesting stability of the F₁ module is

Figure 6. Schematic depicting the complex V (F₁F₀-ATPase) assembly pathway showing known mitochondrial disease genes in red, mtDNA-encoded proteins underlined and assembly factors in italics.

To aid correlation with disease genes, subunits have been labelled according to their gene name. The commonly used protein names are in parenthesis as follows: three copies of the ATP5F1A (α-subunit) and ATP5F1B (β-subunit) are assembled with the aid of the chaperones ATPAF1 and ATPAF2 with later binding of the subunits ATP5F1C (γ-subunit), ATP5F1D (δ-subunit) and ATP5F1E (ε-subunit). The membrane ring composed of ATP5MC1 (c-subunits; also encoded by ATP5MC2 and ATP5MC3) subunits is assembled and joins the pre-complex V prior to the addition of the subunits ATP5PB (b-subunit), ATP5PD (d-subunit), ATP5PF (F6-subunit) and ATP5PO (OSCP). The assembly pathway is followed by integration of ATP5ME (e-subunit), ATP5MF (f-subunit) and ATP5MG (g-subunit) and then by the mtDNA-encoded ATP6 (subunit 6) and ATP8 (subunit 8). The last subunits ATP5MPL (MP68) and ATP5MD (DAPIT) are added to complete the assembly of complex V. IMM, inner mitochondrial membrane; Matrix, mitochondrial matrix.
compromised [299]. For ATPAF2, there is a single known example [301]. In line with the requirement for this protein in assembly of the F1 module, the patient presented with similar symptoms to those with mutations in ATP5F1A, degenerative encephalopathy, severe developmental delay and death in early childhood (Table 13).

The next step in the assembly pathway is the incorporation of the subunits belonging to the central stalk, ATP5F1C (γ-subunit), ATP5F1D (δ-subunit) and ATP5F1E (ε-subunit). Functional studies have shown that loss of any of these subunits leads to similar defects in complex V assembly, lower levels of mature complex and turnover of subunits for the F1 module [302]. ATP5F1C is the key structural molecule connecting the F1 catalytic module to the F0 module and through structural similarities of its coiled–coiled tail with the C-terminal regions of ATPAF1 and ATPAF2, likely displaces the assembly factors during module biogenesis [303,304]. There are no known patients for ATP5F1C, although all three genes encode the same mature protein, the proteins only differing in the sequence of their cleaved mitochondrial targeting signals [307,308]. There are no reported cases of mitochondrial disease linked to mutations in these genes, however, many cases and >5 different variants have been linked to defects in the assembly factor TMEM70 (Table 14) which has been linked with its biogenesis. TMEM70 has been suggested to be required for assembly of the c-subunits into the c-ring [80]. Although this has been argued in the literature [75], it is clear from these studies that TMEM70 is required for the joining of the c-ring to the F1 module, and its absence leads to a severe assembly

| Gene (protein) | Type of genetic variants and protein impact | Clinical presentations and relevant information | Ref. | OMIM |
|---------------|------------------------------------------|-----------------------------------------------|------|------|
| ATP5F1A (α-subunit) | Missense leading to unclear protein impact. | Fatal neonatal encephalopathy, lung hypoplasia or hypertension, may present seizures, heart failure. Decreased CV assembly/activity. | [299,300] | 164360 |
| ATPAF2 (Atp12) | Single known missense leading to unclear protein impact. | Degenerative encephalopathy, severe developmental delay, death in early childhood. Decreased CV assembly/activity. | [301] | 608918 |
| ATP5F1D (δ-subunit) | Single known missense leading to normal protein levels. | Hyperammonemia, lactic acidosis or ketoacidosis, may present cardiomyopathy, delayed speech with survival into childhood. Decreased CV assembly/activity. | [305] | 603150 |
| ATP5F1E (ε-subunit) | Single known missense leading to reduced protein levels. | Neonatal onset lactic acidosis, mild mental retardation, severe peripheral neuropathy with survival into adulthood. Reduced levels of fully assembled CV. | [306] | 606153 |

1Commonly used protein name; S., subunit; A.F., assembly factor.

Assembly of the c-ring

The membrane-embedded c-ring and the central stalk are components of the rotor part of the Complex V and therefore essential for ATP synthesis. In humans, the c-ring consists of eight c-subunits encoded by ATP5MC1, ATP5MC2 and ATP5MC3 (Figure 6). Interestingly, all three genes encode the same mature protein, the proteins only differing in the sequence of their cleaved mitochondrial targeting signals [307,308]. There are no reported cases of mitochondrial disease linked to mutations in these genes, however, many cases and >5 different variants have been linked to defects in the assembly factor TMEM70 (Table 14) which has been linked with its biogenesis. TMEM70 has been suggested to be required for assembly of the c-subunits into the c-ring [80]. Although this has been argued in the literature [75], it is clear from these studies that TMEM70 is required for the joining of the c-ring to the F1 module, and its absence leads to a severe assembly
defect. As a disease gene, TMEM70 is further complicated by variable presentations, depending on the variant patients either present with isolated or combined Complex V or Complex I defects, as well as severe cardiac and neuronal phenotypes (Table 14) [81–84]. Although the precise involvement in Complex I assembly is even less clear, recent studies in gene-edited cells completely lacking TMEM70 protein [75] are consistent with suggesting it has a dual role in assembly of both complexes.

Assembly of the peripheral stalk and F₀ module
The peripheral stalk assembles with the F₁ subcomplex in a two-step manner. The first four subunits to be incorporated are ATP5PB (b-subunit), ATP5PD (d-subunit), ATP5PF (F6-subunit), ATP5PO (OSCP) followed by the membrane-associated subunits ATP5ME (e-subunit), ATP5MF (f-subunit) and ATP5MG (g-subunit) [294,309,310] (Figure 6). In the absence of the c-ring, an intermediate complex V is assembled containing the F₁ catalytic module, the peripheral stalk and the membrane subunits ATP5ME, ATP5MF and ATP5MG [311].

Once the previously formed subcomplex containing the F₁ module and peripheral stalk join with the c-ring, this provides the scaffold necessary for the incorporation of the two mitochondrially-encoded subunits ATP6 (subunit 6) and ATP8 (subunit 8) (Figure 6) [309]. Mutations in both lead to disease (Table 15). More than...
200 \textit{ATP6} cases have been reported with 19 different underlying mutations and a large variability in mutant mtDNA load (reviewed in [312]). Phenotypes are highly variable and both severe infantile Leigh syndrome as well adult onset disease has been reported (Table 15), and as such there is no clear assembly phenotype underpinning mutations in this gene. There are comparatively few reported patients for \textit{ATP8}, however, like for \textit{ATP8} these cases report with a different phenotype likely underpinned by differences in the variant and mutant load. Phenotypes include cardiomyopathy and neuropathy [313] as well as seizures and neuropsychologic decline [314] as well as defects in complex V activity (Table 15). In the case of the former, the fibroblasts from the patient accumulated subcomplexes of unassembled complex V, including a free catalytically active F1 domain, suggesting the protein may be structurally important [313]. Interestingly, \textit{ATP6} and \textit{ATP8} are transcribed as a polycistronic mRNA and there are a few cases known to likely affect both proteins [315,316]. Patients present with severe early onset cardiomyopathy or neurological symptoms, although there have has been one case of a kindred with adult onset cerebellar ataxia and peripheral neuropathy (Table 15). Aside from defects in complex V function, the molecular impacts in these cases are unknown.

Finally, the subunit \textit{ATP5MPL} (MP68), which is required for \textit{ATP6} and \textit{ATP8} stability is incorporated, followed by \textit{ATP5MD} (DAPIT) [309]. A single variant of the latter is known to cause childhood onset [317]. Fibroblasts from patients had no detectable protein and reduced ATP synthesis. Fully assembled complex V has been shown to assemble into dimers [318,319] and, more recently, in tetramers [293]. Interestingly, fibroblasts from \textit{ATP5MD} patients had markedly reduced dimerisation [317], suggesting the protein is involved in this process.

\textbf{Mitochondrial translation and the mitoribosome}

Mitochondrial protein synthesis is a complex process that has its own components such as a mitochondria-specific genetic code, an exclusive set of tRNA and tRNA synthetases and its own ribosome known as the mitoribosome [320]. The composition of ribosomes has considerably changed over the course of evolution, specially the RNA to protein ratio which has reversed from 1:2 protein:RNA in bacteria and cytosolic ribosomes to 2:1 protein:RNA in the mammalian mitoribosome. This explains why almost half of the mitoribosome proteins are mitochondrion-specific and absent from the bacterial ancestor [4]. Like other ribosomes, the mammalian mitoribosome is composed of two subunits, the small (mtSSU) and the large (mtLSU). The mRNA engages with the mtSSU while the mtLSU can anchor itself to the IMM and catalyse translation [321]. The mitoribosome has a total sedimentation coefficient of 55S, composed of the 28S for the isolated mtSSU and the 39S for the mtLSU [322]. The mtSSU consists of the 12S rRNA encoded by the mtDNA, and 30 MRPL mitoribosomal proteins encoded by the nDNA [323]. The mtLSU consists of a tRNA valine (tRNA\textsubscript{val}) [2] and a 16S rRNA, both encoded by the mtDNA, and 52 nuclear-encoded mitoribosomal proteins. The peptidyl transferase centre (PTC) catalyses the formation of the peptide bonds of nascent polypeptides [324] and is located in the internal part of the mitoribosome formed exclusively by the 12S and 16S rRNAs [325,326]. The polypeptide exit tunnel (PET) starts at the PTC and ends at the polypeptide exit site (PES) where the translated polypeptide leaves the mitoribosome [327]. The polypeptide tunnel is an important region that is targeted in bacteria as a binding site for antibiotics [321]. The PET is surrounded by ring composed of bacterial conserved proteins \textit{bL17m} (encoded by the gene \textit{MRPL17}), \textit{uL22m} (\textit{MRPL22}), \textit{uL23m} (\textit{MRPL23}), \textit{uL24m} (\textit{MRPL24}), \textit{uL29m} (\textit{MRPL47}) and the mitochondrial specific \textit{mL45} (\textit{MRPL45}) which promotes the tethering of the mitoribosome to the IMM [321]. The translocation of mitochondrial proteins is mediated by OXA1, which binds newly synthesised polypeptides [162,163] and the mitoribosome [328,329]. The nomenclature for mitoribosomal proteins has recently been overhauled for consistency between cytosolic and mitochondrial ribosomes found across different organisms [330]. To assist readers linking phenotype to function we have chosen to refer to the protein using their HUGO assigned gene name and have listed the revised protein name in parenthesis at first mention.

\textbf{Assembly of the mtSSU}

Even though the mitoribosome has unarguable importance, its assembly pathway has not been studied in as much detail as the assembly of OXPHOS complexes [331]. Recent research has begun to solve the timing of incorporation of mitoribosome protein using pulse and pulse-chase stable isotope labelling with amino acids in cell culture (SILAC) proteomics approaches [331,332], suggesting that the mtSSU displays an early and a late class of incorporation of mitoribosomal proteins, while the mtLSU has an additional intermediate class (Figure 7).
The mitoribosome biogenesis has been suggested to start at or near the mitochondrial nucleoid and present subclusters of assembly proteins for each incorporation class or 'module' in the terminology used for the OXPHOS complexes [331]. The 12S rRNA is stabilised by the RNA chaperone ERAL1 [333,334] and metal-dependent endoribonuclease YBEY [335], and methylated by two proteins, the adenine dimethyltransferase TFB1M [336] and the cytosine methyltransferase NSUN4 [337]. The early mitochondrial proteins participating in mtSSU assembly are mainly localised at the top and bottom of the 12S rRNA core and seem to be grouped into three different clusters [331]. One cluster contains MRPS5 (uS5m), MRPS16 (bS16m), MRPS22 (mS22), MRPS27 (mS27), MRPS34 (mS34) and MRPS35 (PTCD3). A second assembly cluster contains MRPS7 (uS7m), MRPS9 (uS9m), MRPS29 (mS29), MRPS31 (mS31), MRPS35 (mS35) and MRPS39 (mS39). A third cluster of SSU early binding proteins consists of MRPS2 (uS2m), MRPS23 (mS23) and MRPS28 (bS1m). The remaining proteins involved in the early assembly of the mtSSU are MRPS11 (uS11m), MRPS12 (uS12m) and MRPS17 (uS17m) appear to incorporate in an independent manner, with recent studies suggesting that YBEY may incorporate MRPS11 (uS11m) to the nascent mtSSU [335]. The early assembly of mitoribosomal proteins seems to be facilitated by the GTPase NOA1/C4orf14, which was shown to interact with several early assembled mtSSU proteins and the mitochondrial nucleoid [338]. In addition to that, METTL15 was shown to methylate the 12S rRNA at position C839 and be required for the biogenesis of mtSSU with its absence affecting both early assembled proteins MRPS12 (uS12m), MRPS17 (uS17m) and late assembled protein MRPS15 (uS15m), as well as MRPS38 (mS38) [339] which has not yet been assigned an incorporation class [331].

For the later incorporated mtSSU proteins, two assembly clusters seem to be present. One cluster consists of MRPS10 (uS10m), MRPS14 (uS14m), MRPS24 (uS3m) and MRPS33 (mS33) located at the top of the 12S rRNA, and another assembly cluster comprised of MRPS15 (uS15m), MRPS25 (mS25) and MRPS26 (mS26) locating at the bottom of the 12S rRNA with the remaining proteins incorporating in an independent manner [331].

To date, mutations in eleven components and in one assembly factor of the mtSSU have been identified, causing a range of phenotypes, onset and variable survival (Table 16). Interestingly, majority of the disease genes participate in the early stages of the mtSSU assembly (Figure 7) and patients tend to have a combined OXPHOS deficiency depending on tissue type and mutation [340–345]. Several cases of mutations in...
MT-RNR1 (12S rRNA) have been reported to cause hearing loss induced by aminoglycoside exposure (Table 16) with one family also presenting cardiomyopathy [346]. While mutations in ERAL1, the RNA chaperone shown to stabilise 12S rRNA [333,334], have also been shown to cause sensorineural deafness, it has not been linked to aminoglycoside exposure as have mutations in MT-RNR1 [347].

Both MRPS2 and MRPS7 encode proteins assembled early in the mtSSU (uS2m and uS7m, respectively) and mutations in these genes have been reported to cause sensorineural hearing impairment and hypoglycaemia but with survival into adulthood [344,345,348] (Table 16). On the other hand, mutations in MRPS16 (b16m) and MRPS22 (mS22) commonly lead to clinical presentations of fatal lactic acidosis and tubulopathy, with MRPS22 mutations also leading to hypertrophic cardiomyopathy, Leigh-like brain lesions and delayed sexual development [342,349–351]. Interestingly, a homozygous missense mutation in MRPS23 leads to hypoglycaemia and hepatic disease [66], sharing partial phenotypic similarities with other genes encoding early assembled proteins, MRPS2, MRPS7, MRPS16 and MRPS22 (Table 16) (Figure 7). Comparably, mutations in MRPS28 (b1m) were shown to present before birth as intrauterine growth retardation (IUGR) and progress with multisystemic involvement including sensorineural deafness, brain abnormalities, hyperlactatemia and failure to thrive with survival into adulthood [352,353]. The last two genes linked to mitochondrial disease participating in the early assembly of the mtSSU are MRPS34 and PTD3. Mutations in both genes have been shown to cause Leigh or Leigh-like syndrome with variable presentations of hyperlactatemia, optic atrophy, hearing loss, microcephaly and variable OXPHOS defects and survival depending on tissue type and mutation [341,354] (Table 16).

While the majority of mutations in the mtSSU lead to decreased protein levels (Table 16), a missense homozygous mutation was identified in MRPS14 (uS14m) leading to increased levels of corresponding protein, and clinical presentation of lactic acidosis, hypertrophic cardiomyopathy and hypotonia [340]. The unaffected assembly of the mitoribosome suggests that the incorporation of the mutant protein causes impaired mitochondrial translation which was predicted to disrupt the mitoribosome mRNA channel [340]. The other late assembled mtSSU protein linked to disease, MRPS25 (mS25), assembles in a different cluster to MRPS14 (uS14m) [331] and has been linked to a different phenotype including encephalopathy, short stature, muscle fatigue, dystonia, mild elevation of plasma lactate [343].

### Assembly of the mtLSU

The assembly of the mtLSU seems to present an early, an intermediate and a late class of incorporation of proteins (Figure 7) with several subclusters of proteins present at each stage [331]. Assembly factors involved in the biogenesis of the mtLSU have been identified mostly at late stages. MALSU1 is a member of the DUF143 family of proteins of conserved ribosomal silencing factors and is required for mtLSU biogenesis and translation [355–357] (Figure 7). Recently, the structure of an mtLSU intermediate containing MALSU1 was solved [358] revealing the involvement of two other proteins, NDUFAB1, a subunit of complex I with an essential secondary role in mitochondrial function (discussed above) [22], and L0R8F8, the product of a bicistronic transcript that also encodes MID51, a mitochondrial protein involved in morphology [85]. While the function of these new assembly factors is not yet known, they have been suggested to act as caps to prevent premature association of the nascent mtLSU with the mtSSU [358]. At least five other assembly factors have been characterised to be involved in late mtLSU assembly and the formation of the 55S monosome. Three of these are quality control GTPases proposed to prevent premature monosome formation: GTPBP5 (MTG2) [359], GTPBP7/MTG1 [360] and GTPBP10 [361,362], and two others form a heterodimeric complex comprised of NSUN4 and MTERF4 assembly factors which facilitates the monosome formation and enables mtDNA translation [337,363,364].

Once the 55S monosome is assembled, the tRNAval is nestled between two groups of proteins: the early assembled MRPL40 (mL40), MRPL46 (mL46) and MRPL48 (mL48) proteins in one side and the intermediate assembled proteins MRPL18 (mL18), MRPL38 (mL38) and MRPL27 (bL27m) on the other side [4,331]. Although several components required for the assembly of the human mitoribosome have been identified over the years, the lack of assembly factors characterised for the different incorporation clusters and independently incorporated proteins for both mtSSU and mtLSU might suggest that some assembly factors are yet to be discovered.

Even though the mtLSU contains more structural proteins than the mtSSU, there are only four nuclear disease genes associated with the mtLSU, as well as 2 mtDNA-encoded rRNAs, with majority of these participating in the early assembly steps (Figure 7). The majority of mutations associated with defects in the mtLSU are missense mutations leading to a variable phenotype and OXPHOS defect, depending on mutation and
Table 16. Defects in the assembly of the mtSSU

| Gene (RNA/protein name) | A. | S. F. | Types of genetic variants and protein impact | Clinical presentations and relevant information | Ref. | OMIM |
|--------------------------|----|-------|---------------------------------------------|-----------------------------------------------|------|-------|
| MT-RNR1 (12S rRNA)       | X  |       | Transition, transversion and deletion/insertion. | Aminoglycoside-induced hearing loss, incomplete penetrance reported, may include cardiomyopathy. | [346,434,435], and reviewed in [436] | 561000 |
| ERAL1                    | X  |       | Missense leading to decreased protein levels. | Perrault syndrome expressed as sensorineural hearing loss and ovarian dysgenesis with survival into late adulthood. | [347] | 607435 |
| MRPS2 (uS2m)             | X  |       | Missense leading to decreased protein levels. | Sensorineural hearing impairment, mild developmental delay and hypoglycaemia. Variable OXPHOS deficiency depending on the tissue type. Survival into childhood | [344] | 611971 |
| MRPS7 (uS7m)             | X  |       | Missense leading to decreased protein levels. | Congenital sensorineural hearing impairment, lactic acidosis, hypoglycaemia. Variable OXPHOS deficiency depending on the tissue type. Survival into childhood | [345,348] | 611974 |
| MRPS14 (uS14m)           | X  |       | Missense leading to increased protein levels. | Lactic acidosis, hypertrophic cardiomyopathy, hypotonia with survival into childhood. Unaffected mitoribosome assembly but decreased assembly of CI, CIII, CIV and CV suggesting incorporation of the mutant protein causes impaired mitochondrial translation. | [340] | 611978 |
| MRPS16 (bS16m)           | X  |       | Nonsense leading to unclear protein impact but decreased mitochondrial translation. | Fatal lactic acidosis, tubulopathy, hypotonia. Variable OXPHOS deficiency depending on the tissue type. | [342] | 609204 |
| MRPS22 (mS22)            | X  |       | Missense or duplication causing frameshift leading to unclear or decreased protein levels. | Variable phenotypes including fatal lactic acidosis, hypertrophic cardiomyopathy, tubulopathy, dysmorphic features, hypotonia, Leigh-like lesions, hypergonadotropic hypogonadism. Variable OXPHOS deficiency depending on the tissue type. | [349–351] | 605810 |
| MRPS23 (mS23)            | X  |       | Missense leading to unclear protein impact. | Hepatic disease and hypoglycaemia. Combined CI and CIV deficiencies in fibroblasts. | [66] | 611985 |
| MRPS25 (mS25)            | X  |       | Missense leading to decreased protein levels. | Multiple presentations including encephalopathy, short stature, muscle fatigue, dystonia, mild elevation of plasma lactate with survival into adulthood. | [343] | 611987 |
| MRPS28 (bS1m)            | X  |       | Missense and deletion causing frameshift and early termination leading to decreased protein levels. | IUGR, cerebellar atrophy, microcephaly, hyperlactatemia, developmental delay, sensorineural deafness, failure to thrive with survival into adulthood. QIV deficiency in muscle, fibroblasts and liver. | [352,353] | 611990 |
| MRPS34 (mS34)            | X  |       | Intronic, nonsense, missense leading to decreased protein levels. | Leigh or Leigh-like syndrome, mild or hyperlactatemia, may present microcephaly, optic atrophy. Variable OXPHOS defect and survival depending on tissue type and mutation. | [341] | 611994 |
| PTCDO3/ MRPS39 (mS39)    | X  |       | Intronic and insertion causing frameshift leading to decreased protein levels. | Severe Leigh syndrome, optic atrophy, hearing loss. Decreased CI and CIV in fibroblasts. | [354] | 614918 |

1Commonly used protein name; S., subunit; A.F., assembly factor; IUGR, intrauterine growth restriction.
Mutations identified in the 16SrRNA (MT-RNR2) have been reported to cause hypertrophic cardiomyopathy and myopathy with survival into adulthood [365,366], while mutations in the tRNAval (MT-TV) appear to cause a broader spectrum of phenotypes including muscle weakness, hearing and/or visual loss, bilateral cataracts or migraines and muscle weakness, MELAS, severe Leigh or Leigh-like syndrome, hypertrophic cardiomyopathy [367–372] (Table 17). It is not clear if mutations in tRNAval lead to combined defects in mitoribosome assembly and defective polypeptide elongation, although it should be noted that mitoribosomes are able to incorporate tRNAphe when the levels of tRNAval are severely decreased [373]. Despite this implying that the phenotypes are due to defective elongation, the adaptive switch in tRNA composition still leads to impaired mitochondrial translation [374] hinting at a combined defect. Severe phenotypes leading to early death have been associated with MRPL3 (uL3m) and MRPL12.

| Gene (RNA/protein name) | S. | A. F. | Types of genetic variants and protein impact | Clinical presentations and relevant information | Ref. | OMIM |
|-------------------------|----|------|---------------------------------------------|-----------------------------------------------|------|------|
| MT-RNR2 (16S rRNA)      | X  |      | Transition leading to unclear RNA impact.   | Myopathy, hypertrophic cardiomyopathy. Survival into adulthood reported. Decreased ATP production. | [365,366] | 561010 |
| MT-TV (tRNAval)         | X  |      | Transition leading to unclear or decreased tRNAval levels. | Variable phenotype and onset depending on mutation and mutant load with survival into adulthood reported. Predominantly presenting ataxia, hearing and/or visual loss, bilateral cataracts or migraines and muscle weakness, MELAS severe Leigh or Leigh-like syndrome, hypertrophic cardiomyopathy. | [367–372] | 590105 |
| MRPL3 (uL3m)            | X  |      | Missense or deletion leading to unclear or decreased protein levels. | Early onset severe cardiomyopathy, psychomotor retardation, failure to thrive, lactic acidosis. Variable OXPHOS defect depending on tissue type and mutation. | [437,438] | 607118 |
| MRPL12 (bL12m)          | X  |      | Missense leading to decreased protein levels. | Antenatal hypotrophy, tonic seizures, ataxia, hyperlactatemia, failure to thrive. Variable OXPHOS defect depending on tissue type and mutation. | [439] | 602375 |
| MRPL24 (uL24m)          | X  |      | Missense leading to decreased protein levels. | Cerebellar atrophy, choreoathetosis of limbs and face, Wolff–Parkinson–White syndrome, increased lactate in blood with survival into adolescence. Combined CI and CIV defect in muscle. | [377] | 611836 |
| MRPL44 (mL44)           | X  |      | Missense leading to unclear or decreased protein levels. | Predominantly hypertrophic cardiomyopathy, hepatopathy, but also muscle weakness, granular pigmentation of retina, metabolic acidosis. Survival into early adulthood reported. Variable OXPHOS defect depending on tissue type and mutation. | [378,379] | 611849 |

1 Commonly used protein name; S., subunit; A.F., assembly factor; MELAS, mitochondrial encephalopathy, lactic acidosis and stroke-like episodes.
(BL12m) suggesting these proteins play an essential role for mitochondrial translation. In fact, UL3m, encoded by MRPL3 displays extensive binding contact with the 16S rRNA and is one of the earliest assembled proteins, providing an anchor to MRPL39 (ML39) followed by MRPL45 (ML45) [331], the latter known to tether the mitoribosome at the IMM for translation [321]. Recent structural studies also revealed that MRPL12 (BL12m) plays an important role in interacting with mitochondrial elongation factor (EF-G1m) [375] and promoting tRNA translocation on the mitoribosome during translation [376]. On the other hand, mutations in MRPL24 (UL24m) and MRPL44 (ML44) have been associated with severe to mild phenotypes with survival into adolescence and early adulthood [377–379] (Table 17). MRPL24 is a late assembled protein that lacks extensive contact with early assembled proteins and is involved in the formation of the PET [331]. Despite its involvement with the PET, a teenager was recently reported harbouring a homozygous missense variant leading to cerebellar atrophy, choreoathetosis, increased lactate in blood and tachycardia (Wolff–Parkinson–White syndrome) [377]. Finally, four patients have been reported harbouring missense mutations in the gene encoding early assembled protein MRPL44 (ML44). The patients commonly presented with hypertrophic cardiomyopathy with majority presenting stabilisation of their phenotype over the years [378,379].

Conclusion

In this review, we have attempted to catalogue the breadth of genetic and clinical phenotypes associated with impaired assembly of mitochondrial OXPHOS complexes and the mitoribosome. Along with highlighting the intricacy of this system, we hope to have demonstrated the high heterogeneity in clinical presentations that challenges the diagnosis of new patients and the validation of novel disease genes linked to dysfunction in this critical process.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

Funding

We acknowledge funding from the National Health and Medical Research Council (NHMRC Fellowship 1140851 to DAS). DHH is supported by a Melbourne International Research Scholarship and the Mito Foundation PhD Top-up Scholarship.

Open Access

Open access for this article was enabled by the participation of The University of Melbourne in an all-inclusive Read & Publish pilot with Portland Press and the Biochemical Society under a transformative agreement with CAUL.

Acknowledgements

We apologise to those colleagues whose work we could not cite due to space restrictions. We thank Alison Compton, David Thorburn and all members of the Stroud lab for their input.

Abbreviations

COX, cytochrome c oxidase; IMM, inner mitochondrial membrane; LHON, Leber hereditary optic neuropathy; MCIA, mitochondrial complex I intermediate assembly; MRC, mitochondrial respiratory chain; mt, mitochondrial; nDNA, nuclear DNA; OXPHOS, oxidative phosphorylation; PTC, peptidyl transferase centre; VUS, variants of uncertain significance; WES, whole exome; WGS, whole genome.

References

1. Taanman, J.W. (1999) The mitochondrial genome: structure, transcription, translation and replication. Biochim. Biophys. Acta 1410, 103–123. https://doi.org/10.1016/S0005-2728(98)00161-3
2. Brown, A., Arnouts, A., Bai, X.C., Sugimoto, Y., Edwards, P.C., Murshudov, G. et al. (2014) Structure of the large ribosomal subunit from human mitochondria. Science 346, 718–722. https://doi.org/10.1126/science.1258026
3. Greber, B.J., Boehringer, D., Leibundgut, M., Bieri, P., Leitner, A., Schmitz, N. et al. (2014) The complete structure of the large subunit of the mammalian mitochondrial ribosome. Nature 515, 283–286. https://doi.org/10.1038/nature13895
4. Arnouts, A., Brown, A., Toots, J., Scheres, S.H.W. and Ribosome, R.V. (2015) The structure of the human mitochondrial ribosome. Science 348, 95–98. https://doi.org/10.1126/science.aaa11193

© 2020 The Author(s). This is an open access article published by Portland Press Limited on behalf of the Biochemical Society and distributed under the Creative Commons Attribution License 4.0 (CC BY-NC-ND).
334 Uchiumi, T., Ohgaki, K., Yagi, M., Aoki, Y., Sakai, A., Matsumoto, S. et al. (2010) ERAL1 is associated with mitochondrial ribosome and elimination of Bogenhagen, D.F., Ostermeyer-Fay, A.G., Haley, J.D. and Garcia-Diaz, M. (2018) Kinetics and mechanism of mammalian mitochondrial ribosome assembly. Cell Rep. 22, 1935–1944 https://doi.org/10.1016/j.celrep.2018.01.066

337 Schlunzen, F., Zarivach, R., Harms, J., Bashan, A., Tocilj, A., Albrecht, R. et al. (2001) Structural basis for the interaction of antibiotics with the peptidyl transferase centre in eubacteria. Nature 413, 841–842 https://doi.org/10.1038/35101544

339 Jia, L., Dienhart, M., Schramp, M., McCauley, M., Hell, K. and Stuart, R.A. (2003) The complete atomic structure of the large ribosomal subunit at 2.4 A resolution. Mol. Cell 114, 499–515 https://doi.org/10.1016/j.molcel.2003.07.011

341 Galmerti, C.A., Diogli, M., Sartori, I., Uggetti, C., Brega, A., Tartara, A. et al. (2006) Brain pseudotumoral and mental retardation on valproate and mitochondrial DNA mutation. Neurology 67, 1715–1717 https://doi.org/10.1212/01.wnl.0000242882.58086.3a

343 Voss, N.R., Gerstein, M., Steitz, T.A. and Moore, P.B. (2006) The geometry of the ribosomal polypeptide exit tunnel. Mol. Cell 33, 445–456 https://doi.org/10.1016/j.molcel.2006.05.037

346 Fragali, K., Chaussett, A., Serre, V., Acquaviva, C., Bannwarth, S., Rouiller, C. et al. (2019) A novel variant m.8561C>T in the overlapping region of MT-ATP6 and MT-ATP8 in a child with early-onset severe neurological disorders. Mol. Genet. Metab. Rep. 21, 100543 https://doi.org/10.1016/j.ymgmr.2019.100543

350 Barca, E., Ganetzky, R.D., Potturi, P., Juanola-Falgarona, M., Gai, X., Li, D. et al. (2018) USMGS ashkenazi Jewish founder mutation impairs mitochondrial complex V dimerization and ATP synthesis. Hum. Mol. Genet. 27, 3305–3312 https://doi.org/10.1093/hmg/ddy231

355 Arnold, I., Pfeffer, K., Neupert, W., Stuart, R.A. and Schagger, H. (1998) Yeast mitochondrial F1-ATP synthase exists as a dimer: identification of three dimer-specific subunits. EMBO J. 17, 7170–7178 https://doi.org/10.1093/emboj/17.24.7170

357 Hahn, A., Carey, K., Bublitz, M., Mills, D.J., Zickermann, V., Vonck, J. et al. (2016) Structure of a complete ATP synthesis dimer reveals the molecular basis of inner mitochondrial membrane morphology. Mol. Cell 63, 445–456 https://doi.org/10.1016/j.molcel.2016.05.037

360 Diodato, D., Ghezzi, D. and Tinti, V. (2014) The mitochondrial aminocyl tRNA synthetases: genes and syndromes. Int. J. Cell Biol. 2014, 787956 https://doi.org/10.1155/2014/787956

365 Engelmire, R., Pfeffer, S. and Forster, F. (2017) Structure of the human mitochondrial ribosome studied in situ by cryoelectron tomography. Structure 25, 1574–1581.e2 https://doi.org/10.1016/j.str.2017.07.011

369 O’Brien, T.W. (1971) The general occurrence of 55 S ribosomes in mammalian liver mitochondria. J. Biol. Chem. 246, 3409–3417 PMID: 4930061

372 Gaposetty, G. and Thangarajan, R. (2016) Mammalian mitochondrial ribosomal small subunit (MRPS) genes: a putative role in human disease. Gene 58, 1–37 https://doi.org/10.1016/j.gene.2016.05.008

375 Beringer, M. and Podhradsky, M.V. (2007) The ribosomal peptidyl transferase. Mol. Cell 26, 311–321 https://doi.org/10.1016/j.molcel.2007.03.015

378 Ban, N., Nissen, P., Hansen, J., Moore, P.B. and Steitz, T.A. (2006) The complete atomic structure of the large ribosomal subunit at 2.4 A resolution. Science 299, 905–920 https://doi.org/10.1126/science.299.5581.905

383 Voss, N.R., Gerstein, M., Steitz, T.A. and Moore, P.B. (2006) The geometry of the ribosomal polypeptide exit tunnel. J. Mol. Biol. 360, 893–906 https://doi.org/10.1016/j.jmb.2006.05.023

386 Schlunzen, F., Zarvach, R., Hassn, J., Bashan, A., Toclij, A., Albrecht, R. et al. (2001) Structural basis for the interaction of antibiotics with the peptidyl transferase centre in eubacteria. Nature 413, 814–821 https://doi.org/10.1038/35101544

390 Jia, L., Dienhart, M., Schramp, M., McCauley, M., Hell, K. and Stuart, R.A. (2003) Yeast Oxa1 interacts with mitochondrial ribosomes: the importance of the C-terminal region of Oxa1. EMBO J. 22, 6438–6447 https://doi.org/10.1093/emboj/cdg624

393 Sziraczik, G., Ott, M., Bonnefoy, N., Neupert, W. and Herrmann, J.M. (2003) Ribosome binding to the Oxa1 complex facilitates co-translational protein insertion in mitochondria. EMBO J. 22, 6446–6457 https://doi.org/10.1093/emboj/cdg623

396 Geber, B.J. and Ban, N. (2016) Structure and function of the mitochondrial ribosome. Annu. Rev. Biochem. 85, 103–132 https://doi.org/10.1146/annurev-biochem-060815-014343

399 Bogenhagen, D.F., Ostermeyer-Fay, A.G., Hale, J.D. and Garcia-Diaz, M. (2018) Kinetics and mechanism of mammalian mitochondrial ribosome assembly. Cell Rep. 22, 1935–1944 https://doi.org/10.1016/j.celrep.2018.01.066

402 Bogenhagen, D.F., Martin, D.W. and Koller, A. (2014) Initial steps in RNA processing and ribosome assembly occur at mitochondrial DNA nucleoids. Cell Metab. 19, 618–629 https://doi.org/10.1016/j.cmet.2014.03.013

405 Denerlestein, S., Rozanska, A., Wodrow, M., Chrzanska-Lightowers, Z.M. and Lightowers, R.N. (2010) Human ERAL1 is a mitochondrial RNA chaperone involved in the assembly of the 28S small mitochondrial ribosomal subunit. Biochem. J. 430, 551–558 https://doi.org/10.1042/BJ20100757

408 Uchiyama, T., Ohgaki, K., Yagi, M., Aoki, Y., Sakai, A., Matsumoto, S. et al. (2010) ERAL1 is associated with mitochondrial ribosome and elimination of ERAL1 leads to mitochondrial dysfunction and growth retardation. Nucleic Acids Res. 38, 5554–5568 https://doi.org/10.1093/nar/gkq305
Yakubovskaya, E., Guja, K.E., Mejia, E., Castano, S., Hambardzumia, E., Choi, W.S. et al. (2012) Structure of the essential MTERF4:NSUN4 protein complex reveals how an MTERF protein collaborates to facilitate rRNA modification. *Structure* **20**, 1940–1947. https://doi.org/10.1016/j.str.2012.08.027

Coulbault, L., Deslandes, B., Herliczewska, D., Read, M.H., Leporrier, N., Schaeffer, S. et al. (2007) A novel mutation 3090 G>A of the mitochondrial 16S ribosomal RNA associated with myopathy. *Biochem. Biophys. Res. Commun.* **362**, 601–605. https://doi.org/10.1016/j.bbrc.2007.08.040

Liu, Z., Song, Y., Li, D., He, X., Li, S., Wu, B. et al. (2014) The novel mitochondrial 16S rRNA 23361 > C mutation is associated with hypertrophic cardiomyopathy. *J. Med. Genet.* **51**, 176–184. https://doi.org/10.1136/jmedgenet-2013-101818

Chalmers, R.M., Lamont, P.J., Nelson, I., Ellison, D.W., Thomas, N.H., Harding, A.E. et al. (1997) A mitochondrial DNA rRNA(Val) point mutation associated with adult-onset Leigh syndrome. *Neurology* **49**, 589–592. https://doi.org/10.1212/WNL.49.2.589

Tiranti, V., D’Agruma, L., Pareyson, D., Mona, M., Carrara, F., Zelante, L. et al. (1998) A novel mutation in the mitochondrial rRNA(Val) gene associated with a complex neurological presentation. *Ann. Neurol.* **43**, 98–101. https://doi.org/10.1002/ana.410430116

Sacconi, S., Salviati, L., Gooch, C., Bonilla, E., Shanske, S. and DiMauro, S. (2002) Complex neurologic syndrome associated with the G1606A mutation of mitochondrial DNA. *Arch. Neurol.* **59**, 1013–1015. https://doi.org/10.1001/archneur.59.6.1013

Annedondo, J.J., Gallardo, M.E., Garcia-Pavia, P., Domingo, V., Breton, B., Garcia-Silva, M.T. et al. (2012) Mitochondrial tRNA valine as a recurrent target for mutations involved in mitochondrial cardiomyopathies. *Mitochondrion* **12**, 357–362. https://doi.org/10.1016/j.mito.2011.09.010

Fraidakis, M.J., Jardel, C., Allouche, S., Nelson, I., Aure, K., Slama, A. et al. (2014) Phenotypic diversity associated with the MT-TV gene m.1644G > A mutation. *Biophys. J.* **104**, 34–39. https://doi.org/10.1016/j.bpj.2013.04.010

Koripella, R.K., Sharma, M.R., Bhargava, K., Datta, P.P., Kaushal, P.S., Keshavan, P. et al. (2020) Structures of the human mitochondrial translation ribosome bound to EF-G1 reveal distinct features of mitochondrial translation elongation. Nat. Commun. **11**, 3830. https://doi.org/10.1038/s41467-020-17715-2

Sacconi, S., Salviati, L., Gooch, C., Bonilla, E., Shanske, S. and DiMauro, S. (2002) Complex neurologic syndrome associated with the G1606A mutation of mitochondrial DNA. *Arch. Neurol.* **59**, 1013–1015. https://doi.org/10.1001/archneur.59.6.1013
Simon, M.T., Eftekharian, S.S., Stover, A.E., Osborne, A.F., Braftman, B.H., Chang, R.C. et al. (2019) Novel mutations in the mitochondrial complex I assembly gene NDUF5 reveal heterogeneous phenotypes. Mol. Genet. Metab. 126, 53–63 https://doi.org/10.1016/j.ymgme.2018.11.001

Yatsuka, Y., Kishita, Y., Formosa, L.E., Shimura, M., Nozaki, F., Fuji, T. et al. (2020) A homozygous variant in NDUF5 is associated with developmental delay, microcephaly, and epilepsy due to mitochondrial complex I deficiency. Clin. Genet. 98, 155–165 https://doi.org/10.1111/cge.13773

Angelbaut, C., Charif, M., Guene, N., Piro-Megy, C., de Camaret B, M., Procaccio, V. et al. (2015) Mutation in NDUF13/GRIM19 leads to early onset hypotonia, dyskinesia and sensorial deficiencies, and mitochondrial complex I instability. Hum. Mol. Genet. 24, 3938–3955 https://doi.org/10.1093/hmg/ddv133

Gonzalez-Quintana, A., Garcia-Consuegra, I., Belanger-Quintana, A., Serrano-Lorenzo, P., Lucia, A., Blazquez, A. et al. (2020) Novel NDUFA13 mutations associated with isolated complex I deficiency due to a non-coding mutation in complex I assembly factor NDUF5. Hum. Mol. Genet. 29, 4062–4079 https://doi.org/10.1093/hmg/ddz245

Baide-Mairena, H., Gaudio, P., Marti-Sanchez, L., Emperador, S., Sanchez-Montanez, A., Alonso-Luengo, O. et al. (2019) Mutations in the mitochondrial complex I assembly factor NDUF5 cause isolated bilateral striatal necrosis and progressive dystonia in childhood. Mol. Genet. Metab. 126, 250–258 https://doi.org/10.1016/j.ymgme.2019.01.001

Pulkes, T., Loikitza, D., Willis, A.J., Hargreaves, I., Heales, S. and Hanna, M.G. (2005) Nonsense mutations in mitochondrial DNA associated with myalgia and exercise intolerance. Neurology 64, 1091–1092 https://doi.org/10.1212/01.WNL.000014477.33156.55

Hinttala, R., Smeets, R., Mollanen, J.S., Ugalde, C., Uusimaa, J., Miettinen, J.A. et al. (2006) Analysis of mitochondrial DNA sequences in patients with isolated or combined oxidative phosphorylation system deficiency. J. Med. Genet. 43, 881–886 https://doi.org/10.1136/jmg.2006.042168

Taylor, R.W., Singh-Kler, R., Hayes, C.M., Smith, P.E. and Turnbull, D.M. (2001) Progressive mitochondrial disease resulting from a novel missense mutation in the mitochondrial DNA ND3 gene. Ann. Neurol. 50, 104–107 https://doi.org/10.1002/ana.1084

McFarland, R., Kirby, D.M., Fowler, K.J., Ohtake, A., Ryan, M.T., Amor, D.J. et al. (2004) De novo mutations in the mitochondrial ND3 gene as a cause of infantile mitochondrial encephalopathy and complex I deficiency. Ann. Neurol. 55, 58–64 https://doi.org/10.1002/ana.10787

Crimi, M., Papadimitriou, A., Gallabri, S., Palamidou, P., Fortunato, F., Bordoni, A. et al. (2004) A new mitochondrial DNA mutation in ND3 gene causing severe Leigh syndrome with early lethality. Pediatr. Res. 55, 842–846 https://doi.org/10.1203/01.PDR.000017644.73466.68

Sarzi, E., Brown, M.D., Lebon, S., Chretien, D., Munnoch, A., Rotig, A. et al. (2007) A novel recurrent mitochondrial DNA mutation in ND3 gene is associated with isolated complex I deficiency causing Leigh syndrome and dystonia. Am. J. Med. Genet. A 143A, 33–41 https://doi.org/10.1002/ajmg.a.31565

Wang, K., Takahashi, Y., Gao, Z.L., Wang, X.J., Chen, X.W., Goto, J. et al. (2009) Mitochondrial ND3 as the novel causative gene for Leber hereditary optic neuropathy and optic neuropathy. Neurogenetics 10, 337–345 https://doi.org/10.1007/s10408-009-0194-0

Vodopivec, I., Cho, T.A., Rizzo, III, J.F., Fosch, M.P. and Sims, K.B. (2016) Mitochondrial encephalopathy and optic neuropathy due to mt10158 MT-ND3 complex I mutation presenting in an adult patient: case report and review of the literature. Neurologist 21, 61–65 https://doi.org/10.1097/NRL.0000000000000084

Brown, M.D., Torroni, A., Reckord, C.L. and Wallace, D.C. (1995) Phylogenetic analysis of Leber’s hereditary optic neuropathy mitochondrial DNA’s indicates multiple independent occurrences of the mitochondrial disease. Hum. Mutat. 6, 311–325 https://doi.org/10.1002/humu.1380060405

Brown, M.D., Starkovskaya, E., Derbeneva, O., Hosseini, S., Allen, J.C., Mikhailovskaya, I.E. et al. (2002) The role of mtDNA background in disease expression: a new primary LHON mutation associated with Western Eurasian haplogroup. J. Hum. Genet. 47, 130–138 https://doi.org/10.1007/s00439-001-0660-8

Johns, D.R., Neufeld, M.J. and Park, R.D. (1992) An ND-6 mitochondrial DNA mutation associated with Leber hereditary optic neuropathy. Biochem. Biophys. Res. Commun. 187, 1551–1557 https://doi.org/10.1016/0006-291X(92)90479-5

Brown, M.D. and Wallace, D.C. (1994) A mitochondrial DNA mutation at nucleotide pair 14459 of the NADH dehydrogenase subunit 6 gene associated with maternally inherited Leber hereditary optic neuropathy and dystonia. Proc. Natl. Acad. Sci. U.S.A. 91, 6206–6210 https://doi.org/10.1073/pnas.91.13.6206

Raun, K., Wibrand, F., Hansen, F.J., Horn, N., Rosenberg, T. and Schwartz, M. (2001) An mtDNA mutation, 14453G→A, in the NADH dehydrogenase subunit 6 gene associated with mitochondrial EIMAS syndrome. J. Hum. Genet. 46, 805–809 https://doi.org/10.1007/s10045-001-0660-8

Funaolat, B., Reynin, P., Vighetto, A., Ranoux, D., Bonneteuf, J.P., Godinot, C. et al. (2002) Leigh-like encephalopathy complicating Leber’s hereditary optic neuropathy. Ann. Neurol. 52, 374–377 https://doi.org/10.1002/ana.10299

Ugalde, C., Tripets, R.H., Coenen, M.J., van den Heuvel, L.P., Smeets, R., Uusimaa, J., et al. (2003) Impaired complex I assembly in a Leigh syndrome patient with a novel missense mutation in the ND6 gene. Biophys. Res. Commun. 303, 1091–1096 https://doi.org/10.1016/S0006-291X(02)02887-0

Haack, T.B., Danhauser, K., Haberberger, B., Hoser, J., Strecker, V., Boehm, D. et al. (2010) Exome sequencing identifies ACAD9 mutations as a cause of complex I deficiency. Nat. Genet. 42, 1131–1134 https://doi.org/10.1038/ng.706

Dewulf, J.P., Barrea, C., Vincent, M.F., De Laet, C., Van Coster, R., Seneca, S. et al. (2016) Evidence of a wide spectrum of cardiac involvement due to complex I deficiencies, and isolated complex I muscle deficiency. J. Hum. Genet. 99, 217–227 https://doi.org/10.1038/jhmg.2016.05.021

Sanchez-Caballero, L., Puzzenente, B., Bianchi, L., Assouline, Z., Barcia, G., Metodiev, M.D. et al. (2016) Mutations in complex I assembly factor TMEM126B result in muscle weakness and isolated complex I muscle deficiency. Am. J. Hum. Genet. 99, 208–216 https://doi.org/10.1016/j.ajhg.2016.05.022

Wallace, D.C., Singh, G., Lott, M.T., Hodge, J.A., Schurr, T.G., Lezza, A.M. et al. (1988) Mitochondrial DNA mutation associated with Leber’s hereditary optic neuropathy. Science 242, 1427–1430 https://doi.org/10.1126/science.3201231

Ishikawa, Y. and Nakagawa, M. (1991) Clinical correlation of mitochondrial DNA heteroplasmy and Leber’s hereditary optic neuropathy. Jpn. J. Ophthalmol. 35, 259–267 PMID: 1700665

Dechauer, M., Bambeng, C., Claus, D., Zierer, S., Turnbull, D.M. and Taylor, R.W. (2003) Late-onset encephalopathy associated with a C1177A mutation of mitochondrial DNA. Neurology 60, 1357–1359 https://doi.org/10.1212/01.WNL.0000058699.9975.4B

4131
421 Komaki, H., Akanuma, J., Iwata, H., Takahashi, T., Mashima, Y., Nonaka, I. et al. (2003) A novel mtDNA C11777A mutation in Leigh syndrome. *Mitochondrion* **2**, 293–304 https://doi.org/10.1016/S1567-7249(03)00003-5

422 Apataek, D., Račik, B., Brunel-Gallton, C., Henderson, G., Bai, R., Sargent, M.A. et al. (2019) Congenital lactic acidosis, cerebral cysts and pulmonary hypertension in an infant with FOXRED1 related complex I deficiency. *Mol. Genet. Metab. Rep.* **18**, 32–38 https://doi.org/10.1016/j.ymgenr.2018.12.006

423 Pulkes, T., Erson, L., Patterson, V., Siddiqui, A., Wood, N.W., Nelson, I.P. et al. (1999) The mitochondrial DNA G13513A transition in NDIS is associated with a LHON/MELAS overlap syndrome and may be a frequent cause of MELAS. *Ann. Neurol.* **46**, 916–919 https://doi.org/10.1002/1531-8249(199912)46:6<916::AID-ANA16>3.0.CO;2-R

424 Taylor, R.W., Morris, A.A., Hutchinson, M. and Turnbull, D.M. (2002) Leigh disease associated with a novel mitochondrial DNA NDS mutation. *Eur. J. Hum. Genet.* **10**, 141–144 https://doi.org/10.1038/sj.ejhd.5200773

425 Liolitsa, D., Rahman, S., Benton, S., Carr, L.J. and Hanna, M.G. (2003) Is the mitochondrial complex I ND5 gene a hot-spot for MELAS causing mutations? *Ann. Neurol.* **53**, 128–133 https://doi.org/10.1002/ana.10435

426 Abdulhag, U.N., Soiferman, D., Schueler-Furman, O., Miller, C., Shaag, A., Elpeleg, O. et al. (2015) Mitochondrial complex IV deficiency. *Mol. Genet. Metab. Rep.* **3**, 1531-8249(199912)46:6<916::AID-ANA16>3.0.CO;2-R

430 Massa, V., Fernandez-Vizarra, E., Alshahwan, S., Bakhsh, E., Goffrini, P., Ferrero, I. et al. (2008) Severe infantile encephalomyopathy caused by a mutation in COX6B1, a nucleus-encoded subunit of cytochrome c oxidase. *Am. J. Hum. Genet.* **82**, 1281–1289 https://doi.org/10.1093/ajhg.ago008.2009.05.002

431 Higuchi, Y., Okunushi, R., Hara, T., Hashiguchi, A., Yuan, J., Yoshimura, A. et al. (2018) Mutations in COX6B1, a nucleus-encoded subunit of cytochrome c oxidase, cause a mitochondrial syndrome with ataxia, peripheral neuropathy, diabetes mellitus, and hypergonadotropic hypogonadism. *J. Neurol.* **263**, 159–164 https://doi.org/10.1007/s00415-018-8429-2

432 Komaki, H., Akanuma, J., Iwata, H., Takahashi, T., Mashima, Y., Nonaka, I. et al. (2003) A novel mtDNA C11777A mutation in Leigh syndrome. *Mitochondrion* **2**, 293–304 https://doi.org/10.1016/S1567-7249(03)00003-5

433 Kytovuori, L., Lipponen, J., Rusanen, H., Komulainen, T., Martikainen, M.H. and Majamaa, K. (2016) A novel mutation m.8561C > G in MT-ATP6/8 causing a mitochondrial syndrome with ataxia, peripheral neuropathy, diabetes mellitus and histiocytoid cardiomyopathy. *Brain* **139**, 1831–1836 https://doi.org/10.1093/brain/awv423

434 Catteruccia, M., Verrigni, D., Martinelli, D., Torraco, A., Agovino, T., Bonafe, L. et al. (2014) Persistent pulmonary arterial hypertension in the newborn (PPHN): a frequent manifestation of TMEM70 defective patients. *Mol. Genet. Metab. Rep.* **3**, 141–148 https://doi.org/10.1016/j.ymgenr.2014.01.001

435 Bacino, C., Prezant, T.R., Bu, X., Fournier, P. and Fischel-Ghodsian, N. (1995) Susceptibility mutations in the mitochondrial small ribosomal RNA gene in mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes (MELAS). *Brain* **118**, 135–148 https://doi.org/10.1093/brain/118.1.135

436 Gao, Z., Chen, Y. and Guan, M.X. (2017) Mitochondrial DNA mutations associated with aminoglycoside induced deafness. *J. Otol.* **12**, 1–8 https://doi.org/10.1016/j.joto.2017.02.001

437 Galmiche, L., Serre, V., Beinat, M., Assouline, Z., Lebre, A.S., Chretien, D. et al. (2011) Exome sequencing identifies MRPL3 mutation in mitochondrial cardiomyopathy. *Hum. Mutat.* **32**, 1225–1231 https://doi.org/10.1002/humu.21562

438 Bursle, C., Narendra, A., Chuk, R., Cardinal, J., Justo, R., Lewis, B. et al. (2017) COXPD9 an evolving multisystem disease; congenital lactic acidosis, sensorineural hearing loss, hypertrophic cardiomyopathy, cirrhosis and interstitial nephritis. *JIMD Rep.* **34**, 105–109 https://doi.org/10.1007/8894_2016_13

439 Serre, V., Rozanska, A., Beinat, M., Chretien, D., Boddart, N., Munnich, A. et al. (2013) Mutations in mitochondrial ribosomal protein MRPL12 leads to growth retardation, neurological deterioration and mitochondrial translation deficiency. *Biochim. Biophys. Acta* **1832**, 1304–1312 https://doi.org/10.1016/j.bbainsd.2013.04.014
Author/s:  
Hock, DH; Robinson, DRL; Stroud, DA

Title:  
Blackout in the powerhouse: clinical phenotypes associated with defects in the assembly of OXPHOS complexes and the mitoribosome

Date:  
2020-11-01

Citation:  
Hock, D. H., Robinson, D. R. L. & Stroud, D. A. (2020). Blackout in the powerhouse: clinical phenotypes associated with defects in the assembly of OXPHOS complexes and the mitoribosome. BIOCHEMICAL JOURNAL, 477 (21), pp.4085-4132. https://doi.org/10.1042/BCJ20190767.

Persistent Link:  
http://hdl.handle.net/11343/252983

File Description:  
Published version

License:  
CC BY-NC-ND