University Library

MINERVA ACCESS

A gateway to Melbourne's research publications

Minerva Access is the Institutional Repository of The University of Melbourne

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:
2020-11-05

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:
https://hdl.handle.net/11343/252983

License:
CC BY-NC-ND

Biochemical Journal (2020) **477** 4085–4132
https://doi.org/10.1042/BCJ20190767

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 F_1–F_0 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 ($tRNA^{val}$) 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 F_1F_0-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]. The

Received: 1 May 2020
Revised: 29 September 2020
Accepted: 5 October 2020

Version of Record published:
5 November 2020

© 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).

Biochemical Journal (2020) **477** 4085–4132
https://doi.org/10.1042/BCJ20190767

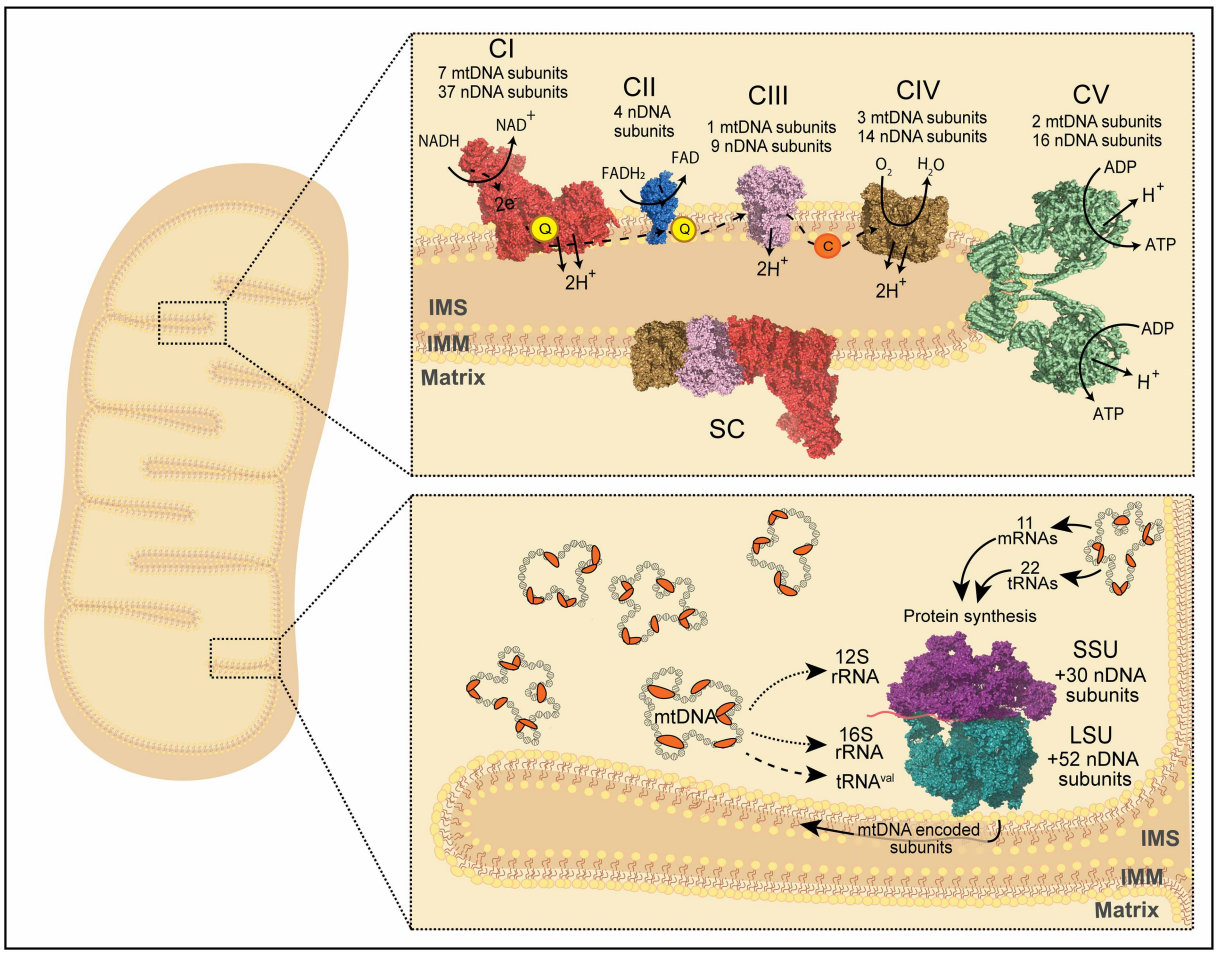


Figure 1. The oxidative phosphorylation (OXPHOS) system and the mitoribosome.

Upper inset, the OXPHOS system consists of complexes I, II, III and IV of the respiratory chain and complex V (the F_1F_o-ATP Synthase). Complexes I, III and IV also exist in large assemblies known as supercomplexes (SC). Subunit compositions of individual complexes are indicated. mtDNA, mitochondrial DNA; nDNA, nuclear DNA; Q, Coenzyme Q; C, Cytochrome c. *Lower inset*, the mitoribosome synthesises the 13 mtDNA-encoded subunits that are present within the OXPHOS complexes. It consists of two major parts, the small subunit (SSU) and large subunit (LSU). The SSU contains one mtDNA-encoded rRNA (12S rRNA) and 30 nDNA encoded proteins. The LSU contains one mtDNA-encoded rRNA (16S rRNA), 52 nDNA encoded proteins and a single mtDNA-encoded $tRNA^{val}$ molecule that has a structural role in mitoribosome. All 22 mtDNA-encoded tRNAs, including $tRNA^{val}$, are involved in the synthesis of the 13 mtDNA-encoded proteins from 11 mRNAs. Two mRNAs, MT-ND4/MT-ND4L and MT-ATP6/MT-ATP8 share open reading frames and the proteins are transcribed as bicistronic elements. Orange elements in mtDNA represent mtDNA associated protein TFAM. IMS, intermembrane space; IMM, inner mitochondrial membrane; Matrix, mitochondrial matrix. Models for complexes I, II, III, IV, V and mitoribosome generated from PDB: 5LDW, 1ZOY, 1BGY, 5B1A, 5ARE and 3J9M, respectively.

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_1F_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

© 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).

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

© 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).

Biochemical Journal (2020) **477** 4085–4132
https://doi.org/10.1042/BCJ20190767

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**, **NDUFV1**, **NDUFV2**. 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

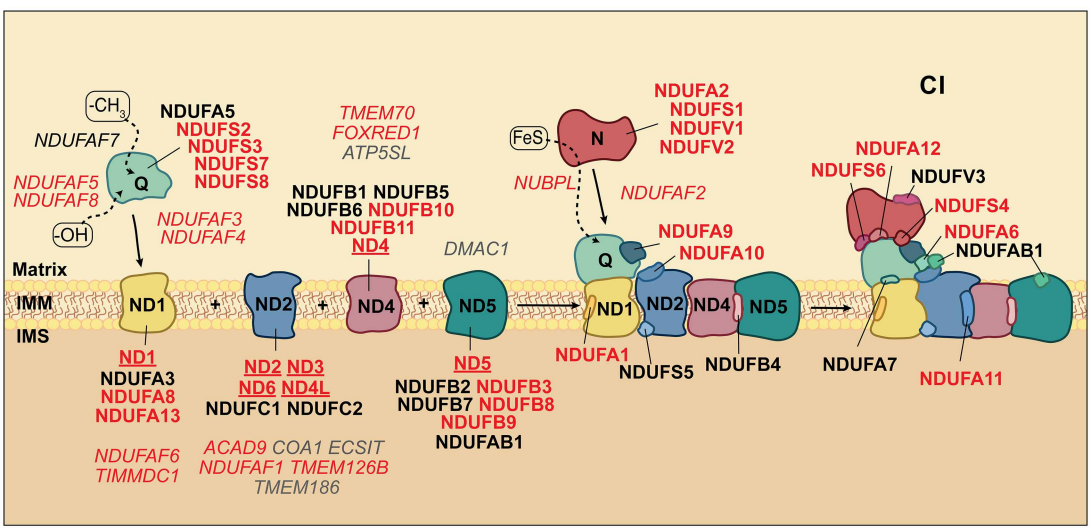


Figure 2. Schematic depicting the complex I assembly pathway showing known mitochondrial disease genes in red, mtDNA-encoded subunits underlined and assembly factors in italics.

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.

© 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).

Biochemical Journal (2020) **477** 4085–4132
https://doi.org/10.1042/BCJ20190767

Table 1. Defects affecting biogenesis of the Q and ND1 modules Part 1 of 2

Gene	S.	A. F.	Types of genetic variants and protein impact	Clinical presentations and relevant information	Ref.	OMIM
Q-module						
NDUFS2	X		Various missense leading to uncertain impact or near absence of protein.	Leigh syndrome, optic atrophy, hypertrophic cardiomyopathy, elevated blood lactate.	[380,381]	602985
NDUFS3	X		Missense leading to uncertain protein impact.	Leigh syndrome, optic atrophy.	[382]	603846
NDUFS7	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
NDUFS8	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, hypertonia, 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
NDUFAF6		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

© 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).

Biochemical Journal (2020) 477 4085–4132
https://doi.org/10.1042/BCJ20190767

Table 1. Defects affecting biogenesis of the Q and ND1 modules Part 2 of 2

Gene	S.	A. 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 **NDUFS3** and **NDUFS3** 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 NDUFAF6 is also required for ND1 biogenesis. While many missense variants in the *NDUFAF6* 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 **NDUFA3**, **NDUFA8** and **NDUFA13** [32] to produce the final Q–ND1 intermediate. Both ***NDUFA8*** and ***NDUFA13*** 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 ***NDUFA8*** and ***NDUFA13*** 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 **NDUFA1** is a subunit of the ND1 module, it is not added to the complex I until late during assembly [32]. Many missense variants in ***NDUFA1***, 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**, **NDUFC1** and **NDUFC2** associate with assembly factors ACAD9, ECSIT, NDUFAF1 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 biscistronic 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

© 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 2. Defects affecting biogenesis of the ND2 module

Gene	S.	A. F.	Types of genetic variants and protein impact	Clinical presentations and relevant information	Ref.	OMIM
MT-ND2	X		Predominantly missense but also nonsense and deletion leading to frameshift and protein impact dependant on variant and mutant mtDNA load.	Variable onset and presentation from Leigh syndrome, LHON, mild exercise intolerance, myalgia with survival into late adulthood reported.	[171,399,400]	516001
MT-ND3	X		Missense and protein impact dependant on variant and mutant mtDNA load.	Variable onset and presentation including severe Leigh syndrome, encephalopathy, lactic acidosis, LHON, hypotonia, ataxia, seizures, dystonia with survival into adulthood reported.	[401–406]	516002
MT-ND4L	X		Missense leading to unclear protein impact.	LHON. Decreased CI activity.	[407,408]	516004
MT-ND6	X		Missense and protein impact dependant on variant and mutant mtDNA load.	Most common presentation of LHON and dystonia with variable onset, but also MELAS with survival into childhood and severe Leigh syndrome or adult onset Leigh-like syndrome.	[409–413]	516006
NDUFA9	X		Missense leading to decreased protein levels.	Variable phenotype of childhood-onset of progressive dystonia developing neuropathy and Leigh syndrome without acidosis in adulthood or severe respiratory and metabolic acidosis, retinitis pigmentosa and early death.	[64,69]	603834
NDUFA10	X		Predominantly missense but also insertion leading to decreased protein levels.	Leigh syndrome combined with hypertrophic cardiomyopathy, hypotonia.	[65,66]	603835
NDUFA11	X		Missense and intronic with unclear protein impact.	Variable presentation from mild late-onset myopathy to fatal infantile lactic acidosis, encephalocardiomyopathy, hypotonia, bilateral optic atrophy	[67,68]	612638
NDUFAF1		X	Missense leading to decreased or unclear protein levels.	Hypertrophic cardiomyopathy, failure to thrive, developmental delay, lactic acidosis, hypotonia, leukodystrophy.	[55–57]	606934
ACAD9		X	Predominantly missense but also duplication leading to frameshift with unclear protein impact.	Variable phenotype from mild growth retardation, exercise intolerance, cardiac hypertrophy surviving to adulthood to severe hypertrophic cardiomyopathy, encephalopathy and lactic acidosis.	[414,415]	611103
TMEM126B		X	Missense but also nonsense (frameshift) leading to unclear protein impact.	Most common presentation of myalgia and exercise intolerance with survival to adulthood but also hypertrophic cardiomyopathy, renal tubular acidosis and severe muscle weakness. Decreased CI assembly.	[416,417]	615533

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

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

© 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).

Biochemical Journal (2020) 477 4085–4132
https://doi.org/10.1042/BCJ20190767

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 biscistronic 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 CX_nC 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-

© 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).

Biochemical Journal (2020) **477** 4085–4132
https://doi.org/10.1042/BCJ20190767

Table 3. Defects affecting biogenesis of the ND4 and ND5 modules Part 1 of 2

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

© 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).

Biochemical Journal (2020) 477 4085–4132
https://doi.org/10.1042/BCJ20190767

Table 3. Defects affecting biogenesis of the ND4 and ND5 modules Part 2 of 2

Gene	S.	A. F.	Types of genetic variants and protein impact	Clinical presentations and relevant information	Ref.	OMIM
			leading to decreased protein levels	respiratory failure, cardiac hypertrophy, hypotonia, failure to thrive		
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.

throughput sequencing of a complex I deficient cohort [77] and since then many patients have been characterised [77–79]. The phenotype is variable, with both severe (Leigh syndrome, cardiomyopathy) and mild cases having been identified, presumably dependent on the severity of the particular variant(s). Interestingly, FOXRED is an FAD-dependent oxidoreductase, and although it is not clear if FAD binding is important in its function, Rendon and co-workers [79] identified a patient with relatively mild symptoms (epilepsy and severe psychomotor retardation) that suffered a combined complexes I and II defect, suggesting FOXRED1 may be involved in the biogenesis of multiple flavoprotein containing complexes. TMEM70 is an assembly factor implicated in the assembly of both complexes I and V, with functional studies showing that its loss leads to accumulation 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 **NDUFAB1**. **NDUFAB1** 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 **NDUFAB1** in the gene-edited HEK293T model system leads to cell death (whereas 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 **NDUFAB1** 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 the presence of 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 **NDUFV1** and **NDUFV2** 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

© 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 4. Defects affecting biogenesis of the N-module

Gene	S.	A. 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		Intronic, missense and deletion leading to uncertain or decreased protein level.	Severe presentation of fatal lactic acidosis, Leigh syndrome.	[112–114]	603848
NDUFV1	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
NDUFV2	X		Intronic deletion and insertion causing protein frameshift leading to decreased protein levels.	Hypertrophic cardiomyopathy and encephalopathy, Leigh syndrome, hypotonia.	[100–102]	600532
NDUFAF2		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 factor NUBPL has a CXXC motif that binds iron–sulfur clusters and is thought to aid their delivery to **NDUFS1** and **NDUFV1**. 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 **NDUFV1** remains partially functional.

The subunits **NDUFA6**, **NDUFA7**, **NDUFA11**, **NDUFA12**, **NDUFS4**, **NDUFS6**, **NDUFV3** and the other copy of **NDUFAB1**, associated with LYR motif containing **NDUFA6**, seems to be incorporated at later

© 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).

Biochemical Journal (2020) 477 4085–4132
https://doi.org/10.1042/BCJ20190767

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 ***NDUFA6*** [106], ***NDUFA12*** [107], ***NDUFS4*** [108–111], and ***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 ***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 *NDUFAF2* contain this partially built assembly as a stable intermediate [22,105]. NDUFAF2 is thought to have evolved through duplication of the **NDUFA12** gene [116], and the structure of the intermediate isolated from a fungal **NDUFS6** knockout model (lacking the N-module) shows NDUFAF2 occupying the site usually taken by **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, **NDUFA12**, **NDUFS4** and **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 ***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). ***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 ***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 ***SDHB*** and ***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 **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 ***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

© 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 5. Defects affecting biogenesis of complex II

Gene	S.	A. F.	Types of genetic variants and protein impact	Clinical presentations and relevant information	Ref.	OMIM
SDHA	X		Predominantly missense but also intronic and nonsense leading to unclear or decreased protein levels.	Majority presenting Leigh syndrome and may also present leukodystrophy, dystonia, ataxia, optic atrophy, lactic acidosis, cardiomyopathy.	[121–129]	600857
SDHB[1]	X		Single known homozygous missense variant with possible reduced penetrance but leading to decreased protein levels.	Leukoencephalopathy, hypotonia or virtually asymptomatic, minor brain lesions.	[127,130]	185470
SDHD	X		Predominantly missense but also disruption of stop codon and protein extension leading unclear or decreased protein levels.	Fatal hypertrophic cardiomyopathy or encephalomyopathy, developmental delay and lactic acidosis. Symptoms developed after viral infection.	[131,132]	602690
SDHAF1		X	Missense with unclear protein impact.	Infantile leukodystrophy, spastic quadriparesis, lactate and succinate accumulation in the brain. Decreased CII activity/ assembly.	[143]	612848

[1]Possible VUS, see text for detail. S., subunit; A.F., assembly factor.

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

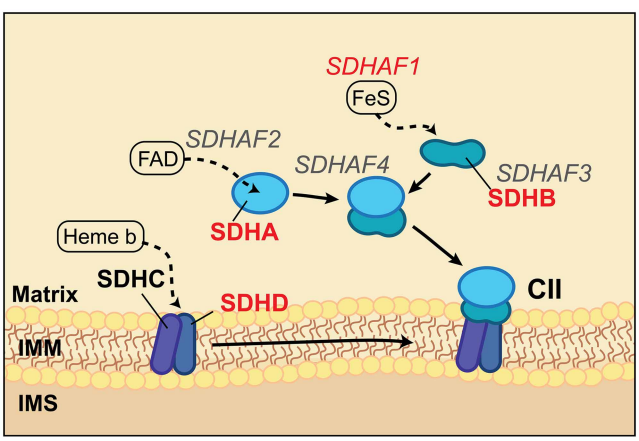


Figure 3. Schematic depicting the complex II assembly pathway showing known mitochondrial disease genes in red and assembly factors in italics.

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.

© 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).

Biochemical Journal (2020) **477** 4085–4132
https://doi.org/10.1042/BCJ20190767

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_1 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 ($CIII_2$), with each monomer being composed of one mtDNA-encoded subunit (**CYB**) and nine nuclear-encoded subunits, **CYC1**, **UQCRC1**, **UQCRC2**, **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 mitoribosomes and its co-translational insertion into the IMM (Figure 4) [158]. For efficient translation, the

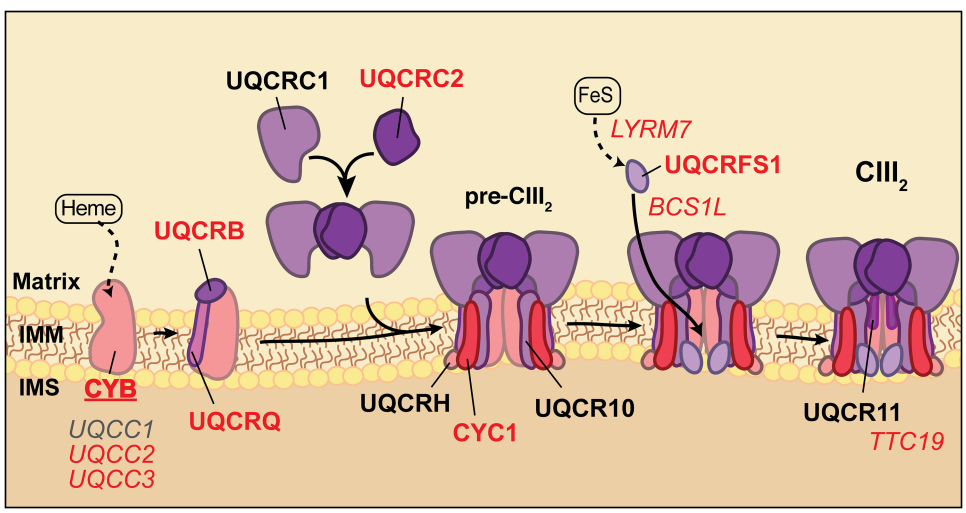


Figure 4. Schematic depicting the complex III assembly pathway showing known mitochondrial disease genes in red, mtDNA-encoded subunits underlined and assembly factors in italics.

CYB is joined by UQCRB and UQCRQ. The subcomplex joins with a tetramer of UQCRC1 and UQCRC2, CYC1, UQCRH and UQCR10, resulting in the first dimeric intermediate, pre-$CIII_2$. UQCRFS1 receives the iron–sulfur cluster from the assembly factor LYRM7 and is assembled into the pre-$CIII_2$ 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.

© 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).

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, athetosis, 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.

© 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).

Biochemical Journal (2020) 477 4085–4132
https://doi.org/10.1042/BCJ20190767

Following membrane integration of **CYB**, the first of two heme b molecules is incorporated at the b_L 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 b_H 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 **UQCRC1** and **UQCRC2** 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-$CIII_2$ (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-$CIII_2$ [158]. ***UQCRC2*** 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 hyperglycemia, hyperammonemia followed by normal development in childhood or early adulthood. Isolated CIII deficiency	[184]	123980
UQCRC2	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.

© 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).

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.

© 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).

Biochemical Journal (2020) **477** 4085–4132
https://doi.org/10.1042/BCJ20190767

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

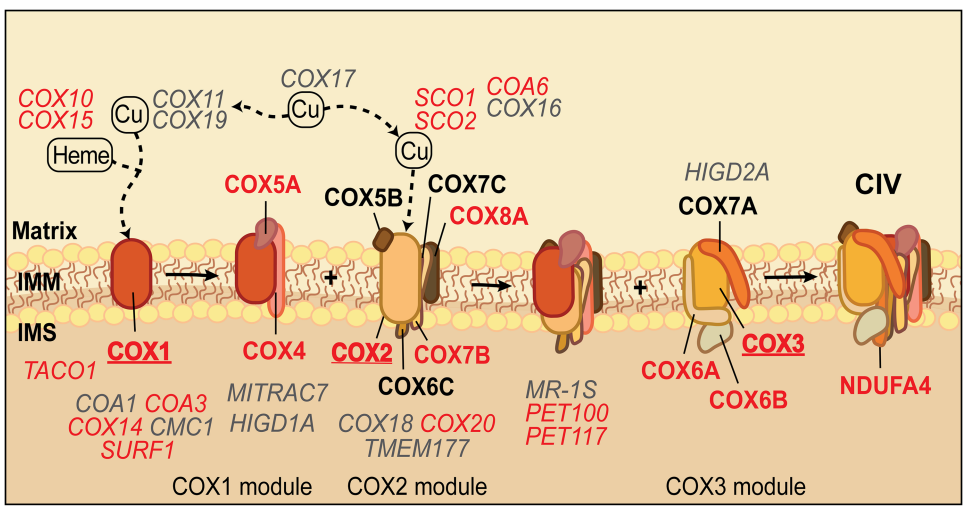


Figure 5. Schematic depicting the complex IV assembly pathway showing known mitochondrial disease genes in red, mtDNA-encoded proteins underlined and assembly factors in italics.

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.

© 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).

survival [216,222,223]. Importantly, TACO1 patients have overlapping phenotypes to those with disease caused by mutations in **COX1** (Table 9). Patients with mutations in ***COX1 (MT-CO1)*** tend to survive into adulthood and present with phenotypes common to other mtDNA-encoded genes such as Leigh syndrome, lactic acidosis, hearing loss and myopathy, although other presentations including recurrent myoglobinuria and cerebellar ataxia have also been reported, and indeed overlap with patients with mutations in other mtDNA-encoded complex IV subunits [224–226]. Mutations in *COA3* have also been identified as the cause of a relatively mild phenotype of peripheral neuropathy, with exercise intolerance and short stature without clear involvement of heart, liver or brain [227], while a homozygous mutation in its assembly partner, *COX14*, leads to a severe phenotype of respiratory distress, lactic acidosis, hypertrophic cardiomyopathy, brain hypertrophy, microphthalmia and ketonuria [218]. The mild phenotype of the COA3 patient compared with the severe phenotype of COX14 patients might not be explained by residual protein levels as both patients showed almost or totally absent COX14 protein [218,227] and reduced COA3 levels [227]. This might suggest that although these two assembly factors are involved at the same step in complex IV assembly their precise function might differ.

The insertion of heme A is a two-step process performed by COX10, which converts heme B to heme O, and COX15, which converts heme O to heme A [228]. The heme A group is thought to be delivered to **COX1** by SURF1 based on its ability to stoichiometrically bind heme A [229,230]. This step is thought to occur after dissociation of CMC1, which coincides with the binding of nuclear-encoded subunits **COX4** and **COX5A** [221] and the formation of the **COX1** module (Figure 5). Patients have been identified harbouring mutations in both ***COX4*** isoform encoding genes, with clinical phenotypes shown to differ depending on which isoform is affected. For example, variants in the ubiquitously expressed ***COX4I1*** [231] cause short stature, increase in chromosomal breaks and a phenotype similar to Fanconi anaemia [232], as well as more severe presentations of developmental delay, short stature and seizures, resembling Leigh syndrome in two siblings [233]. The two severely affected siblings also share compound heterozygous missense variants in *MDN1*, which is a AAA ATPase involved in cytosolic ribosomal biogenesis [234,235]. Despite the lack of functional studies to confirm pathogenicity of the *MDN1* variants, their involvement in the more severe phenotype cannot be discarded and might account for their combined OXPHOS deficiency, especially given that their unaffected sibling has inherited only one *MDN1* variant [233]. Likewise, ***COX4I2*** is highly expressed in lungs [231] and in the pancreas [236] with mutations in this gene leading to exocrine pancreatic insufficiency, dyserythropoietic anaemia, calvarial hyperostosis and failure to thrive, without a complex IV defect detected in fibroblasts [236] (Table 9). Taken together, the distinct expression patterns of ***COX4I1*** and ***COX4I2*** seem to correlate with their generalised or tissue-specific presentations. The third **COX1** module subunit, **COX5A**, has also been linked to disease, with mutations in ***COX5A*** leading to early onset pulmonary hypertension, brain abnormalities and lactic acidosis [237] (Table 9). Mutations in the assembly factors *COX10* and *COX15* have been reported to cause variable severe phenotypes including lactic acidosis, Leigh syndrome, hypertrophic cardiomyopathy and hypotonia [238–241], while mutations in *SURF1*, one of the most common causes of Leigh syndrome (reviewed in [242], has also been associated with other severe phenotypes such as Charcot–Marie–Tooth disease, rapidly progressive encephalopathy, ataxia, hypotonia, lactic acidosis and early death (Table 9). Analysis of patient-derived fibroblasts harbouring null mutation in *SURF1* revealed a rapidly degraded monomer and an accumulation of a complex IV subassembly, while a fully assembled complex IV was present in supercomplexes — higher order structures comprised of complexes I, III and IV [11]. Interestingly this phenomenon may be tissue specific, as analysis of different tissues from a patient harbouring truncating mutations in *SURF1* revealed decreased levels of fully assembled complex IV in heart, brain and muscle with increased accumulation of complex IV subassemblies in heart and muscle, but absent from brain [243].

The copper biosynthesis and insertion in mammalian cells has mostly been extrapolated from yeast studies. In this pathway, the Cu_B 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].

© 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).

Biochemical Journal (2020) **477** 4085–4132
https://doi.org/10.1042/BCJ20190767

Table 9. Defects affecting assembly of COX1 module Part 1 of 2

Gene	S.	A. F.	Types of genetic variants and protein impact	Clinical presentations and relevant information	Ref.	OMIM
MT-CO1 (*COX1*)	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, ketonuria, 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. Missense, nonsense,	Most common cause of Leigh syndrome associated with CIV deficiency but variable severe phenotypes including Charcot-Marie-Tooth disease,	(Reviewed in [242])	185620

Continued

© 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 9. Defects affecting assembly of COX1 module Part 2 of 2

Gene	S.	A. F.	Types of genetic variants and protein impact	Clinical presentations and relevant information	Ref.	OMIM
			insertions, deletions, intronic.	rapidly progressive encephalopathy, ataxia, hypotonia, lactic acidosis and early death.		
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.

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 (Cu_A) 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 disulfide 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).

The timing of the incorporation of the mammalian subunits **COX5B**, **COX6C**, **COX7B**, **COX7C** and **COX8A** to the **COX2** module remain unclear as well as the precise functions of associated assembly factors PET100, PET117 and MR-1S [215,273–275]. Despite their unclear roles, functional studies suggest the interaction between PET117, MR-1S and complex IV subunits are mediated by PET100 [215]. At this step, mutations in ***COX7B*** and ***COX8A*** and the genes encoding assembly factors *PET100* and *PET117* have been linked to diseases with variable presentations [274,276–280] (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

© 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).

Biochemical Journal (2020) **477** 4085–4132
https://doi.org/10.1042/BCJ20190767

Table 10. Defects affecting assembly of COX2 module Part 1 of 2

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		Intronic 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		Intronic 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

Continued

© 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 Part 2 of 2

Gene	S.	A. F.	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.

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].

© 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).

Biochemical Journal (2020) **477** 4085–4132
https://doi.org/10.1042/BCJ20190767

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, cardiomyopathy, isolated CIV deficiency in muscle and absent from undifferentiated myoblast.	[288]	602009
COX6B1	X		Missense leading to decreased protein levels.	Severe infantile encephalomyopathy, cardiomyopathy, lactic acidosis. CIV deficiency detected in muscle and mildly in 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.

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.

© 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).

Biochemical Journal (2020) **477** 4085–4132
https://doi.org/10.1042/BCJ20190767

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

Gene (alias)	S.	A. F.	Types of genetic variants and protein impact	Clinical presentations and relevant information	Ref.	OMIM
COA7		X	Predominantly missense but also intronic 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 F_1 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 F_1 module is

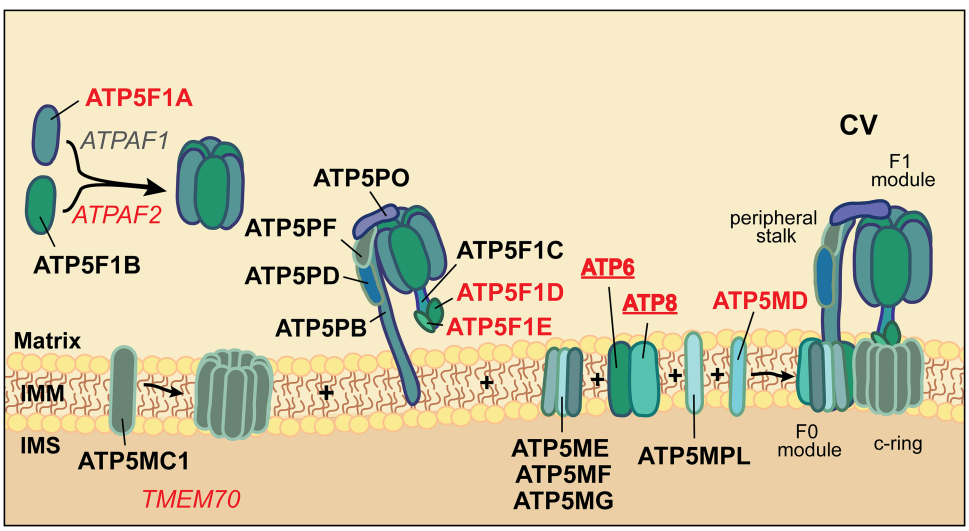


Figure 6. Schematic depicting the complex V (F_1F_0-ATPase) assembly pathway showing known mitochondrial disease genes in red, mtDNA-encoded proteins underlined and assembly factors in italics.

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 ATP5F1C1 (γ-subunit), ATP5F1D (δ-subunit) and ATP5F1E (ε-subunit). The membrane ring composed of ATP5MC1 (c-subunits; also encoded by *ATP5MC2* and *ATP5MC2*) 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.

© 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).

Biochemical Journal (2020) 477 4085–4132
https://doi.org/10.1042/BCJ20190767

Table 13. Defects in the F_1 catalytic module and central stalk

Gene (protein[1])	S.	A. F.	Types of genetic variants and protein impact	Clinical presentations and relevant information	Ref.	OMIM
ATP5F1A (α-subunit)	X		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*)		X	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)	X		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)	X		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

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

compromised [299]. For *ATPAF2*, there is a single known example [301]. In line with the requirement for this protein in assembly of the F_1 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 F_1 module [302]. **ATP5F1C** is the key structural molecule connecting the F_1 catalytic module to the F_0 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 a few cases have been reported for **ATP5F1D** and **ATP5F1E** [305,306]. Although patients present with similar, relatively mild phenotypes in line with expectations based on functional studies (Table 13), the molecular underpinnings are different. In the case of **ATP5F1D**, the patient had normal levels of the mutant protein, whereas other subunits of the F_1 module were destabilised [305]. In the case of **ATP5F1E**, the patient had reduced levels of protein, though retained a fully assembled complex (including the mutant protein) albeit at lower levels than controls [306]. Both patients had similar net effects on total levels of assembled Complex V and function (Table 13).

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 F_1 module, and its absence leads to a severe assembly

© 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 14. Defects affecting the c-ring

Gene	S.	A.F.	Types of genetic variants and protein impact	Clinical presentations and relevant information	Ref.	OMIM
TMEM70		X	Multiple. Intronic, insertion, deletion, duplication, nonsense, missense leading to unclear or absent protein levels.	Variable phenotype including hypertrophic cardiomyopathy, lactic acidosis, hyperammonemia, persistent pulmonary hypertension, encephalocardiomyopathy, neonatal hypotonia or hypertonia, facial dysmosphism, bilateral cataracts, leukoencephalopathy. Presentations vary from normal to defective isolated (CI or CIII) or combined (CI + CIII) OXPHOS activities in muscle or fibroblasts.	[81,82,84,432]	612418

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

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 F_0 module

The peripheral stalk assembles with the F_1 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 F_1 catalytic module, the peripheral stalk and the membrane subunits **ATP5ME**, **ATP5MF** and **ATP5MG** [311].

Once the previously formed subcomplex containing the F_1 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

Table 15. Defects in the Fo module

Gene (protein[1])	S.	A.F.	Types of genetic variants and protein impact	Clinical presentations and relevant information	Ref.	OMIM
MT-ATP6 (subunit 6)	X		Several missense variants with protein levels dependant on variant and mutant mtDNA load.	Most commonly presented as Leigh syndrome, NARP, spinocerebellar ataxia but also Charcot-Marie-Tooth, hypertrophic cardiomyopathy, lactic acidosis. Higher heteroplasmic levels correlate with earlier-onset phenotypes. Often but not always show ATP synthesis rate.	Reviewed in [312]	516060
MT-ATP8 (subunit 8)	X		Missense or nonsense leading to unclear protein impact.	Hypertrophic cardiomyopathy and neuropathy with survival into adolescence. Decreased CV assembly/activity. Alternately has presented with reversible cognitive dysfunctions with seizures and brain pseudoatrophy.	[313–316,433]	516070
ATP5MD (DAPIT)	X		Single known intronic variant leading to undetectable protein levels.	Leigh syndrome, developmental regression after febrile illness. Survival into childhood. Decreased proportion of CV dimer.	[317]	615204

[1]Commonly used protein name; S., subunit; A.F., assembly factor; NARP, neuropathy, ataxia and retinitis pigmentosa.

© 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).

Biochemical Journal (2020) 477 4085–4132
https://doi.org/10.1042/BCJ20190767

200 ***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 ***ATP8***, however, like for ***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 F_1 domain, suggesting the protein may be structurally important [313]. Interestingly, **ATP6** and **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 **ATP5MPL (MP68)**, which is required for **ATP6** and **ATP8** stability is incorporated, followed by **ATP5MD (DAPIT)** [309]. A single variant of the latter is known to cause Leigh syndrome with 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 ***ATP5MD*** patients had markedly reduced dimerisation [317], suggesting the protein is involved in this process.

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 MRP mitoribosomal proteins encoded by the nDNA [323]. The mtLSU consists of a tRNA valine ($tRNA^{val}$) [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 **bL17m** (encoded by the gene ***MRPL17***), **uL22m** (***MRPL22***), **uL23m** (***MRPL23***), **uL24m** (***MRPL24***), **uL29m** (***MRPL47***) and the mitochondrial specific **mL45** (***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.

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).

© 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).

Biochemical Journal (2020) **477** 4085–4132
https://doi.org/10.1042/BCJ20190767

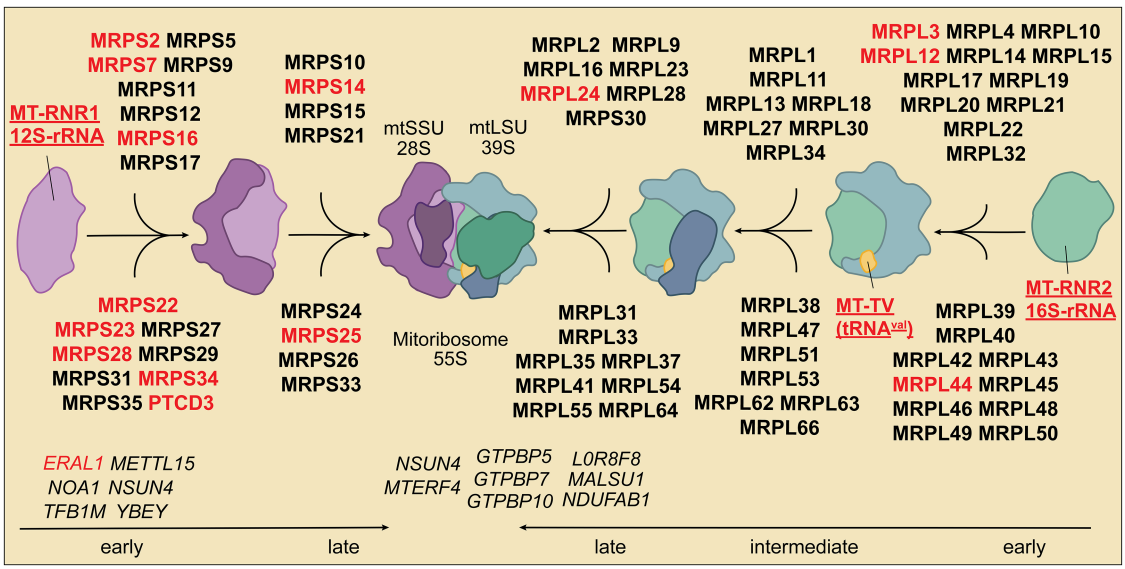


Figure 7. Schematic model depicting the mitoribosome assembly pathway based on data from [331].To aid correlation with disease genes, subunits have been labelled according to their gene name (outlined in 332). The incorporation stage of MRPS6 (bS6M), MRPS18C (bS18M), MRPS37 (mS37), MRPS38 (mS38), MRPL36 (bL36m) and MRPL52 (ml52) remains unclear. Known mitochondrial disease genes in red, mtDNA-encoded rRNAs and tRNA underlined, assembly factors in italics. The mtSSU proteins are incorporated at early or late stage in the assembly pathway while the mtLSU proteins are incorporated at early, intermediate or late stages. Mitoribosome small subunit, mtSSU; mitoribosome large subunit, mtLSU.

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 **MRPS18B** (**mS40**). 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 seem 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

© 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).

Biochemical Journal (2020) 477 4085–4132
https://doi.org/10.1042/BCJ20190767

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*** (**bS16m**) 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*** (**bS1m**) 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 *PTCD3*. 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 $tRNA^{val}$ 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

© 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 16. Defects in the assembly of the mtSSU

Gene (RNA/ protein name[1])	S.	A. 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. CIV 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
PTCD3/ 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

[1]Commonly used protein name; S., subunit; A.F., assembly factor; IUGR, intrauterine growth restriction.

© 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).

Biochemical Journal (2020) **477** 4085–4132
https://doi.org/10.1042/BCJ20190767

Table 17. Defects in the assembly of the mtLSU

Gene (RNA/ protein name[1])	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 ($tRNA^{val}$)	X		Transition leading to unclear or decreased $tRNA^{val}$ 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.

tissue type analysed (Table 17). 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 $tRNA^{val}$ (*MT-TV*) appear to cause a broader spectrum of phenotypes including muscle weakness, hearing and visual loss, MELAS, Leigh syndrome and hypertrophic cardiomyopathy [367–372] (Table 17). It is not clear if mutations in $tRNA^{val}$ lead to combined defects in mitoribosome assembly and defective polypeptide elongation, although it should be noted that mitoribosomes are able to incorporate $tRNA^{phe}$ when the levels of $tRNA^{val}$ are severely decreased [373]. Despite this implying that the phenotypes are due to defective elongation, the adaptative 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***

© 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).

(**bL12m**) suggesting these proteins play an essential 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-$G1_{mt}$) [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., Amunts, 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 Amunts, 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.aaa1193

© 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).

Biochemical Journal (2020) **477** 4085–4132
https://doi.org/10.1042/BCJ20190767

5 Frazier, A.E., Thorburn, D.R. and Compton, A.G. (2019) Mitochondrial energy generation disorders: genes, mechanisms, and clues to pathology. *J. Biol. Chem.* **294**, 5386–5395 https://doi.org/10.1074/jbc.R117.809194
6 Gorman, G.S., Chinnery, P.F., DiMauro, S., Hirano, M., Koga, Y., McFarland, R. et al. (2016) Mitochondrial diseases. *Nat. Rev. Dis. Primers* **2**, 16080 https://doi.org/10.1038/nrdp.2016.80
7 Hallberg, B.M. and Larsson, N.G. (2014) Making proteins in the powerhouse. *Cell Metab.* **20**, 226–240 https://doi.org/10.1016/j.cmet.2014.07.001
8 Siira, S.J., Shearwood, A.J., Bracken, C.P., Rackham, O. and Filipovska, A. (2017) Defects in RNA metabolism in mitochondrial disease. *Int. J. Biochem. Cell Biol.* **85**, 106–113 https://doi.org/10.1016/j.biocel.2017.02.003
9 Jackson, T.D., Palmer, C.S. and Stojanovski, D. (2018) Mitochondrial diseases caused by dysfunctional mitochondrial protein import. *Biochem. Soc. Trans.* **46**, 1225–1238 https://doi.org/10.1042/BST20180239
10 Pfanner, N., Warscheid, B. and Wiedemann, N. (2019) Mitochondrial proteins: from biogenesis to functional networks. *Nat. Rev. Mol. Cell Biol.* **20**, 267–284 https://doi.org/10.1038/s41580-018-0092-0
11 Schagger, H. and Pfeiffer, K. (2000) Supercomplexes in the respiratory chains of yeast and mammalian mitochondria. *EMBO J.* **19**, 1777–1783 https://doi.org/10.1093/emboj/19.8.1777
12 Hirst, J. (2018) Open questions: respiratory chain supercomplexes-why are they there and what do they do? *BMC Biol.* **16**, 111 https://doi.org/10.1186/s12915-018-0577-5
13 Thompson, K., Collier, J.J., Glasgow, R.I.C., Robertson, F.M., Pyle, A., Blakely, E.L. et al. (2020) Recent advances in understanding the molecular genetic basis of mitochondrial disease. *J. Inherit. Metab. Dis.* **43**, 36–50 https://doi.org/10.1002/jimd.12104
14 McCormick, E.M., Zolkipli-Cunningham, Z. and Falk, M.J. (2018) Mitochondrial disease genetics update: recent insights into the molecular diagnosis and expanding phenotype of primary mitochondrial disease. *Curr. Opin. Pediatr.* **30**, 714–724 https://doi.org/10.1097/MOP.0000000000000686
15 Rahman, J. and Rahman, S. (2018) Mitochondrial medicine in the omics era. *Lancet* **391**, 2560–2574 https://doi.org/10.1016/S0140-6736(18)30727-X
16 Lott, M.T., Leipzig, J.N., Derbeneva, O., Xie, H.M., Chalkia, D., Sarmady, M. et al. (2013) mtDNA variation and analysis using mitomap and mitomaster. *Curr. Protoc. Bioinform.* **44**, 1.23.1–26 https://doi.org/10.1002/0471250953.bi0123s44
17 Taylor, R.W., Pyle, A., Griffin, H., Blakely, E.L., Duff, J., He, L. et al. (2014) Use of whole-exome sequencing to determine the genetic basis of multiple mitochondrial respiratory chain complex deficiencies. *J. Am. Med. Assoc.* **312**, 68–77 https://doi.org/10.1001/jama.2014.7184
18 Wortmann, S.B., Koolen, D.A., Smeitink, J.A., van den Heuvel, L. and Rodenburg, R.J. (2015) Whole exome sequencing of suspected mitochondrial patients in clinical practice. *J. Inherit. Metab. Dis.* **38**, 437–443 https://doi.org/10.1007/s10545-015-9823-y
19 Pronicka, E., Piekutowska-Abramczuk, D., Ciara, E., Trubicka, J., Rokicki, D., Karkucinska-Wieckowska, A. et al. (2016) New perspective in diagnostics of mitochondrial disorders: two years' experience with whole-exome sequencing at a national paediatric centre. *J. Transl. Med.* **14**, 174 https://doi.org/10.1186/s12967-016-0930-9
20 Theunissen, T.E.J., Nguyen, M., Kamps, R., Hendrickx, A.T., Sallevelt, S., Gottschalk, R.W.H. et al. (2018) Whole exome sequencing is the preferred strategy to identify the genetic defect in patients with a probable or possible mitochondrial cause. *Front. Genet.* **9**, 400 https://doi.org/10.3389/fgene.2018.00400
21 Schon, K.R., Ratnaike, T., van den Ameele, J., Horvath, R. and Chinnery, P.F. (2020) Mitochondrial diseases: a diagnostic revolution. *Trends Genet.* **36**, 702–717 https://doi.org/10.1016/j.tig.2020.06.009
22 Stroud, D.A., Surgenor, E.E., Formosa, L.E., Reljic, B., Frazier, A.E., Dibley, M.G. et al. (2016) Accessory subunits are integral for assembly and function of human mitochondrial complex I. *Nature* **538**, 123–126 https://doi.org/10.1038/nature19754
23 Braschi, B., Denny, P., Gray, K., Jones, T., Seal, R., Tweedie, S. et al. (2019) Genenames.org: the HGNC and VGNC resources in 2019. *Nucleic Acids Res.* **47**(D1), D786–D92 https://doi.org/10.1093/nar/gky930
24 Zhu, J., Vinothkumar, K.R. and Hirst, J. (2016) Structure of mammalian respiratory complex I. *Nature* **536**, 354–358 https://doi.org/10.1038/nature19095
25 Fiedorczuk, K., Letts, J.A., Degliesposti, G., Kaszuba, K., Skehel, M. and Sazanov, L.A. (2016) Atomic structure of the entire mammalian mitochondrial complex I. *Nature* **538**, 406–410 https://doi.org/10.1038/nature19794
26 Sharma, L.K., Lu, J. and Bai, Y. (2009) Mitochondrial respiratory complex I: structure, function and implication in human diseases. *Curr. Med. Chem.* **16**, 1266–1277 https://doi.org/10.2174/092986709787846578
27 Smeitink, J.A., Loeffen, J.L., Triepels, R.H., Smeets, R.J., Trijbels, J.M. and van den Heuvel, L.P. (1998) Nuclear genes of human complex I of the mitochondrial electron transport chain: state of the art. *Hum. Mol. Genet.* **7**, 1573–1579 https://doi.org/10.1093/hmg/7.10.1573
28 Brandt, U. (2006) Energy converting NADH:quinone oxidoreductase (complex I). *Annu. Rev. Biochem.* **75**, 69–92 https://doi.org/10.1146/annurev.biochem.75.103004.142539
29 Vinothkumar, K.R., Zhu, J. and Hirst, J. (2014) Architecture of mammalian respiratory complex I. *Nature* **515**, 80–84 https://doi.org/10.1038/nature13686
30 Letts, J.A. and Sazanov, L.A. (2015) Gaining mass: the structure of respiratory complex I-from bacterial towards mitochondrial versions. *Curr. Opin. Struct. Biol.* **33**, 135–145 https://doi.org/10.1016/j.sbi.2015.08.008
31 Berrisford, J.M., Baradaran, R. and Sazanov, L.A. (2016) Structure of bacterial respiratory complex I. *Biochim. Biophys. Acta* **1857**, 892–901 https://doi.org/10.1016/j.bbabio.2016.01.012
32 Guerrero-Castillo, S., Baertling, F., Kownatzki, D., Wessels, H.J., Arnold, S., Brandt, U. et al. (2017) The assembly pathway of mitochondrial respiratory chain complex I. *Cell Metab.* **25**, 128–139 https://doi.org/10.1016/j.cmet.2016.09.002
33 Rendon O, Z., Neiva L, S., Sasarman, F. and Shoubridge, E.A. (2014) The arginine methyltransferase NDUFAF7 is essential for complex I assembly and early vertebrate embryogenesis. *Hum. Mol. Genet.* **23**, 5159–5170 https://doi.org/10.1093/hmg/ddu239
34 Rhein, V.F., Carroll, J., Ding, S., Fearnley, I.M. and Walker, J.E. (2016) NDUFAF5 hydroxylates NDUFS7 at an early stage in the assembly of human complex I. *J. Biol. Chem.* **291**, 14851–14860 https://doi.org/10.1074/jbc.M116.734970
35 Rhein, V.F., Carroll, J., Ding, S., Fearnley, I.M. and Walker, J.E. (2013) NDUFAF7 methylates arginine 85 in the NDUFS2 subunit of human complex I. *J. Biol. Chem.* **288**, 33016–33026 https://doi.org/10.1074/jbc.M113.518803
36 Sugiana, C., Pagliarini, D.J., McKenzie, M., Kirby, D.M., Salemi, R., Abu-Amero, K.K. et al. (2008) Mutation of C20orf7 disrupts complex I assembly and causes lethal neonatal mitochondrial disease. *Am. J. Hum. Genet.* **83**, 468–478 https://doi.org/10.1016/j.ajhg.2008.09.009

 © 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).

37 Floyd, B.J., Wilkerson, E.M., Veling, M.T., Minogue, C.E., Xia, C., Beebe, E.T. et al. (2016) Mitochondrial protein interaction mapping identifies regulators of respiratory chain function. *Mol. Cell* **63**, 621–632 https://doi.org/10.1016/j.molcel.2016.06.033
38 Alston, C.L., Veling, M.T., Heidler, J., Taylor, L.S., Alaimo, J.T., Sung, A.Y. et al. (2020) Pathogenic Bi-allelic mutations in NDUFAF8 cause leigh syndrome with an isolated complex I deficiency. *Am. J. Hum. Genet.* **106**, 92–101 https://doi.org/10.1016/j.ajhg.2019.12.001
39 Carilla-Latorre, S., Annesley, S.J., Munoz-Braceras, S., Fisher, P.R. and Escalante, R. (2013) Ndufaf5 deficiency in the Dictyostelium model: new roles in autophagy and development. *Mol. Biol. Cell* **24**, 1519–1528 https://doi.org/10.1091/mbc.e12-11-0796
40 Carilla-Latorre, S., Gallardo, M.E., Annesley, S.J., Calvo-Garrido, J., Grana, O., Accari, S.L. et al. (2010) Mida is a putative methyltransferase that is required for mitochondrial complex I function. *J. Cell Sci.* **123**(Pt 10), 1674–1683 https://doi.org/10.1242/jcs.066076
41 Saada, A., Vogel, R.O., Hoefs, S.J., van den Brand, M.A., Wessels, H.J., Willems, P.H. et al. (2009) Mutations in NDUFAF3 (C3ORF60), encoding an NDUFAF4 (C6ORF66)-interacting complex I assembly protein, cause fatal neonatal mitochondrial disease. *Am. J. Hum. Genet.* **84**, 718–727 https://doi.org/10.1016/j.ajhg.2009.04.020
42 Baertling, F., Sanchez-Caballero, L., van den Brand, M.A.M., Wintjes, L.T., Brink, M., van den Brandt, F.A. et al. (2017) NDUFAF4 variants are associated with leigh syndrome and cause a specific mitochondrial complex I assembly defect. *Eur. J. Hum. Genet.* **25**, 1273–1277 https://doi.org/10.1038/ejhg.2017.133
43 Howell, N., Bindoff, L.A., McCullough, D.A., Kubacka, I., Poulton, J., Mackey, D. et al. (1991) Leber hereditary optic neuropathy: identification of the same mitochondrial ND1 mutation in six pedigrees. *Am. J. Hum. Genet.* **49**, 939–950 PMID: 1928099
44 Valentino, M.L., Barboni, P., Ghelli, A., Bucchi, L., Rengo, C., Achilli, A. et al. (2004) The ND1 gene of complex I is a mutational hot spot for Leber's hereditary optic neuropathy. *Ann. Neurol.* **56**, 631–641 https://doi.org/10.1002/ana.20236
45 Kirby, D.M., McFarland, R., Ohtake, A., Dunning, C., Ryan, M.T., Wilson, C. et al. (2004) Mutations of the mitochondrial ND1 gene as a cause of MELAS. *J. Med. Genet.* **41**, 784–789 https://doi.org/10.1136/jmg.2004.020537
46 Kremer, L.S., Bader, D.M., Mertes, C., Kopajtich, R., Pichler, G., Iuso, A. et al. (2017) Genetic diagnosis of Mendelian disorders via RNA sequencing. *Nat. Commun.* **8**, 15824 https://doi.org/10.1038/ncomms15824
47 McKenzie, M., Tucker, E.J., Compton, A.G., Lazarou, M., George, C., Thorburn, D.R. et al. (2011) Mutations in the gene encoding C8orf38 block complex I assembly by inhibiting production of the mitochondria-encoded subunit ND1. *J. Mol. Biol.* **414**, 413–426 https://doi.org/10.1016/j.jmb.2011.10.012
48 Fernandez-Moreira, D., Ugalde, C., Smeets, R., Rodenburg, R.J., Lopez-Laso, E., Ruiz-Falco, M.L. et al. (2007) X-linked NDUFA1 gene mutations associated with mitochondrial encephalomyopathy. *Ann. Neurol.* **61**, 73–83 https://doi.org/10.1002/ana.21036
49 Formosa, L.E., Muellner-Wong, L., Reljic, B., Sharpe, A.J., Jackson, T.D., Beilharz, T.H. et al. (2020) Dissecting the roles of mitochondrial complex I intermediate assembly complex factors in the biogenesis of complex I. *Cell Rep.* **31**, 107541 https://doi.org/10.1016/j.celrep.2020.107541
50 Mick, D.U., Dennerlein, S., Wiese, H., Reinhold, R., Pacheu-Grau, D., Lorenzi, I. et al. (2012) MITRAC links mitochondrial protein translocation to respiratory-chain assembly and translational regulation. *Cell* **151**, 1528–1541 https://doi.org/10.1016/j.cell.2012.11.053
51 Wang, C., Richter-Dennerlein, R., Pacheu-Grau, D., Liu, F., Zhu, Y., Dennerlein, S. et al. (2020) MITRAC15/COA1 promotes mitochondrial translation in a ND2 ribosome-nascent chain complex. *EMBO Rep.* **21**, e48833 https://doi.org/10.15252/embr.201948833
52 Anderson, S., Bankier, A.T., Barrell, B.G., de Bruijn, M.H., Coulson, A.R., Drouin, J. et al. (1981) Sequence and organization of the human mitochondrial genome. *Nature* **290**, 457–465 https://doi.org/10.1038/290457a0
53 Kuffner, R., Rohr, A., Schmiede, A., Krull, C. and Schulte, U. (1998) Involvement of two novel chaperones in the assembly of mitochondrial NADH: Ubiquinone oxidoreductase (complex I). *J. Mol. Biol.* **283**, 409–417 https://doi.org/10.1006/jmbi.1998.2114
54 Vogel, R.O., Janssen, R.J., Ugalde, C., Grovenstein, M., Huijbens, R.J., Visch, H.J. et al. (2005) Human mitochondrial complex I assembly is mediated by NDUFAF1. *FEBS J.* **272**, 5317–5326 https://doi.org/10.1111/j.1742-4658.2005.04928.x
55 Dunning, C.J., McKenzie, M., Sugiana, C., Lazarou, M., Silke, J., Connelly, A. et al. (2007) Human CIA30 is involved in the early assembly of mitochondrial complex I and mutations in its gene cause disease. *EMBO J.* **26**, 3227–3237 https://doi.org/10.1038/sj.emboj.7601748
56 Fassone, E., Taanman, J.W., Hargreaves, I.P., Sebire, N.J., Cleary, M.A., Burch, M. et al. (2011) Mutations in the mitochondrial complex I assembly factor NDUFAF1 cause fatal infantile hypertrophic cardiomyopathy. *J. Med. Genet.* **48**, 691–697 https://doi.org/10.1136/jmedgenet-2011-100340
57 Wu, L., Peng, J., Ma, Y., He, F., Deng, X., Wang, G. et al. (2016) Leukodystrophy associated with mitochondrial complex I deficiency due to a novel mutation in the NDUFAF1 gene. Mitochondrial DNA A DNA Mapp. *Seq. Anal.* **27**, 1034–1037 https://doi.org/10.3109/19401736.2014.926543
58 Ensenauer, R., He, M., Willard, J.M., Goetzman, E.S., Corydon, T.J., Vandahl, B.B. et al. (2005) Human acyl-CoA dehydrogenase-9 plays a novel role in the mitochondrial beta-oxidation of unsaturated fatty acids. *J. Biol. Chem.* **280**, 32309–32316 https://doi.org/10.1074/jbc.M504460200
59 He, M., Rutledge, S.L., Kelly, D.R., Palmer, C.A., Murdoch, G., Majumder, N. et al. (2007) A new genetic disorder in mitochondrial fatty acid beta-oxidation: ACAD9 deficiency. *Am. J. Hum. Genet.* **81**, 87–103 https://doi.org/10.1086/519219
60 Nouws, J., Nijtmans, L., Houten, S.M., van den Brand, M., Huynen, M., Venselaar, H. et al. (2010) Acyl-CoA dehydrogenase 9 is required for the biogenesis of oxidative phosphorylation complex I. *Cell Metab.* **12**, 283–294 https://doi.org/10.1016/j.cmet.2010.08.002
61 Repp, B.M., Mastantuono, E., Alston, C.L., Schiff, M., Haack, T.B., Rotig, A. et al. (2018) Clinical, biochemical and genetic spectrum of 70 patients with ACAD9 deficiency: is riboflavin supplementation effective? *Orphanet. J. Rare Dis.* **13**, 120 https://doi.org/10.1186/s13023-018-0784-8
62 Nouws, J., Te Brinke, H., Nijtmans, L.G. and Houten, S.M. (2014) ACAD9, a complex I assembly factor with a moonlighting function in fatty acid oxidation deficiencies. *Hum. Mol. Genet.* **23**, 1311–1319 https://doi.org/10.1093/hmg/ddt521
63 Heide, H., Bleier, L., Steger, M., Ackermann, J., Drose, S., Schwamb, B. et al. (2012) Complexome profiling identifies TMEM126B as a component of the mitochondrial complex I assembly complex. *Cell Metab.* **16**, 538–549 https://doi.org/10.1016/j.cmet.2012.08.009
64 van den Bosch, B.J., Gerards, M., Sluiter, W., Stegmann, A.P., Jongen, E.L., Hellebrekers, D.M. et al. (2012) Defective NDUFA9 as a novel cause of neonatally fatal complex I disease. *J. Med. Genet.* **49**, 10–15 https://doi.org/10.1136/jmedgenet-2011-100466
65 Hoefs, S.J., van Spronsen, F.J., Lenssen, E.W., Nijtmans, L.G., Rodenburg, R.J., Smeitink, J.A. et al. (2011) NDUFA10 mutations cause complex I deficiency in a patient with Leigh disease. *Eur. J. Hum. Genet.* **19**, 270–274 https://doi.org/10.1038/ejhg.2010.204
66 Kohda, M., Tokuzawa, Y., Kishita, Y., Nyuzuki, H., Moriyama, Y., Mizuno, Y. et al. (2016) A comprehensive genomic analysis reveals the genetic landscape of mitochondrial respiratory chain complex deficiencies. *PLoS Genet.* **12**, e1005679 https://doi.org/10.1371/journal.pgen.1005679
67 Berger, I., Hershkovitz, E., Shaag, A., Edvardson, S., Saada, A. and Elpeleg, O. (2008) Mitochondrial complex I deficiency caused by a deleterious NDUFA11 mutation. *Ann. Neurol.* **63**, 405–408 https://doi.org/10.1002/ana.21332

© 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).

68 Peverelli, L., Legati, A., Lamantea, E., Nasca, A., Lerario, A., Galimberti, V. et al. (2019) New missense variants of NDUFA11 associated with late-onset myopathy. *Muscle Nerve* **60**, E11–E14 https://doi.org/10.1002/mus.26511
69 Baertling, F., Sanchez-Caballero, L., van den Brand, M.A.M., Fung, C.W., Chan, S.H., Wong, V.C. et al. (2018) NDUFA9 point mutations cause a variable mitochondrial complex I assembly defect. *Clin. Genet.* **93**, 111–118 https://doi.org/10.1111/cge.13089
70 Hudson, G., Carelli, V., Spruijt, L., Gerards, M., Mowbray, C., Achilli, A. et al. (2007) Clinical expression of Leber hereditary optic neuropathy is affected by the mitochondrial DNA-haplogroup background. *Am. J. Hum. Genet.* **81**, 228–233 https://doi.org/10.1086/519394
71 Jia, X., Li, S., Xiao, X., Guo, X. and Zhang, Q. (2006) Molecular epidemiology of mtDNA mutations in 903 Chinese families suspected with Leber hereditary optic neuropathy. *J. Hum. Genet.* **51**, 851–856 https://doi.org/10.1007/s10038-006-0032-2
72 Friederich, M.W., Erdogan, A.J., Coughlin, II, C.R., Elos, M.T., Jiang, H., O'Rourke, C.P. et al. (2017) Mutations in the accessory subunit NDUFB10 result in isolated complex I deficiency and illustrate the critical role of intermembrane space import for complex I holoenzyme assembly. *Hum. Mol. Genet.* **26**, 702–716 https://doi.org/10.1093/hmg/ddw431
73 van Rahden, V.A., Fernandez-Vizarra, E., Alawi, M., Brand, K., Fellmann, F., Horn, D. et al. (2015) Mutations in NDUFB11, encoding a complex I component of the mitochondrial respiratory chain, cause microphthalmia with linear skin defects syndrome. *Am. J. Hum. Genet.* **96**, 640–650 https://doi.org/10.1016/j.ajhg.2015.02.002
74 Torraco, A., Bianchi, M., Verrigni, D., Gelmetti, V., Riley, L., Niceta, M. et al. (2017) A novel mutation in NDUFB11 unveils a new clinical phenotype associated with lactic acidosis and sideroblastic anemia. *Clin. Genet.* **91**, 441–447 https://doi.org/10.1111/cge.12790
75 Sanchez-Caballero, L., Elurbe, D.M., Baertling, F., Guerrero-Castillo, S., van den Brand, M., van Strien, J. et al. (2020) TMEM70 functions in the assembly of complexes I and V. *Biochim. Biophys. Acta Bioenerg.* **1861**, 148202 https://doi.org/10.1016/j.bbabio.2020.148202
76 Formosa, L.E., Mimaki, M., Frazier, A.E., McKenzie, M., Stait, T.L., Thorburn, D.R. et al. (2015) Characterization of mitochondrial FOXRED1 in the assembly of respiratory chain complex I. *Hum. Mol. Genet.* **24**, 2952–2965 https://doi.org/10.1093/hmg/ddv058
77 Calvo, S.E., Tucker, E.J., Compton, A.G., Kirby, D.M., Crawford, G., Burtt, N.P. et al. (2010) High-throughput, pooled sequencing identifies mutations in NUBPL and FOXRED1 in human complex I deficiency. *Nat. Genet.* **42**, 851–858 https://doi.org/10.1038/ng.659
78 Fassone, E., Duncan, A.J., Taanman, J.W., Pagnamenta, A.T., Sadowski, M.I., Holand, T. et al. (2010) FOXRED1, encoding an FAD-dependent oxidoreductase complex-I-specific molecular chaperone, is mutated in infantile-onset mitochondrial encephalopathy. *Hum. Mol. Genet.* **19**, 4837–4847 https://doi.org/10.1093/hmg/ddq414
79 Rendon O, Z., Antonicka, H., Horvath, R. and Shoubridge, E.A. (2016) A mutation in the flavin adenine dinucleotide-dependent oxidoreductase FOXRED1 results in cell-type-specific assembly defects in oxidative phosphorylation complexes I and II. *Mol. Cell. Biol.* **36**, 2132–2140 https://doi.org/10.1128/MCB.00066-16
80 Kovalcikova, J., Vrbacky, M., Pecina, P., Tauchmannova, K., Nuskova, H., Kaplanova, V. et al. (2019) TMEM70 facilitates biogenesis of mammalian ATP synthase by promoting subunit c incorporation into the rotor structure of the enzyme. *FASEB J.* **33**, 14103–14117 https://doi.org/10.1096/fj.201900685RR
81 Diodato, D., Invernizzi, F., Lamantea, E., Fagiolari, G., Parini, R., Menni, F. et al. (2015) Common and novel TMEM70 mutations in a cohort of Italian patients with mitochondrial encephalocardiomyopathy. *JIMD Rep.* **15**, 71–78 https://doi.org/10.1007/8904_2014_300
82 Cizkova, A., Stranecky, V., Mayr, J.A., Tesarova, M., Havlickova, V., Paul, J. et al. (2008) TMEM70 mutations cause isolated ATP synthase deficiency and neonatal mitochondrial encephalocardiomyopathy. *Nat. Genet.* **40**, 1288–1290 https://doi.org/10.1038/ng.246
83 Jonckheere, A.I., Huigsloot, M., Lammens, M., Jansen, J., van den Heuvel, L.P., Spiekerkoetter, U. et al. (2011) Restoration of complex V deficiency caused by a novel deletion in the human TMEM70 gene normalizes mitochondrial morphology. *Mitochondrion* **11**, 954–963 https://doi.org/10.1016/j.mito.2011.08.012
84 Spiegel, R., Khayat, M., Shalev, S.A., Horovitz, Y., Mandel, H., Hershkovitz, E. et al. (2011) TMEM70 mutations are a common cause of nuclear encoded ATP synthase assembly defect: further delineation of a new syndrome. *J. Med. Genet.* **48**, 177–182 https://doi.org/10.1136/jmg.2010.084608
85 Dibley, M.G., Formosa, L.E., Lyu, B., Reljic, B., McGann, D., Muellner-Wong, L. et al. (2020) The mitochondrial acyl-carrier protein interaction network highlights important roles for LYRM family members in complex I and mitoribosome assembly. *Mol. Cell Proteom.* **19**, 65–77 https://doi.org/10.1074/mcp.RA119.001784
86 Chol, M., Lebon, S., Benit, P., Chretien, D., de Lonlay, P., Goldenberg, A. et al. (2003) The mitochondrial DNA G13513A MELAS mutation in the NADH dehydrogenase 5 gene is a frequent cause of Leigh-like syndrome with isolated complex I deficiency. *J. Med. Genet.* **40**, 188–191 https://doi.org/10.1136/jmg.40.3.188
87 Shanske, S., Coku, J., Lu, J., Ganesh, J., Krishna, S., Tanji, K. et al. (2008) The G13513A mutation in the ND5 gene of mitochondrial DNA as a common cause of MELAS or Leigh syndrome: evidence from 12 cases. *Arch. Neurol.* **65**, 368–372 https://doi.org/10.1001/archneurol.2007.67
88 Calvo, S.E., Compton, A.G., Hershman, S.G., Lim, S.C., Lieber, D.S., Tucker, E.J. et al. (2012) Molecular diagnosis of infantile mitochondrial disease with targeted next-generation sequencing. *Sci. Transl. Med.* **4**, 118ra10 https://doi.org/10.1126/scitranslmed.3003310
89 Haack, T.B., Haberberger, B., Frisch, E.M., Wieland, T., Iuso, A., Gorza, M. et al. (2012) Molecular diagnosis in mitochondrial complex I deficiency using exome sequencing. *J. Med. Genet.* **49**, 277–283 https://doi.org/10.1136/jmedgenet-2012-100846
90 Alston, C.L., Howard, C., Olahova, M., Hardy, S.A., He, L., Murray, P.G. et al. (2016) A recurrent mitochondrial p.Trp22Arg NDUFB3 variant causes a distinctive facial appearance, short stature and a mild biochemical and clinical phenotype. *J. Med. Genet.* **53**, 634–641 https://doi.org/10.1136/jmedgenet-2015-103576
91 Piekutowska-Abramczuk, D., Assouline, Z., Matakovic, L., Feichtinger, R.G., Konarikova, E., Jurkiewicz, E. et al. (2018) NDUFB8 mutations cause mitochondrial complex I deficiency in individuals with Leigh-like encephalomyopathy. *Am. J. Hum. Genet.* **102**, 460–467 https://doi.org/10.1016/j.ajhg.2018.01.008
92 Haack, T.B., Madignier, F., Herzer, M., Lamantea, E., Danhauser, K., Invernizzi, F. et al. (2012) Mutation screening of 75 candidate genes in 152 complex I deficiency cases identifies pathogenic variants in 16 genes including NDUFB9. *J. Med. Genet.* **49**, 83–89 https://doi.org/10.1136/jmedgenet-2011-100577
93 Hoefs, S.J., Dieteren, C.E., Distelmaier, F., Janssen, R.J., Epplen, A., Swarts, H.G. et al. (2008) NDUFA2 complex I mutation leads to Leigh disease. *Am. J. Hum. Genet.* **82**, 1306–1315 https://doi.org/10.1016/j.ajhg.2008.05.007

© 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).

94 Perrier, S., Gauquelin, L., Tetreault, M., Tran, L.T., Webb, N., Srour, M. et al. (2018) Recessive mutations in NDUFA2 cause mitochondrial leukoencephalopathy. *Clin. Genet.* **93**, 396–400 https://doi.org/10.1111/cge.13126
95 Benit, P., Chretien, D., Kadhom, N., de Lonlay-Debeney, P., Cormier-Daire, V., Cabral, A. et al. (2001) Large-scale deletion and point mutations of the nuclear NDUFV1 and NDUFS1 genes in mitochondrial complex I deficiency. *Am. J. Hum. Genet.* **68**, 1344–1352 https://doi.org/10.1086/320603
96 Martin, M.A., Blazquez, A., Gutierrez-Solana, L.G., Fernandez-Moreira, D., Briones, P., Andreu, A.L. et al. (2005) Leigh syndrome associated with mitochondrial complex I deficiency due to a novel mutation in the NDUFS1 gene. *Arch. Neurol.* **62**, 659–661 https://doi.org/10.1001/archneur.62.4.659
97 Hoefs, S.J., Skjeldal, O.H., Rodenburg, R.J., Nedregaard, B., van Kaauwen, E.P., Spiekerkotter, U. et al. (2010) Novel mutations in the NDUFS1 gene cause low residual activities in human complex I deficiencies. *Mol. Genet. Metab.* **100**, 251–256 https://doi.org/10.1016/j.ymgme.2010.03.015
98 Ferreira, M., Torraco, A., Rizza, T., Fattori, F., Meschini, M.C., Castana, C. et al. (2011) Progressive cavitating leukoencephalopathy associated with respiratory chain complex I deficiency and a novel mutation in NDUFS1. *Neurogenetics* **12**, 9–17 https://doi.org/10.1007/s10048-010-0265-2
99 Schuelke, M., Smeitink, J., Mariman, E., Loeffen, J., Plecko, B., Trijbels, F. et al. (1999) Mutant NDUFV1 subunit of mitochondrial complex I causes leukodystrophy and myoclonic epilepsy. *Nat. Genet.* **21**, 260–261 https://doi.org/10.1038/6772
100 Benit, P., Beugnot, R., Chretien, D., Giurgea, I., De Lonlay-Debeney, P., Issartel, J.P. et al. (2003) Mutant NDUFV2 subunit of mitochondrial complex I causes early onset hypertrophic cardiomyopathy and encephalopathy. *Hum. Mutat.* **21**, 582–586 https://doi.org/10.1002/humu.10225
101 Pagniez-Mammeri, H., Lombes, A., Brivet, M., Ogier-de Baulny, H., Landrieu, P., Legrand, A. et al. (2009) Rapid screening for nuclear genes mutations in isolated respiratory chain complex I defects. *Mol. Genet. Metab.* **96**, 196–200 https://doi.org/10.1016/j.ymgme.2008.12.003
102 Cameron, J.M., MacKay, N., Feigenbaum, A., Tarnopolsky, M., Blaser, S., Robinson, B.H. et al. (2015) Exome sequencing identifies complex I NDUFV2 mutations as a novel cause of Leigh syndrome. *Eur. J. Paediatr. Neurol.* **19**, 525–532 https://doi.org/10.1016/j.ejpn.2015.05.002
103 Sheftel, A.D., Stehling, O., Pierik, A.J., Netz, D.J., Kerscher, S., Elsasser, H.P. et al. (2009) Human ind1, an iron-sulfur cluster assembly factor for respiratory complex I. *Mol. Cell. Biol.* **29**, 6059–6073 https://doi.org/10.1128/MCB.00817-09
104 Kevelam, S.H., Rodenburg, R.J., Wolf, N.I., Ferreira, P., Lunsing, R.J., Nijtmans, L.G. et al. (2013) NUBPL mutations in patients with complex I deficiency and a distinct MRI pattern. *Neurology* **80**, 1577–1583 https://doi.org/10.1212/WNL.0b013e31828f1914
105 Lazarou, M., McKenzie, M., Ohtake, A., Thorburn, D.R. and Ryan, M.T. (2007) Analysis of the assembly profiles for mitochondrial- and nuclear-DNA-encoded subunits into complex I. *Mol. Cell. Biol.* **27**, 4228–4237 https://doi.org/10.1128/MCB.00074-07
106 Alston, C.L., Heidler, J., Dibley, M.G., Kremer, L.S., Taylor, L.S., Fratter, C. et al. (2018) Bi-allelic mutations in NDUFA6 establish its role in early-onset isolated mitochondrial complex I deficiency. *Am. J. Hum. Genet.* **103**, 592–601 https://doi.org/10.1016/j.ajhg.2018.08.013
107 Ostergaard, E., Rodenburg, R.J., van den Brand, M., Thomsen, L.L., Duno, M., Batbayli, M. et al. (2011) Respiratory chain complex I deficiency due to NDUFA12 mutations as a new cause of Leigh syndrome. *J. Med. Genet.* **48**, 737–740 https://doi.org/10.1136/jmg.2011.088856
108 van den Heuvel, L., Ruitenbeek, W., Smeets, R., Gelman-Kohan, Z., Elpeleg, O., Loeffen, J. et al. (1998) Demonstration of a new pathogenic mutation in human complex I deficiency: a 5-bp duplication in the nuclear gene encoding the 18-kD (AQDQ) subunit. *Am. J. Hum. Genet.* **62**, 262–268 https://doi.org/10.1086/301716
109 Budde, S.M., van den Heuvel, L.P., Janssen, A.J., Smeets, R.J., Buskens, C.A., DeMeirleir, L. et al. (2000) Combined enzymatic complex I and III deficiency associated with mutations in the nuclear encoded NDUFS4 gene. *Biochem. Biophys. Res. Commun.* **275**, 63–68 https://doi.org/10.1006/bbrc.2000.3257
110 Benit, P., Steffann, J., Lebon, S., Chretien, D., Kadhom, N., de Lonlay, P. et al. (2003) Genotyping microsatellite DNA markers at putative disease loci in inbred/multiplex families with respiratory chain complex I deficiency allows rapid identification of a novel nonsense mutation (IVS1nt -1) in the NDUFS4 gene in Leigh syndrome. *Hum. Genet.* **112**, 563–566 https://doi.org/10.1007/s00439-002-0884-2
111 Anderson, S.L., Chung, W.K., Frezzo, J., Papp, J.C., Ekstein, J., DiMauro, S. et al. (2008) A novel mutation in NDUFS4 causes Leigh syndrome in an Ashkenazi Jewish family. *J. Inherit. Metab. Dis.* **31**, S461–S467 https://doi.org/10.1007/s10545-008-1049-9
112 Kirby, D.M., Salemi, R., Sugiana, C., Ohtake, A., Parry, L., Bell, K.M. et al. (2004) NDUFS6 mutations are a novel cause of lethal neonatal mitochondrial complex I deficiency. *J. Clin. Invest.* **114**, 837–845 https://doi.org/10.1172/JCI20683
113 Spiegel, R., Shaag, A., Mandel, H., Reich, D., Penyakov, M., Hujeirat, Y. et al. (2009) Mutated NDUFS6 is the cause of fatal neonatal lactic acidemia in Caucasus Jews. *Eur. J. Hum. Genet.* **17**, 1200–1203 https://doi.org/10.1038/ejhg.2009.24
114 Rouzier, C., Chaussenot, A., Fragaki, K., Serre, V., Ait-El-Mkadem, S., Richelme, C. et al. (2019) NDUFS6 related Leigh syndrome: a case report and review of the literature. *J. Hum. Genet.* **64**, 637–645 https://doi.org/10.1038/s10038-019-0594-4
115 Adjobo-Hermans, M.J.W., de Haas, R., Willems, P., Wojtala, A., van Emst-de Vries, S.E., Wagenaars, J.A. et al. (2020) NDUFS4 deletion triggers loss of NDUFA12 in Ndufs4(-/-) mice and Leigh syndrome patients: a stabilizing role for NDUFAF2. *Biochim. Biophys. Acta Bioenerg.* **1861**, 148213 https://doi.org/10.1016/j.bbabio.2020.148213
116 Barghuti, F., Elian, K., Gomori, J.M., Shaag, A., Edvardson, S., Saada, A. et al. (2008) The unique neuroradiology of complex I deficiency due to NDUFA12L defect. *Mol. Genet. Metab.* **94**, 78–82 https://doi.org/10.1016/j.ymgme.2007.11.013
117 Parey, K., Haapanen, O., Sharma, V., Kofeler, H., Zullig, T., Prinz, S. et al. (2019) High-resolution cryo-EM structures of respiratory complex I: mechanism, assembly, and disease. *Sci. Adv.* **5**, eaax9484 https://doi.org/10.1126/sciadv.aax9484
118 Hoefs, S.J., Dieteren, C.E., Rodenburg, R.J., Naess, K., Bruhn, H., Wibom, R. et al. (2009) Baculovirus complementation restores a novel NDUFAF2 mutation causing complex I deficiency. *Hum. Mutat.* **30**, E728–E736 https://doi.org/10.1002/humu.21037
119 Herzer, M., Koch, J., Prokisch, H., Rodenburg, R., Rauscher, C., Radauer, W. et al. (2010) Leigh disease with brainstem involvement in complex I deficiency due to assembly factor NDUFAF2 defect. *Neuropediatrics* **41**, 30–34 https://doi.org/10.1055/s-0030-1255062
120 Cecchini, G. (2003) Function and structure of complex II of the respiratory chain. *Annu. Rev. Biochem.* **72**, 77–109 https://doi.org/10.1146/annurev.biochem.72.121801.161700
121 Bourgeron, T., Rustin, P., Chretien, D., Birch-Machin, M., Bourgeois, M., Viegas-Pequignot, E. et al. (1995) Mutation of a nuclear succinate dehydrogenase gene results in mitochondrial respiratory chain deficiency. *Nat. Genet.* **11**, 144–149 https://doi.org/10.1038/ng1095-144
122 Taylor, R.W., Birch-Machin, M.A., Schaefer, J., Taylor, L., Shakir, R., Ackrell, B.A. et al. (1996) Deficiency of complex II of the mitochondrial respiratory chain in late-onset optic atrophy and ataxia. *Ann. Neurol.* **39**, 224–232 https://doi.org/10.1002/ana.410390212
123 Parfait, B., Chretien, D., Rotig, A., Marsac, C., Munnich, A. and Rustin, P. (2000) Compound heterozygous mutations in the flavoprotein gene of the respiratory chain complex II in a patient with Leigh syndrome. *Hum. Genet.* **106**, 236–243 https://doi.org/10.1007/s004399900218

© 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).

124 Birch-Machin, M.A., Taylor, R.W., Cochran, B., Ackrell, B.A. and Turnbull, D.M. (2000) Late-onset optic atrophy, ataxia, and myopathy associated with a mutation of a complex II gene. *Ann. Neurol.* **48**, 330–335 https://doi.org/10.1002/1531-8249(200009)48:3<330::AID-ANA7>3.0.CO;2-A
125 Van Coster, R., Seneca, S., Smet, J., Van Hecke, R., Gerlo, E., Devreese, B. et al. (2003) Homozygous Gly555Glu mutation in the nuclear-encoded 70 kDa flavoprotein gene causes instability of the respiratory chain complex II. *Am. J. Med. Genet. A* **120A**, 13–18 https://doi.org/10.1002/ajmg.a.10202
126 Horvath, R., Abicht, A., Holinski-Feder, E., Laner, A., Gempel, K., Prokisch, H. et al. (2006) Leigh syndrome caused by mutations in the flavoprotein (Fp) subunit of succinate dehydrogenase (SDHA). *J. Neurol. Neurosurg. Psychiatry* **77**, 74–76 https://doi.org/10.1136/jnnp.2005.067041
127 Alston, C.L., Davison, J.E., Meloni, F., van der Westhuizen, F.H., He, L., Hornig-Do, H.T. et al. (2012) Recessive germline SDHA and SDHB mutations causing leukodystrophy and isolated mitochondrial complex II deficiency. *J. Med. Genet.* **49**, 569–577 https://doi.org/10.1136/jmedgenet-2012-101146
128 Renkema, G.H., Wortmann, S.B., Smeets, R.J., Venselaar, H., Antoine, M., Visser, G. et al. (2015) SDHA mutations causing a multisystem mitochondrial disease: novel mutations and genetic overlap with hereditary tumors. *Eur. J. Hum. Genet.* **23**, 202–209 https://doi.org/10.1038/ejhg.2014.80
129 Courage, C., Jackson, C.B., Hahn, D., Euro, L., Nuoffer, J.M., Gallati, S. et al. (2017) SDHA mutation with dominant transmission results in complex II deficiency with ocular, cardiac, and neurologic involvement. *Am. J. Med. Genet. A* **173**, 225–230 https://doi.org/10.1002/ajmg.a.37986
130 Ardissone, A., Invernizzi, F., Nasca, A., Moroni, I., Farina, L. and Ghezzi, D. (2015) Mitochondrial leukoencephalopathy and complex II deficiency associated with a recessive SDHB mutation with reduced penetrance. *Mol. Genet. Metab. Rep.* **5**, 51–54 https://doi.org/10.1016/j.ymgmr.2015.10.006
131 Alston, C.L., Ceccatelli Berti, C., Blakely, E.L., Olahova, M., He, L., McMahon, C.J. et al. (2015) A recessive homozygous p.Asp92Gly SDHD mutation causes prenatal cardiomyopathy and a severe mitochondrial complex II deficiency. *Hum. Genet.* **134**, 869–879 https://doi.org/10.1007/s00439-015-1568-z
132 Jackson, C.B., Nuoffer, J.M., Hahn, D., Prokisch, H., Haberberger, B., Gautschi, M. et al. (2014) Mutations in SDHD lead to autosomal recessive encephalomyopathy and isolated mitochondrial complex II deficiency. *J. Med. Genet.* **51**, 170–175 https://doi.org/10.1136/jmedgenet-2013-101932
133 Hao, H.X., Khalimonchuk, O., Schraders, M., Dephoure, N., Bayley, J.P., Kunst, H. et al. (2009) SDH5, a gene required for flavination of succinate dehydrogenase, is mutated in paraganglioma. *Science* **325**, 1139–1142 https://doi.org/10.1126/science.1175689
134 Kim, H.J., Jeong, M.Y., Na, U. and Winge, D.R. (2012) Flavinylation and assembly of succinate dehydrogenase are dependent on the C-terminal tail of the flavoprotein subunit. *J. Biol. Chem.* **287**, 40670–40679 https://doi.org/10.1074/jbc.M112.405704
135 Bezawork-Geleta, A., Wen, H., Dong, L., Yan, B., Vider, J., Boukalova, S. et al. (2018) Alternative assembly of respiratory complex II connects energy stress to metabolic checkpoints. *Nat. Commun.* **9**, 2221 https://doi.org/10.1038/s41467-018-04603-z
136 Bezawork-Geleta, A., Dong, L., Rohlena, J. and Neuzil, J. (2016) The assembly factor sdhaf2 is dispensable for flavination of the catalytic subunit of mitochondrial complex II in breast cancer cells. *J. Biol. Chem.* **291**, 21414–21420 https://doi.org/10.1074/jbc.C116.755017
137 Maher, M.J., Herath, A.S., Udagedara, S.R., Dougan, D.A. and Truscott, K.N. (2018) Crystal structure of bacterial succinate:quinone oxidoreductase flavoprotein SdhA in complex with its assembly factor SdhE. *Proc. Natl. Acad. Sci. U.S.A.* **115**, 2982–2987 https://doi.org/10.1073/pnas.1800195115
138 Van Vranken, J.G., Bricker, D.K., Dephoure, N., Gygi, S.P., Cox, J.E., Thummel, C.S. et al. (2014) SDHAF4 promotes mitochondrial succinate dehydrogenase activity and prevents neurodegeneration. *Cell Metab.* **20**, 241–252 https://doi.org/10.1016/j.cmet.2014.05.012
139 Maio, N., Ghezzi, D., Verrigni, D., Rizza, T., Bertini, E., Martinelli, D. et al. (2016) Disease-causing SDHAF1 mutations impair transfer of Fe-S clusters to SDHB. *Cell Metab.* **23**, 292–302 https://doi.org/10.1016/j.cmet.2015.12.005
140 Maio, N., Singh, A., Uhrigshardt, H., Saxena, N., Tong, W.H. and Rouault, T.A. (2014) Cochaperone binding to LYR motifs confers specificity of iron sulfur cluster delivery. *Cell Metab.* **19**, 445–457 https://doi.org/10.1016/j.cmet.2014.01.015
141 Na, U., Yu, W., Cox, J., Bricker, D.K., Brockmann, K., Rutter, J. et al. (2014) The LYR factors SDHAF1 and SDHAF3 mediate maturation of the iron-sulfur subunit of succinate dehydrogenase. *Cell Metab.* **20**, 253–266 https://doi.org/10.1016/j.cmet.2014.05.014
142 Dwight, T., Na, U., Kim, E., Zhu, Y., Richardson, A.L., Robinson, B.G. et al. (2017) Analysis of SDHAF3 in familial and sporadic pheochromocytoma and paraganglioma. *BMC Cancer* **17**, 497 https://doi.org/10.1186/s12885-017-3486-z
143 Ghezzi, D., Goffrini, P., Uziel, G., Horvath, R., Klopstock, T., Lochmuller, H. et al. (2009) SDHAF1, encoding a LYR complex-II specific assembly factor, is mutated in SDH-defective infantile leukoencephalopathy. *Nat. Genet.* **41**, 654–656 https://doi.org/10.1038/ng.378
144 Lemarie, A. and Grimm, S. (2009) Mutations in the heme b-binding residue of SDHC inhibit assembly of respiratory chain complex II in mammalian cells. *Mitochondrion* **9**, 254–260 https://doi.org/10.1016/j.mito.2009.03.004
145 Oyedotun, K.S., Sit, C.S. and Lemire, B.D. (2007) The *Saccharomyces cerevisiae* succinate dehydrogenase does not require heme for ubiquinone reduction. *Biochim. Biophys. Acta* **1767**, 1436–1445 https://doi.org/10.1016/j.bbabio.2007.09.008
146 Gault M, D., Mandelker, D., DeLair, D., Stewart, C.R., Kemel, Y., Sheehan, M.R. et al. (2018) Germline SDHA mutations in children and adults with cancer. *Cold Spring Harb. Mol. Case Stud.* **4**, a002584 https://doi.org/10.1101/mcs.a002584
147 Huang, Y., Wang, L.A., Xie, Q., Pang, J., Wang, L., Yi, Y. et al. (2018) Germline SDHB and SDHD mutations in pheochromocytoma and paraganglioma patients. *Endocr. Connect.* **7**, 1217–1225 https://doi.org/10.1530/EC-18-0325
148 Muller, U., Troidl, C. and Niemann, S. (2005) SDHC mutations in hereditary paraganglioma/pheochromocytoma. *Fam. Cancer* **4**, 9–12 https://doi.org/10.1007/s10689-004-0621-1
149 Bayley, J.P., Kunst, H.P., Cascon, A., Sampietro, M.L., Gaal, J., Korpershoek, E. et al. (2010) SDHAF2 mutations in familial and sporadic paraganglioma and phaeochromocytoma. *Lancet Oncol.* **11**, 366–372 https://doi.org/10.1016/S1470-2045(10)70007-3
150 Gill, A.J. (2018) Succinate dehydrogenase (SDH)-deficient neoplasia. *Histopathology* **72**, 106–116 https://doi.org/10.1111/his.13277
151 Dalla Pozza, E., Dando, I., Pacchiana, R., Liboi, E., Scupoli, M.T., Donadelli, M. et al. (2020) Regulation of succinate dehydrogenase and role of succinate in cancer. *Semin. Cell Dev. Biol.* **98**, 4–14 https://doi.org/10.1016/j.semcdb.2019.04.013
152 Bezawork-Geleta, A., Rohlena, J., Dong, L., Pacak, K. and Neuzil, J. (2017) Mitochondrial complex II: at the crossroads. *Trends Biochem. Sci.* **42**, 312–325 https://doi.org/10.1016/j.tibs.2017.01.003
153 Fernandez-Vizarra, E. and Zeviani, M. (2015) Nuclear gene mutations as the cause of mitochondrial complex III deficiency. *Front. Genet.* **6**, 134 https://doi.org/10.3389/fgene.2015.00134
154 Schagger, H., Link, T.A., Engel, W.D. and von Jagow, G. (1986) Isolation of the eleven protein subunits of the bc1 complex from beef heart. *Methods Enzymol.* **126**, 224–237 https://doi.org/10.1016/S0076-6879(86)26024-3

© 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).

155 Iwata, S., Lee, J.W., Okada, K., Lee, J.K., Iwata, M., Rasmussen, B. et al. (1998) Complete structure of the 11-subunit bovine mitochondrial cytochrome bc1 complex. *Science* **281**, 64–71 https://doi.org/10.1126/science.281.5373.64
156 Cooley, J.W. (2013) Protein conformational changes involved in the cytochrome bc1 complex catalytic cycle. *Biochim. Biophys. Acta* **1827**, 1340–1345 https://doi.org/10.1016/j.bbabio.2013.07.007
157 Zara, V., Conte, L. and Trumpower, B.L. (2009) Biogenesis of the yeast cytochrome bc1 complex. *Biochim. Biophys. Acta* **1793**, 89–96 https://doi.org/10.1016/j.bbamcr.2008.04.011
158 Ndi, M., Marin-Buera, L., Salvatori, R., Singh, A.P. and Ott, M. (2018) Biogenesis of the bc1 complex of the mitochondrial respiratory chain. *J. Mol. Biol.* **430**, 3892–3905 https://doi.org/10.1016/j.jmb.2018.04.036
159 Hunte, C., Koepke, J., Lange, C., Rossmanith, T. and Michel, H. (2000) Structure at 2.3 A resolution of the cytochrome bc(1) complex from the yeast *Saccharomyces cerevisiae* co-crystallized with an antibody Fv fragment. *Structure* **8**, 669–684 https://doi.org/10.1016/S0969-2126(00)00152-0
160 Tucker, E.J., Wanschers, B.F., Szklarczyk, R., Mountford, H.S., Wijeyeratne, X.W., van den Brand, M.A. et al. (2013) Mutations in the UQCC1-interacting protein, UQCC2, cause human complex III deficiency associated with perturbed cytochrome b protein expression. *PLoS Genet.* **9**, e1004034 https://doi.org/10.1371/journal.pgen.1004034
161 Gruschke, S., Kehrein, K., Rompler, K., Grone, K., Israel, L., Imhof, A. et al. (2011) Cbp3-Cbp6 interacts with the yeast mitochondrial ribosomal tunnel exit and promotes cytochrome b synthesis and assembly. *J. Cell Biol.* **193**, 1101–1114 https://doi.org/10.1083/jcb.201103132
162 Thompson, K., Mai, N., Olahova, M., Scialo, F., Formosa, L.E., Stroud, D.A. et al. (2018) OXA1L mutations cause mitochondrial encephalopathy and a combined oxidative phosphorylation defect. *EMBO Mol. Med.* **10**, e9060 https://doi.org/10.15252/emmm.201809060
163 Hell, K., Neupert, W. and Stuart, R.A. (2001) Oxa1p acts as a general membrane insertion machinery for proteins encoded by mitochondrial DNA. *EMBO J.* **20**, 1281–1288 https://doi.org/10.1093/emboj/20.6.1281
164 Dumoulin, R., Sagnol, I., Ferlin, T., Bozon, D., Stepien, G. and Mousson, B. (1996) A novel gly290asp mitochondrial cytochrome b mutation linked to a complex III deficiency in progressive exercise intolerance. *Mol. Cell. Probes.* **10**, 389–391 https://doi.org/10.1006/mcpr.1996.0053
165 Andreu, A.L., Hanna, M.G., Reichmann, H., Bruno, C., Penn, A.S., Tanji, K. et al. (1999) Exercise intolerance due to mutations in the cytochrome b gene of mitochondrial DNA. *N. Engl. J. Med.* **341**, 1037–1044 https://doi.org/10.1056/NEJM199909303411404
166 Keightley, J.A., Anitori, R., Burton, M.D., Quan, F., Buist, N.R. and Kennaway, N.G. (2000) Mitochondrial encephalomyopathy and complex III deficiency associated with a stop-codon mutation in the cytochrome b gene. *Am. J. Hum. Genet.* **67**, 1400–1410 https://doi.org/10.1086/316900
167 Legros, F., Chatzoglou, E., Frachon, P., De Baulny H, O., Laforet, P., Jardel, C. et al. (2001) Functional characterization of novel mutations in the human cytochrome b gene. *Eur. J. Hum. Genet.* **9**, 510–518 https://doi.org/10.1038/sj.ejhg.5200678
168 Wibrand, F., Ravn, K., Schwartz, M., Rosenberg, T., Horn, N. and Vissing, J. (2001) Multisystem disorder associated with a missense mutation in the mitochondrial cytochrome b gene. *Ann. Neurol.* **50**, 540–543 https://doi.org/10.1002/ana.1224
169 Schuelke, M., Krude, H., Finckh, B., Mayatepek, E., Janssen, A., Schmelz, M. et al. (2002) Septo-optic dysplasia associated with a new mitochondrial cytochrome b mutation. *Ann. Neurol.* **51**, 388–392 https://doi.org/10.1002/ana.10151
170 Johns, D.R. and Neufeld, M.J. (1991) Cytochrome b mutations in Leber hereditary optic neuropathy. *Biochem. Biophys. Res. Commun.* **181**, 1358–1364 https://doi.org/10.1016/0006-291X(91)92088-2
171 Brown, M.D., Voljavec, A.S., Lott, M.T., Torroni, A., Yang, C.C. and Wallace, D.C. (1992) Mitochondrial DNA complex I and III mutations associated with Leber's hereditary optic neuropathy. *Genetics* **130**, 163–173 PMID: 1732158
172 Johns, D.R. and Neufeld, M.J. (1993) Cytochrome c oxidase mutations in Leber hereditary optic neuropathy. *Biochem. Biophys. Res. Commun.* **196**, 810–815 https://doi.org/10.1006/bbrc.1993.2321
173 Heher, K.L. and Johns, D.R. (1993) A maculopathy associated with the 15257 mitochondrial DNA mutation. *Arch. Ophthalmol.* **111**, 1495–1499 https://doi.org/10.1001/archopht.1993.01090110061024
174 Huoponen, K., Lamminen, T., Juvonen, V., Aula, P., Nikoskelainen, E. and Savontaus, M.L. (1993) The spectrum of mitochondrial DNA mutations in families with Leber hereditary optic neuroretinopathy. *Hum. Genet.* **92**, 379–384 https://doi.org/10.1007/BF01247339
175 Feichtinger, R.G., Brunner-Krainz, M., Alhaddad, B., Wortmann, S.B., Kovacs-Nagy, R., Stojakovic, T. et al. (2017) Combined respiratory chain deficiency and UQCC2 mutations in neonatal encephalomyopathy: defective supercomplex assembly in complex III deficiencies. *Oxidative Med. Cell. longev.* **2017**, 7202589 https://doi.org/10.1155/2017/7202589
176 Acin-Perez, R., Bayona-Bafaluy, M.P., Fernandez-Silva, P., Moreno-Loshuertos, R., Perez-Martos, A., Bruno, C. et al. (2004) Respiratory complex III is required to maintain complex I in mammalian mitochondria. *Mol. Cell* **13**, 805–815 https://doi.org/10.1016/S1097-2765(04)00124-8
177 Hildenbeutel, M., Hegg, E.L., Stephan, K., Gruschke, S., Meunier, B. and Ott, M. (2014) Assembly factors monitor sequential hemylation of cytochrome b to regulate mitochondrial translation. *J. Cell Biol.* **205**, 511–524 https://doi.org/10.1083/jcb.201401009
178 Wanschers, B.F., Szklarczyk, R., van den Brand, M.A., Jonckheere, A., Suijskens, J., Smeets, R. et al. (2014) A mutation in the human CBP4 ortholog UQCC3 impairs complex III assembly, activity and cytochrome b stability. *Hum. Mol. Genet.* **23**, 6356–6365 https://doi.org/10.1093/hmg/ddu357
179 Haut, S., Brivet, M., Touati, G., Rustin, P., Lebon, S., Garcia-Cazorla, A. et al. (2003) A deletion in the human QP-C gene causes a complex III deficiency resulting in hypoglycaemia and lactic acidosis. *Hum. Genet.* **113**, 118–122 https://doi.org/10.1007/s00439-003-0946-0
180 Barel, O., Shorer, Z., Flusser, H., Ofir, R., Narkis, G., Finer, G. et al. (2008) Mitochondrial complex III deficiency associated with a homozygous mutation in UQCRQ. *Am. J. Hum. Genet.* **82**, 1211–1216 https://doi.org/10.1016/j.ajhg.2008.03.020
181 Stephan, K. and Ott, M. (2020) Timing of dimerization of the bc1 complex during mitochondrial respiratory chain assembly. *Biochim. Biophys. Acta* **1861**, 148177 https://doi.org/10.1016/j.bbabio.2020.148177
182 Miyake, N., Yano, S., Sakai, C., Hatakeyama, H., Matsushima, Y., Shiina, M. et al. (2013) Mitochondrial complex III deficiency caused by a homozygous UQCRC2 mutation presenting with neonatal-onset recurrent metabolic decompensation. *Hum. Mutat.* **34**, 446–452 https://doi.org/10.1002/humu.22257
183 Gaignard, P., Eyer, D., Lebigot, E., Oliveira, C., Therond, P., Boutron, A. et al. (2017) UQCRC2 mutation in a patient with mitochondrial complex III deficiency causing recurrent liver failure, lactic acidosis and hypoglycemia. *J. Hum. Genet.* **62**, 729–731 https://doi.org/10.1038/jhg.2017.22
184 Gaignard, P., Menezes, M., Schiff, M., Bayot, A., Rak, M., de Baulny H, O. et al. (2013) Mutations in CYC1, encoding cytochrome c1 subunit of respiratory chain complex III, cause insulin-responsive hyperglycemia. *Am. J. Hum. Genet.* **93**, 384–389 https://doi.org/10.1016/j.ajhg.2013.06.015

© 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).

Biochemical Journal (2020) **477** 4085–4132
https://doi.org/10.1042/BCJ20190767

185 Sanchez, E., Lobo, T., Fox, J.L., Zeviani, M., Winge, D.R. and Fernandez-Vizarra, E. (2013) LYRM7/MZM1L is a UQCRFS1 chaperone involved in the last steps of mitochondrial complex III assembly in human cells. *Biochim. Biophys. Acta* **1827**, 285–293 https://doi.org/10.1016/j.bbabio.2012.11.003
186 Maio, N., Kim, K.S., Singh, A. and Rouault, T.A. (2017) A single adaptable cochaperone-scaffold complex delivers nascent iron-sulfur clusters to mammalian respiratory chain complexes I-III. *Cell Metab.* **25**, 945–953.e6 https://doi.org/10.1016/j.cmet.2017.03.010
187 Wagener, N., Ackermann, M., Funes, S. and Neupert, W. (2011) A pathway of protein translocation in mitochondria mediated by the AAA-ATPase Bcs1. *Mol. Cell* **44**, 191–202 https://doi.org/10.1016/j.molcel.2011.07.036
188 Tang, W.K., Borgnia, M.J., Hsu, A.L., Esser, L., Fox, T., de Val, N. et al. (2020) Structures of AAA protein translocase Bcs1 suggest translocation mechanism of a folded protein. *Nat. Struct. Mol. Biol.* **27**, 202–209 https://doi.org/10.1038/s41594-020-0373-0
189 Kater, L., Wagener, N., Berninghausen, O., Becker, T., Neupert, W. and Beckmann, R. (2020) Structure of the Bcs1 AAA-ATPase suggests an airlock-like translocation mechanism for folded proteins. *Nat. Struct. Mol. Biol.* **27**, 142–149 https://doi.org/10.1038/s41594-019-0364-1
190 Atkinson, A., Smith, P., Fox, J.L., Cui, T.-Z., Khalimonchuk, O. and Winge, D.R. (2011) The LYR protein Mzm1 functions in the insertion of the Rieske Fe/S protein in yeast mitochondria. *Mol. Cell. Biol.* **31**, 3988–3996 https://doi.org/10.1128/MCB.05673-11
191 Cui, T.-Z., Smith, P.M., Fox, J.L., Khalimonchuk, O. and Winge, D.R. (2012) Late-stage maturation of the Rieske Fe/S protein: Mzm1 stabilizes Rip1 but does not facilitate its translocation by the AAA ATPase Bcs1. *Mol. Cell. Biol.* **32**, 4400–4409 https://doi.org/10.1128/MCB.00441-12
192 Dallabona, C., Abbink, T.E.M., Carrozzo, R., Torraco, A., Legati, A., van Berkel, C.G.M. et al. (2016) LYRM7 mutations cause a multifocal cavitating leukoencephalopathy with distinct MRI appearance. *Brain* **139**, 782–794 https://doi.org/10.1093/brain/aww392
193 Hempel, M., Kremer, L.S., Tsiakas, K., Alhaddad, B., Haack, T.B., Löbel, U. et al. (2017) LYRM7 - associated complex III deficiency: a clinical, molecular genetic, MR tomographic, and biochemical study. *Mitochondrion* **37**, 55–61 https://doi.org/10.1016/j.mito.2017.07.001
194 Invernizzi, F., Tigano, M., Dallabona, C., Donnini, C., Ferrero, I., Cremonte, M. et al. (2013) A homozygous mutation in LYRM7/MZM1L associated with early onset encephalopathy, lactic acidosis, and severe reduction of mitochondrial complex III activity. *Hum. Mutat.* **34**, 1619–1622 https://doi.org/10.1002/humu.22441
195 Gusic, M., Schottmann, G., Feichtinger, R.G., Du, C., Scholz, C., Wagner, M. et al. (2020) Bi-Allelic UQCRFS1 variants are associated with mitochondrial complex III deficiency, cardiomyopathy, and alopecia totalis. *Am. J. Hum. Genet.* **106**, 102–111 https://doi.org/10.1016/j.ajhg.2019.12.005
196 Visapaa, I., Fellman, V., Vesa, J., Dasvarma, A., Hutton, J.L., Kumar, V. et al. (2002) GRACILE syndrome, a lethal metabolic disorder with iron overload, is caused by a point mutation in BCS1L. *Am. J. Hum. Genet.* **71**, 863–876 https://doi.org/10.1086/342773
197 Blazquez, A., Gil-Borlado, M.C., Moran, M., Verdu, A., Cazorla-Calleja, M.R., Martin, M.A. et al. (2009) Infantile mitochondrial encephalomyopathy with unusual phenotype caused by a novel BCS1L mutation in an isolated complex III-deficient patient. *Neuromuscul. Disord.* **19**, 143–146 https://doi.org/10.1016/j.nmd.2008.11.016
198 de Lonlay, P., Valnot, I., Barrientos, A., Gorbatyuk, M., Tzagoloff, A., Taanman, J.W. et al. (2001) A mutant mitochondrial respiratory chain assembly protein causes complex III deficiency in patients with tubulopathy, encephalopathy and liver failure. *Nat. Genet.* **29**, 57–60 https://doi.org/10.1038/ng706
199 De Meirleir, L., Seneca, S., Damis, E., Sepulchre, B., Hoorens, A., Gerlo, E. et al. (2003) Clinical and diagnostic characteristics of complex III deficiency due to mutations in the BCS1L gene. *Am. J. Med. Genet. A* **121A**, 126–131 https://doi.org/10.1002/ajmg.a.20171
200 Fernandez-Vizarra, E., Bugiani, M., Goffrini, P., Carrara, F., Farina, L., Procopio, E. et al. (2007) Impaired complex III assembly associated with BCS1L gene mutations in isolated mitochondrial encephalopathy. *Hum. Mol. Genet.* **16**, 1241–1252 https://doi.org/10.1093/hmg/ddm072
201 Hinson, J.T., Fantin, V.R., Schonberger, J., Breivik, N., Siem, G., McDonough, B. et al. (2007) Missense mutations in the BCS1L gene as a cause of the Bjornstad syndrome. *N. Engl. J. Med.* **356**, 809–819 https://doi.org/10.1056/NEJMoa055262
202 Lynn, A.M., King, R.I., Mackay, R.J., Florkowski, C.M. and Wilson, C.J. (2012) BCS1L gene mutation presenting with GRACILE-like syndrome and complex III deficiency. *Ann. Clin. Biochem.* **49**(Pt 2), 201–203 https://doi.org/10.1258/acb.2011.011180
203 Ghezzi, D., Arzuffi, P., Zordan, M., Da Re, C., Lamperti, C., Benna, C. et al. (2011) Mutations in TTC19 cause mitochondrial complex III deficiency and neurological impairment in humans and flies. *Nat. Genet.* **43**, 259–263 https://doi.org/10.1038/ng.761
204 Bottani, E., Cerutti, R., Harbour, M.E., Ravaglia, S., Dogan, S.A., Giordano, C. et al. (2017) TTC19 plays a husbandry role on UQCRFS1 turnover in the biogenesis of mitochondrial respiratory complex III. *Mol. Cell* **67**, 96–105.e4 https://doi.org/10.1016/j.molcel.2017.06.001
205 Mordaunt, D.A., Jolley, A., Balasubramaniam, S., Thorburn, D.R., Mountford, H.S., Compton, A.G. et al. (2015) Phenotypic variation of TTC19-deficient mitochondrial complex III deficiency: a case report and literature review. *Am. J. Med. Genet. A* **167**, 1330–1336 https://doi.org/10.1002/ajmg.a.36968
206 Habibzadeh, P., Inaloo, S., Silawi, M., Dastsooz, H., Farazi Fard, M.A., Sadeghipour, F. et al. (2019) A novel TTC19 mutation in a patient with neurological, psychological, and gastrointestinal impairment. *Front. Neurol.* **10**, 944 https://doi.org/10.3389/fneur.2019.00944
207 Conboy, E., Selcen, D., Brodsky, M., Gavrilova, R. and Ho, M.L. (2018) Novel homozygous variant in TTC19 causing mitochondrial complex III deficiency with recurrent stroke-like episodes: expanding the phenotype. *Semin. Pediatr. Neurol.* **26**, 16–20 https://doi.org/10.1016/j.spen.2018.04.003
208 Nogueira, C., Barros, J., Sa, M.J., Azevedo, L., Taipa, R., Torraco, A. et al. (2013) Novel TTC19 mutation in a family with severe psychiatric manifestations and complex III deficiency. *Neurogenetics* **14**, 153–160 https://doi.org/10.1007/s10048-013-0361-1
209 Rak, M., Benit, P., Chretien, D., Bouchereau, J., Schiff, M., El-Khoury, R. et al. (2016) Mitochondrial cytochrome c oxidase deficiency. *Clin. Sci. (Lond)* **130**, 393–407 https://doi.org/10.1042/CS20150707
210 Timon-Gomez, A., Nyvltova, E., Abriata, L.A., Vila, A.J., Hosler, J. and Barrientos, A. (2018) Mitochondrial cytochrome c oxidase biogenesis: recent developments. *Semin. Cell Dev. Biol.* **76**, 163–178 https://doi.org/10.1016/j.semcdb.2017.08.055
211 Tsukihara, T., Aoyama, H., Yamashita, E., Tomizaki, T., Yamaguchi, H., Shinzawa-Itoh, K. et al. (1996) The whole structure of the 13-subunit oxidized cytochrome c oxidase at 2.8 A. *Science* **272**, 1136–1144 https://doi.org/10.1126/science.272.5265.1136
212 Balsa, E., Marco, R., Perales-Clemente, E., Szklarczyk, R., Calvo, E., Landazuri, M.O. et al. (2012) NDUFA4 is a subunit of complex IV of the mammalian electron transport chain. *Cell Metab.* **16**, 378–386 https://doi.org/10.1016/j.cmet.2012.07.015
213 Sinkler, C.A., Kalpage, H., Shay, J., Lee, I., Malek, M.H., Grossman, L.I. et al. (2017) Tissue- and condition-specific isoforms of mammalian cytochrome c oxidase subunits: from function to human disease. *Oxid. Med. Cell Longev.* **2017**, 1534056 https://doi.org/10.1155/2017/1534056
214 Hock, D.H., Reljic, B., Ang, C.S., Muellner-Wong, L., Mountford, H.S., Compton, A.G. et al. (2020) HIGD2A is required for assembly of the COX3 module of human mitochondrial complex IV. *Mol. Cell Proteom.* **19**, 1145–1160 https://doi.org/10.1074/mcp.RA120.002076
215 Vidoni, S., Harbour, M.E., Guerrero-Castillo, S., Signes, A., Ding, S., Fearnley, I.M. et al. (2017) MR-1S interacts with PET100 and PET117 in module-based assembly of human cytochrome c oxidase. *Cell Rep.* **18**, 1727–1738 https://doi.org/10.1016/j.celrep.2017.01.044

 © 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).

216 Weraarpachai, W., Antonicka, H., Sasarman, F., Seeger, J., Schrank, B., Kolesar, J.E. et al. (2009) Mutation in TACO1, encoding a translational activator of COX I, results in cytochrome c oxidase deficiency and late-onset Leigh syndrome. *Nat. Genet.* **41**, 833–837 https://doi.org/10.1038/ng.390
217 Richman, T.R., Spahr, H., Ermer, J.A., Davies, S.M., Viola, H.M., Bates, K.A. et al. (2016) Loss of the RNA-binding protein TACO1 causes late-onset mitochondrial dysfunction in mice. *Nat. Commun.* **7**, 11884 https://doi.org/10.1038/ncomms11884
218 Weraarpachai, W., Sasarman, F., Nishimura, T., Antonicka, H., Aure, K., Rotig, A. et al. (2012) Mutations in C12orf62, a factor that couples COX I synthesis with cytochrome c oxidase assembly, cause fatal neonatal lactic acidosis. *Am. J. Hum. Genet.* **90**, 142–151 https://doi.org/10.1016/j.ajhg.2011.11.027
219 Szklarczyk, R., Wanschers, B.F., Cuypers, T.D., Esseling, J.J., Riemersma, M., van den Brand, M.A. et al. (2012) Iterative orthology prediction uncovers new mitochondrial proteins and identifies C12orf62 as the human ortholog of COX14, a protein involved in the assembly of cytochrome c oxidase. *Genome Biol.* **13**, R12 https://doi.org/10.1186/gb-2012-13-2-r12
220 Clemente, P., Peralta, S., Cruz-Bermudez, A., Echevarria, L., Fontanesi, F., Barrientos, A. et al. (2013) hCOA3 stabilizes cytochrome c oxidase 1 (COX1) and promotes cytochrome c oxidase assembly in human mitochondria. *J. Biol. Chem.* **288**, 8321–8331 https://doi.org/10.1074/jbc.M112.422220
221 Bourens, M. and Barrientos, A. (2017) A CMC1-knockout reveals translation-independent control of human mitochondrial complex IV biogenesis. *EMBO Rep.* **18**, 477–494 https://doi.org/10.15252/embr.201643103
222 Seeger, J., Schrank, B., Pyle, A., Stucka, R., Lorcher, U., Muller-Ziermann, S. et al. (2010) Clinical and neuropathological findings in patients with TACO1 mutations. *Neuromuscul. Disord.* **20**, 720–724 https://doi.org/10.1016/j.nmd.2010.06.017
223 Makrythanasis, P., Nelis, M., Santoni, F.A., Guipponi, M., Vannier, A., Bena, F. et al. (2014) Diagnostic exome sequencing to elucidate the genetic basis of likely recessive disorders in consanguineous families. *Hum. Mutat.* **35**, 1203–1210 https://doi.org/10.1002/humu.22617
224 Bruno, C., Martinuzzi, A., Tang, Y., Andreu, A.L., Pallotti, F., Bonilla, E. et al. (1999) A stop-codon mutation in the human mtDNA cytochrome c oxidase I gene disrupts the functional structure of complex IV. *Am. J. Hum. Genet.* **65**, 611–620 https://doi.org/10.1086/302546
225 Karadimas, C.L., Greenstein, P., Sue, C.M., Joseph, J.T., Tanji, K., Haller, R.G. et al. (2000) Recurrent myoglobinuria due to a nonsense mutation in the COX I gene of mitochondrial DNA. *Neurology* **55**, 644–649 https://doi.org/10.1212/WNL.55.5.644
226 Poole, O.V., Everett, C.M., Gandhi, S., Marino, S., Bugiardini, E., Woodward, C. et al. (2019) Adult-onset Leigh syndrome linked to the novel stop codon mutation m.6579G > A in MT-CO1. *Mitochondrion* **47**, 294–297 https://doi.org/10.1016/j.mito.2019.02.004
227 Ostergaard, E., Weraarpachai, W., Ravn, K., Born, A.P., Jonson, L., Duno, M. et al. (2015) Mutations in COA3 cause isolated complex IV deficiency associated with neuropathy, exercise intolerance, obesity, and short stature. *J. Med. Genet.* **52**, 203–207 https://doi.org/10.1136/jmedgenet-2014-102914
228 Soto, I.C., Fontanesi, F., Liu, J. and Barrientos, A. (2012) Biogenesis and assembly of eukaryotic cytochrome c oxidase catalytic core. *Biochim. Biophys. Acta* **1817**, 883–897 https://doi.org/10.1016/j.bbabio.2011.09.005
229 Bundschuh, F.A., Hoffmeier, K. and Ludwig, B. (2008) Two variants of the assembly factor Surf1 target specific terminal oxidases in Paracoccus denitrificans. *Biochim. Biophys. Acta* **1777**, 1336–1343 https://doi.org/10.1016/j.bbabio.2008.05.448
230 Bundschuh, F.A., Hannappel, A., Anderka, O. and Ludwig, B. (2009) Surf1, associated with Leigh syndrome in humans, is a heme-binding protein in bacterial oxidase biogenesis. *J. Biol. Chem.* **284**, 25735–25741 https://doi.org/10.1074/jbc.M109.040295
231 Huttemann, M., Kadenbach, B. and Grossman, L.I. (2001) Mammalian subunit IV isoforms of cytochrome c oxidase. *Gene* **267**, 111–123 https://doi.org/10.1016/S0378-1119(01)00385-7
232 Abu-Libdeh, B., Douiev, L., Amro, S., Shahrour, M., Ta-Shma, A., Miller, C. et al. (2017) Mutation in the COX4I1 gene is associated with short stature, poor weight gain and increased chromosomal breaks, simulating Fanconi anemia. *Eur. J. Hum. Genet.* **25**, 1142–1146 https://doi.org/10.1038/ejhg.2017.112
233 Pillai, N.R., AlDhaheri, N.S., Ghosh, R., Lim, J., Streff, H., Nayak, A. et al. (2019) Biallelic variants in COX4I1 associated with a novel phenotype resembling Leigh syndrome with developmental regression, intellectual disability, and seizures. *Am. J. Med. Genet. A* **179**, 2138–2143 https://doi.org/10.1002/ajmg.a.61288
234 Garbarino, J.E. and Gibbons, I.R. (2002) Expression and genomic analysis of midasin, a novel and highly conserved AAA protein distantly related to dynein. *BMC Genom.* **3**, 18 https://doi.org/10.1186/1471-2164-3-18
235 Raman, N., Weir, E. and Muller, S. (2016) The AAA ATPase MDN1 acts as a SUMO-targeted regulator in mammalian pre-ribosome remodeling. *Mol. Cell* **64**, 607–615 https://doi.org/10.1016/j.molcel.2016.09.039
236 Shteyer, E., Saada, A., Shaag, A., Al-Hijawi, F.A., Kidess, R., Revel-Vilk, S. et al. (2009) Exocrine pancreatic insufficiency, dyserythropoeitic anemia, and calvarial hyperostosis are caused by a mutation in the COX4I2 gene. *Am. J. Hum. Genet.* **84**, 412–417 https://doi.org/10.1016/j.ajhg.2009.02.006
237 Baertling, F., Al-Murshedi, F., Sanchez-Caballero, L., Al-Senaidi, K., Joshi, N.P., Venselaar, H. et al. (2017) Mutation in mitochondrial complex IV subunit COX5A causes pulmonary arterial hypertension, lactic acidemia, and failure to thrive. *Hum. Mutat.* **38**, 692–703 https://doi.org/10.1002/humu.23210
238 Valnot, I., von Kleist-Retzow, J.C., Barrientos, A., Gorbatyuk, M., Taanman, J.W., Mehaye, B. et al. (2000) A mutation in the human heme A: farnesyltransferase gene (COX10) causes cytochrome c oxidase deficiency. *Hum. Mol. Genet.* **9**, 1245–1249 https://doi.org/10.1093/hmg/9.8.1245
239 Antonicka, H., Leary, S.C., Guercin, G.H., Agar, J.N., Horvath, R., Kennaway, N.G. et al. (2003) Mutations in COX10 result in a defect in mitochondrial heme A biosynthesis and account for multiple, early-onset clinical phenotypes associated with isolated COX deficiency. *Hum. Mol. Genet.* **12**, 2693–2702 https://doi.org/10.1093/hmg/ddg284
240 Antonicka, H., Mattman, A., Carlson, C.G., Glerum, D.M., Hoffbuhr, K.C., Leary, S.C. et al. (2003) Mutations in COX15 produce a defect in the mitochondrial heme biosynthetic pathway, causing early-onset fatal hypertrophic cardiomyopathy. *Am. J. Hum. Genet.* **72**, 101–114 https://doi.org/10.1086/345489
241 Bugiani, M., Tiranti, V., Farina, L., Uziel, G. and Zeviani, M. (2005) Novel mutations in COX15 in a long surviving Leigh syndrome patient with cytochrome c oxidase deficiency. *J. Med. Genet.* **42**, e28 https://doi.org/10.1136/jmg.2004.029926
242 Wedatilake, Y., Brown, R.M., McFarland, R., Yaplito-Lee, J., Morris, A.A., Champion, M. et al. (2013) SURF1 deficiency: a multi-centre natural history study. *Orphanet. J. Rare Dis.* **8**, 96 https://doi.org/10.1186/1750-1172-8-96
243 Stiburek, L., Vesela, K., Hansikova, H., Pecina, P., Tesarova, M., Cerna, L. et al. (2005) Tissue-specific cytochrome c oxidase assembly defects due to mutations in SCO2 and SURF1. *Biochem. J.* **392**(Pt 3), 625–632 https://doi.org/10.1042/BJ20050807
244 Glerum, D.M., Shtanko, A. and Tzagoloff, A. (1996) Characterization of COX17, a yeast gene involved in copper metabolism and assembly of cytochrome oxidase. *J. Biol. Chem.* **271**, 14504–14509 https://doi.org/10.1074/jbc.271.24.14504

© 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).

Biochemical Journal (2020) **477** 4085–4132
https://doi.org/10.1042/BCJ20190767

245 Cobine, P.A., Pierrel, F. and Winge, D.R. (2006) Copper trafficking to the mitochondrion and assembly of copper metalloenzymes. *Biochim. Biophys. Acta* **1763**, 759–772 https://doi.org/10.1016/j.bbamcr.2006.03.002
246 Banci, L., Bertini, I., Cantini, F., Ciofi-Baffoni, S., Gonnelli, L. and Mangani, S. (2004) Solution structure of Cox11, a novel type of beta-immunoglobulin-like fold involved in CuB site formation of cytochrome c oxidase. *J. Biol. Chem.* **279**, 34833–34839 https://doi.org/10.1074/jbc.M403655200
247 Bode, M., Woellhaf, M.W., Bohnert, M., van der Laan, M., Sommer, F., Jung, M. et al. (2015) Redox-regulated dynamic interplay between Cox19 and the copper-binding protein Cox11 in the intermembrane space of mitochondria facilitates biogenesis of cytochrome c oxidase. *Mol. Biol. Cell* **26**, 2385–2401 https://doi.org/10.1091/mbc.E14-11-1526
248 Dennerlein, S., Oeljeklaus, S., Jans, D., Hellwig, C., Bareth, B., Jakobs, S. et al. (2015) MITRAC7 acts as a COX1-specific chaperone and reveals a checkpoint during cytochrome c oxidase assembly. *Cell Rep.* **12**, 1644–1655 https://doi.org/10.1016/j.celrep.2015.08.009
249 Hayashi, T., Asano, Y., Shintani, Y., Aoyama, H., Kioka, H., Tsukamoto, O. et al. (2015) Higd1a is a positive regulator of cytochrome c oxidase. *Proc. Natl. Acad. Sci. U.S.A.* **112**, 1553–1558 https://doi.org/10.1073/pnas.1419767112
250 Protasoni, M., Perez-Perez, R., Lobo-Jarne, T., Harbour, M.E., Ding, S., Penas, A. et al. (2020) Respiratory supercomplexes act as a platform for complex III-mediated maturation of human mitochondrial complexes I and IV. *EMBO J.* **39**, e102817 https://doi.org/10.15252/embj.2019102817
251 Bourens, M., Boulet, A., Leary, S.C. and Barrientos, A. (2014) Human COX20 cooperates with SCO1 and SCO2 to mature COX2 and promote the assembly of cytochrome c oxidase. *Hum. Mol. Genet.* **23**, 2901–2913 https://doi.org/10.1093/hmg/ddu003
252 Vissing, C.R., Duno, M., Olesen, J.H., Rafiq, J., Risom, L., Christensen, E. et al. (2013) Recurrent myoglobinuria and deranged acylcarnitines due to a mutation in the mtDNA MT-CO2 gene. *Neurology* **80**, 1908–1910 https://doi.org/10.1212/WNL.0b013e3182929fb2
253 Zierz, C.M., Baty, K., Blakely, E.L., Hopton, S., Falkous, G., Schaefer, A.M. et al. (2019) A novel pathogenic variant in MT-CO2 causes an isolated mitochondrial complex IV deficiency and late-onset cerebellar ataxia. *J. Clin. Med.* **8**, 789 https://doi.org/10.3390/jcm8060789
254 Roos, S., Sofou, K., Hedberg-Oldfors, C., Kollberg, G., Lindgren, U., Thomsen, C. et al. (2019) Mitochondrial complex IV deficiency caused by a novel frameshift variant in MT-CO2 associated with myopathy and perturbed acylcarnitine profile. *Eur. J. Hum. Genet.* **27**, 331–335 https://doi.org/10.1038/s41431-018-0286-0
255 Wong, L.J., Dai, P., Tan, D., Lipson, M., Grix, A., Sifry-Platt, M. et al. (2001) Severe lactic acidosis caused by a novel frame-shift mutation in mitochondrial-encoded cytochrome c oxidase subunit II. *Am. J. Med. Genet.* **102**, 95–99 https://doi.org/10.1002/1096-8628(20010722)102:1<95::AID-AJMG1412>3.0.CO;2-U
256 Szklarczyk, R., Wanschers, B.F., Nijtmans, L.G., Rodenburg, R.J., Zschocke, J., Dikow, N. et al. (2013) A mutation in the FAM36A gene, the human ortholog of COX20, impairs cytochrome c oxidase assembly and is associated with ataxia and muscle hypotonia. *Hum. Mol. Genet.* **22**, 656–667 https://doi.org/10.1093/hmg/dds473
257 Doss, S., Lohmann, K., Seibler, P., Arns, B., Klopstock, T., Zuhlke, C. et al. (2014) Recessive dystonia-ataxia syndrome in a Turkish family caused by a COX20 (FAM36A) mutation. *J. Neurol.* **261**, 207–212 https://doi.org/10.1007/s00415-013-7177-7
258 Otero, M.G., Tiongson, E., Diaz, F., Haude, K., Panzer, K., Collier, A. et al. (2019) Novel pathogenic COX20 variants causing dysarthria, ataxia, and sensory neuropathy. *Ann. Clin. Transl. Neurol.* **6**, 154–160 https://doi.org/10.1002/acn3.661
259 Xu, H., Ji, T., Lian, Y., Wang, S., Chen, X., Li, S. et al. (2019) Observation of novel COX20 mutations related to autosomal recessive axonal neuropathy and static encephalopathy. *Hum. Genet.* **138**, 749–756 https://doi.org/10.1007/s00439-019-02026-4
260 Banci, L., Bertini, I., Ciofi-Baffoni, S., Hadjiloi, T., Martinelli, M. and Palumaa, P. (2008) Mitochondrial copper(I) transfer from Cox17 to Sco1 is coupled to electron transfer. *Proc. Natl. Acad. Sci. U.S.A.* **105**, 6803–6808 https://doi.org/10.1073/pnas.0800019105
261 Morgada, M.N., Abriata, L.A., Cefaro, C., Gajda, K., Banci, L. and Vila, A.J. (2015) Loop recognition and copper-mediated disulfide reduction underpin metal site assembly of CuA in human cytochrome oxidase. *Proc. Natl. Acad. Sci. U.S.A.* **112**, 11771–11776 https://doi.org/10.1073/pnas.1505056112
262 Stroud, D.A., Maher, M.J., Lindau, C., Vogtle, F.N., Frazier, A.E., Surgenor, E. et al. (2015) COA6 is a mitochondrial complex IV assembly factor critical for biogenesis of mtDNA-encoded COX2. *Hum. Mol. Genet.* **24**, 5404–5415 https://doi.org/10.1093/hmg/ddv265
263 Bourens, M. and Barrientos, A. (2017) Human mitochondrial cytochrome c oxidase assembly factor COX18 acts transiently as a membrane insertase within the subunit 2 maturation module. *J. Biol. Chem.* **292**, 7774–7783 https://doi.org/10.1074/jbc.M117.778514
264 Aich, A., Wang, C., Chowdhury, A., Ronsor, C., Pacheu-Grau, D., Richter-Dennerlein, R. et al. (2018) COX16 promotes COX2 metallation and assembly during respiratory complex IV biogenesis. *eLife* **7**, e32572 https://doi.org/10.7554/eLife.32572
265 Lorenzi, I., Oeljeklaus, S., Aich, A., Ronsor, C., Callegari, S., Dudek, J. et al. (2018) The mitochondrial TMEM177 associates with COX20 during COX2 biogenesis. *Biochim. Biophys. Acta Mol. Cell Res.* **1865**, 323–333 https://doi.org/10.1016/j.bbamcr.2017.11.010
266 Valnot, I., Osmond, S., Gigarel, N., Mehaye, B., Amiel, J., Cormier-Daire, V. et al. (2000) Mutations of the SCO1 gene in mitochondrial cytochrome c oxidase deficiency with neonatal-onset hepatic failure and encephalopathy. *Am. J. Hum. Genet.* **67**, 1104–1109 https://doi.org/10.1016/S0002-9297(07)62940-1
267 Stiburek, L., Vesela, K., Hansikova, H., Hulkova, H. and Zeman, J. (2009) Loss of function of Sco1 and its interaction with cytochrome c oxidase. *Am. J. Physiol. Cell Physiol.* **296**, C1218–C1226 https://doi.org/10.1152/ajpcell.00564.2008
268 Papadopoulou, L.C., Sue, C.M., Davidson, M.M., Tanji, K., Nishino, I., Sadlock, J.E. et al. (1999) Fatal infantile cardioencephalomyopathy with COX deficiency and mutations in SCO2, a COX assembly gene. *Nat. Genet.* **23**, 333–337 https://doi.org/10.1038/15513
269 Salviati, L., Sacconi, S., Rasalan, M.M., Kronn, D.F., Braun, A., Canoll, P. et al. (2002) Cytochrome c oxidase deficiency due to a novel SCO2 mutation mimics Werdnig-Hoffmann disease. *Arch. Neurol.* **59**, 862–865 https://doi.org/10.1001/archneur.59.5.862
270 Verdijk, R.M., de Krijger, R., Schoonderwoerd, K., Tiranti, V., Smeets, H., Govaerts, L.C. et al. (2008) Phenotypic consequences of a novel SCO2 gene mutation. *Am. J. Med. Genet. A* **146A**, 2822–2827 https://doi.org/10.1002/ajmg.a.32523
271 Ghosh, A., Trivedi, P.P., Timbalia, S.A., Griffin, A.T., Rahn, J.J., Chan, S.S. et al. (2014) Copper supplementation restores cytochrome c oxidase assembly defect in a mitochondrial disease model of COA6 deficiency. *Hum. Mol. Genet.* **23**, 3596–3606 https://doi.org/10.1093/hmg/ddu069
272 Baertling, F., AMvdB, M., Hertecant, J.L., Al-Shamsi, A., PvdH, L., Distelmaier, F. et al. (2015) Mutations in COA6 cause cytochrome c oxidase deficiency and neonatal hypertrophic cardiomyopathy. *Hum. Mutat.* **36**, 34–38 https://doi.org/10.1002/humu.22715
273 Church, C., Goehring, B., Forsha, D., Wazny, P. and Poyton, R.O. (2005) A role for Pet100p in the assembly of yeast cytochrome c oxidase: interaction with a subassembly that accumulates in a pet100 mutant. *J. Biol. Chem.* **280**, 1854–1863 https://doi.org/10.1074/jbc.M410726200

© 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).

274 Lim, S.C., Smith, K.R., Stroud, D.A., Compton, A.G., Tucker, E.J., Dasvarma, A. et al. (2014) A founder mutation in PET100 causes isolated complex IV deficiency in Lebanese individuals with Leigh syndrome. *Am. J. Hum. Genet.* **94**, 209–222 https://doi.org/10.1016/j.ajhg.2013.12.015
275 Taylor, N.G., Swenson, S., Harris, N.J., Germany, E.M., Fox, J.L. and Khalimonchuk, O. (2017) The assembly factor Pet117 couples heme a synthase activity to cytochrome oxidase assembly. *J. Biol. Chem.* **292**, 1815–1825 https://doi.org/10.1074/jbc.M116.766980
276 Zvulunov, A., Kachko, L., Manor, E., Shinwell, E. and Carmi, R. (1998) Reticulolinear aplasia cutis congenita of the face and neck: a distinctive cutaneous manifestation in several syndromes linked to Xp22. *Br. J. Dermatol.* **138**, 1046–1052 https://doi.org/10.1046/j.1365-2133.1998.02277.x
277 Indrieri, A., van Rahden, V.A., Tiranti, V., Morleo, M., Iaconis, D., Tammaro, R. et al. (2012) Mutations in COX7B cause microphthalmia with linear skin lesions, an unconventional mitochondrial disease. *Am. J. Hum. Genet.* **91**, 942–949 https://doi.org/10.1016/j.ajhg.2012.09.016
278 Hallmann, K., Kudin, A.P., Zsurka, G., Kornblum, C., Reimann, J., Stuve, B. et al. (2016) Loss of the smallest subunit of cytochrome c oxidase, COX8A, causes Leigh-like syndrome and epilepsy. *Brain* **139**, 338–345 https://doi.org/10.1093/brain/awv357
279 Olahova, M., Haack, T.B., Alston, C.L., Houghton, J.A., He, L., Morris, A.A. et al. (2015) A truncating PET100 variant causing fatal infantile lactic acidosis and isolated cytochrome c oxidase deficiency. *Eur. J. Hum. Genet.* **23**, 935–939 https://doi.org/10.1038/ejhg.2014.214
280 Renkema, G.H., Visser, G., Baertling, F., Wintjes, L.T., Wolters, V.M., van Montfrans, J. et al. (2017) Mutated PET117 causes complex IV deficiency and is associated with neurodevelopmental regression and medulla oblongata lesions. *Hum. Genet.* **136**, 759–769 https://doi.org/10.1007/s00439-017-1794-7
281 Ogunjimi, E.O., Pokalsky, C.N., Shroyer, L.A. and Prochaska, L.J. (2000) Evidence for a conformational change in subunit III of bovine heart mitochondrial cytochrome c oxidase. *J. Bioenerg. Biomembr.* **32**, 617–626 https://doi.org/10.1023/A:1005678729157
282 Chen, Y.C., Taylor, E.B., Dephoure, N., Heo, J.M., Tonhato, A., Papandreou, I. et al. (2012) Identification of a protein mediating respiratory supercomplex stability. *Cell Metab.* **15**, 348–360 https://doi.org/10.1016/j.cmet.2012.02.006
283 Tiranti, V., Corona, P., Greco, M., Taanman, J.W., Carrara, F., Lamantea, E. et al. (2000) A novel frameshift mutation of the mtDNA COIII gene leads to impaired assembly of cytochrome c oxidase in a patient affected by Leigh-like syndrome. *Hum. Mol. Genet.* **9**, 2733–2742 https://doi.org/10.1093/hmg/9.18.2733
284 Horvath, R., Scharfe, C., Hoeltzenbein, M., Do, B.H., Schroder, C., Warzok, R. et al. (2002) Childhood onset mitochondrial myopathy and lactic acidosis caused by a stop mutation in the mitochondrial cytochrome c oxidase III gene. *J. Med. Genet.* **39**, 812–816 https://doi.org/10.1136/jmg.39.11.812
285 Manfredi, G., Schon, E.A., Moraes, C.T., Bonilla, E., Berry, G.T., Sladky, J.T. et al. (1995) A new mutation associated with MELAS is located in a mitochondrial DNA polypeptide-coding gene. *Neuromuscul. Disord.* **5**, 391–398 https://doi.org/10.1016/0960-8966(94)00079-O
286 Keightley, J.A., Hoffbuhr, K.C., Burton, M.D., Salas, V.M., Johnston, W.S., Penn, A.M. et al. (1996) A microdeletion in cytochrome c oxidase (COX) subunit III associated with COX deficiency and recurrent myoglobinuria. *Nat. Genet.* **12**, 410–416 https://doi.org/10.1038/ng0496-410
287 Tamiya, G., Makino, S., Hayashi, M., Abe, A., Numakura, C., Ueki, M. et al. (2014) A mutation of COX6A1 causes a recessive axonal or mixed form of Charcot-Marie-Tooth disease. *Am. J. Hum. Genet.* **95**, 294–300 https://doi.org/10.1016/j.ajhg.2014.07.013
288 Inoue, M., Uchino, S., Iida, A., Noguchi, S., Hayashi, S., Takahashi, T. et al. (2019) COX6A2 variants cause a muscle-specific cytochrome c oxidase deficiency. *Ann. Neurol.* **86**, 193–202 https://doi.org/10.1002/ana.25517
289 Taanman, J.W., Hall, R.E., Tang, C., Marusich, M.F., Kennaway, N.G. and Capaldi, R.A. (1993) Tissue distribution of cytochrome c oxidase isoforms in mammals. Characterization with monoclonal and polyclonal antibodies. *Biochim. Biophys. Acta* **1225**, 95–100 https://doi.org/10.1016/0925-4439(93)90128-N
290 Zong, S., Wu, M., Gu, J., Liu, T., Guo, R. and Yang, M. (2018) Structure of the intact 14-subunit human cytochrome c oxidase. *Cell Res.* **28**, 1026–1034 https://doi.org/10.1038/s41422-018-0071-1
291 Pitceathly, R.D., Rahman, S., Wedatilake, Y., Polke, J.M., Cirak, S., Foley, A.R. et al. (2013) NDUFA4 mutations underlie dysfunction of a cytochrome c oxidase subunit linked to human neurological disease. *Cell Rep.* **3**, 1795–1805 https://doi.org/10.1016/j.celrep.2013.05.005
292 Lyons A, M., Ardissone, A., Reyes, A., Robinson, A.J., Moroni, I., Ghezzi, D. et al. (2016) COA7 (C1orf163/RESA1) mutations associated with mitochondrial leukoencephalopathy and cytochrome c oxidase deficiency. *J. Med. Genet.* **53**, 846–849 https://doi.org/10.1136/jmedgenet-2016-104194
293 Gu, J., Zhang, L., Zong, S., Guo, R., Liu, T., Yi, J. et al. (2019) Cryo-EM structure of the mammalian ATP synthase tetramer bound with inhibitory protein IF1. *Science* **364**, 1068–1075 https://doi.org/10.1126/science.aaw4852
294 Jonckheere, A.I., Smeitink, J.A. and Rodenburg, R.J. (2012) Mitochondrial ATP synthase: architecture, function and pathology. *J. Inherit. Metab. Dis.* **35**, 211–225 https://doi.org/10.1007/s10545-011-9382-9
295 Ackerman, S.H. (2002) Atp11p and Atp12p are chaperones for F(1)-ATPase biogenesis in mitochondria. *Biochim. Biophys. Acta* **1555**, 101–105 https://doi.org/10.1016/S0005-2728(02)00262-1
296 Wang, Z.G. and Ackerman, S.H. (2000) The assembly factor Atp11p binds to the beta-subunit of the mitochondrial F(1)-ATPase. *J. Biol. Chem.* **275**, 5767–5772 https://doi.org/10.1074/jbc.275.8.5767
297 Wang, Z.G., Sheluho, D., Gatti, D.L. and Ackerman, S.H. (2000) The alpha-subunit of the mitochondrial F(1) ATPase interacts directly with the assembly factor Atp12p. *EMBO J.* **19**, 1486–1493 https://doi.org/10.1093/emboj/19.7.1486
298 Lefebvre-Legendre, L., Salin, B., Schaeffer, J., Brethes, D., Dautant, A., Ackerman, S.H. et al. (2005) Failure to assemble the alpha 3 beta 3 subcomplex of the ATP synthase leads to accumulation of the alpha and beta subunits within inclusion bodies and the loss of mitochondrial cristae in *Saccharomyces cerevisiae*. *J. Biol. Chem.* **280**, 18386–18392 https://doi.org/10.1074/jbc.M410789200
299 Jonckheere, A.I., Renkema, G.H., Bras, M., van den Heuvel, L.P., Hoischen, A., Gilissen, C. et al. (2013) A complex V ATP5A1 defect causes fatal neonatal mitochondrial encephalopathy. *Brain* **136**(Pt 5), 1544–1554 https://doi.org/10.1093/brain/awt086
300 Lieber, D.S., Calvo, S.E., Shanahan, K., Slate, N.G., Liu, S., Hershman, S.G. et al. (2013) Targeted exome sequencing of suspected mitochondrial disorders. *Neurology* **80**, 1762–1770 https://doi.org/10.1212/WNL.0b013e3182918c40
301 De Meirleir, L., Seneca, S., Lissens, W., De Clercq, I., Eyskens, F., Gerlo, E. et al. (2004) Respiratory chain complex V deficiency due to a mutation in the assembly gene ATP12. *J. Med. Genet.* **41**, 120–124 https://doi.org/10.1136/jmg.2003.012047
302 Pecina, P., Nuskova, H., Karbanova, V., Kaplanova, V., Mracek, T. and Houstek, J. (2018) Role of the mitochondrial ATP synthase central stalk subunits gamma and delta in the activity and assembly of the mammalian enzyme. *Biochim. Biophys. Acta Bioenerg.* **1859**, 374–381 https://doi.org/10.1016/j.bbabio.2018.02.007

© 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).

303 Ludlam, A., Brunzelle, J., Pribyl, T., Xu, X., Gatti, D.L. and Ackerman, S.H. (2009) Chaperones of F1-ATPase. *J. Biol. Chem.* **284**, 17138–17146 https://doi.org/10.1074/jbc.M109.002568
304 Ruhle, T. and Leister, D. (2015) Assembly of F1F0-ATP synthases. *Biochim. Biophys. Acta* **1847**, 849–860 https://doi.org/10.1016/j.bbabio.2015.02.005
305 Olahova, M., Yoon, W.H., Thompson, K., Jangam, S., Fernandez, L., Davidson, J.M. et al. (2018) Biallelic mutations in ATP5F1D, which encodes a subunit of ATP synthase, cause a metabolic disorder. *Am. J. Hum. Genet.* **102**, 494–504 https://doi.org/10.1016/j.ajhg.2018.01.020
306 Mayr, J.A., Havlickova, V., Zimmermann, F., Magler, I., Kaplanova, V., Jesina, P. et al. (2010) Mitochondrial ATP synthase deficiency due to a mutation in the ATP5E gene for the F1 epsilon subunit. *Hum. Mol. Genet.* **19**, 3430–3439 https://doi.org/10.1093/hmg/ddq254
307 Yan, W.L., Lerner, T.J., Haines, J.L. and Gusella, J.F. (1994) Sequence analysis and mapping of a novel human mitochondrial ATP synthase subunit 9 cDNA (ATP5G3). *Genomics* **24**, 375–377 https://doi.org/10.1006/geno.1994.1631
308 Dyer, M.R. and Walker, J.E. (1993) Sequences of members of the human gene family for the c subunit of mitochondrial ATP synthase. *Biochem. J.* **293**(Pt1), 51–64 https://doi.org/10.1042/bj2930051
309 He, J., Ford, H.C., Carroll, J., Douglas, C., Gonzales, E., Ding, S. et al. (2018) Assembly of the membrane domain of ATP synthase in human mitochondria. *Proc. Natl. Acad. Sci. U.S.A.* **115**, 2988–2993 https://doi.org/10.1073/pnas.1722086115
310 Signes, A. and Fernandez-Vizarra, E. (2018) Assembly of mammalian oxidative phosphorylation complexes I-V and supercomplexes. *Essays Biochem.* **62**, 255–270 https://doi.org/10.1042/EBC20170098
311 He, J., Ford, H.C., Carroll, J., Ding, S., Fearnley, I.M. and Walker, J.E. (2017) Persistence of the mitochondrial permeability transition in the absence of subunit c of human ATP synthase. *Proc. Natl. Acad. Sci. U.S.A.* **114**, 3409–3414 https://doi.org/10.1073/pnas.1702357114
312 Ganetzky, R.D., Stendel, C., McCormick, E.M., Zolkipli-Cunningham, Z., Goldstein, A.C., Klopstock, T. et al. (2019) MT-ATP6 mitochondrial disease variants: phenotypic and biochemical features analysis in 218 published cases and cohort of 14 new cases. *Hum. Mutat.* **40**, 499–515 https://doi.org/10.1002/humu.23723
313 Jonckheere, A.I., Hogeveen, M., Nijtmans, L., van den Brand, M., Janssen, A., Diepstra, H. et al. (2009) A novel mitochondrial ATP8 gene mutation in a patient with apical hypertrophic cardiomyopathy and neuropathy. *BMJ Case Rep.* **2009**, bcr07.2008.0504 https://doi.org/10.1136/bcr.07.2008.0504
314 Galimberti, C.A., Diegoli, M., Sartori, I., Uggetti, C., Brega, A., Tartara, A. et al. (2006) Brain pseudoatrophy and mental regression on valproate and a mitochondrial DNA mutation. *Neurology* **67**, 1715–1717 https://doi.org/10.1212/01.wnl.0000242882.58086.9a
315 Ware, S.M., El-Hassan, N., Kahler, S.G., Zhang, Q., Ma, Y.W., Miller, E. et al. (2009) Infantile cardiomyopathy caused by a mutation in the overlapping region of mitochondrial ATPase 6 and 8 genes. *J. Med. Genet.* **46**, 308–314 https://doi.org/10.1136/jmg.2008.063149
316 Fragaki, K., Chaussenot, A., Serre, V., Acquaviva, C., Bannwarth, S., Rouzier, 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 signs. *Mol. Genet. Metab. Rep.* **21**, 100543 https://doi.org/10.1016/j.ymgmr.2019.100543
317 Barca, E., Ganetzky, R.D., Potluri, P., Juanola-Falgarona, M., Gai, X., Li, D. et al. (2018) USMG5 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
318 Arnold, I., Pfeiffer, K., Neupert, W., Stuart, R.A. and Schagger, H. (1998) Yeast mitochondrial F1F0-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
319 Hahn, A., Parey, K., Bublitz, M., Mills, D.J., Zickermann, V., Vonck, J. et al. (2016) Structure of a complete ATP synthase 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
320 Diodato, D., Ghezzi, D. and Tiranti, V. (2014) The mitochondrial aminoacyl tRNA synthetases: genes and syndromes. *Int. J. Cell Biol.* **2014**, 787956 https://doi.org/10.1155/2014/787956
321 Englmeier, 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
322 O'Brien, T.W. (1971) The general occurrence of 55 S ribosomes in mammalian liver mitochondria. *J. Biol. Chem.* **246**, 3409–3417 PMID: 4930061
323 Gopisetty, G. and Thangarajan, R. (2016) Mammalian mitochondrial ribosomal small subunit (MRPS) genes: a putative role in human disease. *Gene* **589**, 27–35 https://doi.org/10.1016/j.gene.2016.05.008
324 Beringer, M. and Rodnina, M.V. (2007) The ribosomal peptidyl transferase. *Mol. Cell* **26**, 311–321 https://doi.org/10.1016/j.molcel.2007.03.015
325 Ban, N., Nissen, P., Hansen, J., Moore, P.B. and Steitz, T.A. (2000) The complete atomic structure of the large ribosomal subunit at 2.4 A resolution. *Science* **289**, 905–920 https://doi.org/10.1126/science.289.5481.905
326 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
327 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**, 814–821 https://doi.org/10.1038/35101544
328 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
329 Szyrach, 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**, 6448–6457 https://doi.org/10.1093/emboj/cdg623
330 Greber, 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
331 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
332 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
333 Dennerlein, S., Rozanska, A., Wydro, M., Chrzanowska-Lightowlers, Z.M. and Lightowlers, 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
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 ERAL1 leads to mitochondrial dysfunction and growth retardation. *Nucleic Acids Res.* **38**, 5554–5568 https://doi.org/10.1093/nar/gkq305

© 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).

335 Summer, S., Smirnova, A., Gabriele, A., Toth, U., Fasemore, A.M., Forstner, K.U. et al. (2020) YBEY is an essential biogenesis factor for mitochondrial ribosomes. *Nucleic Acids Res.* **48**, 9762–9786 https://doi.org/10.1093/nar/gkaa148
336 Metodiev, M.D., Lesko, N., Park, C.B., Camara, Y., Shi, Y., Wibom, R. et al. (2009) Methylation of 12S rRNA is necessary for in vivo stability of the small subunit of the mammalian mitochondrial ribosome. *Cell Metab.* **9**, 386–397 https://doi.org/10.1016/j.cmet.2009.03.001
337 Metodiev, M.D., Spahr, H., Loguercio Polosa, P., Meharg, C., Becker, C., Altmueller, J. et al. (2014) NSUN4 is a dual function mitochondrial protein required for both methylation of 12S rRNA and coordination of mitoribosomal assembly. *PLoS Genet.* **10**, e1004110 https://doi.org/10.1371/journal.pgen.1004110
338 He, J., Cooper, H.M., Reyes, A., Di Re, M., Kazak, L., Wood, S.R. et al. (2012) Human C4orf14 interacts with the mitochondrial nucleoid and is involved in the biogenesis of the small mitochondrial ribosomal subunit. *Nucleic Acids Res.* **40**, 6097–6108 https://doi.org/10.1093/nar/gks257
339 Van Haute, L., Hendrick, A.G., D'Souza, A.R., Powell, C.A., Rebelo-Guiomar, P., Harbour, M.E. et al. (2019) METTL15 introduces N4-methylcytidine into human mitochondrial 12S rRNA and is required for mitoribosome biogenesis. *Nucleic Acids Res.* **47**, 10267–10281 https://doi.org/10.1093/nar/gkz735
340 Jackson, C.B., Huemer, M., Bolognini, R., Martin, F., Szinnai, G., Donner, B.C. et al. (2019) A variant in MRPS14 (uS14 m) causes perinatal hypertrophic cardiomyopathy with neonatal lactic acidosis, growth retardation, dysmorphic features and neurological involvement. *Hum. Mol. Genet.* **28**, 639–649 https://doi.org/10.1093/hmg/ddy374
341 Lake, N.J., Webb, B.D., Stroud, D.A., Richman, T.R., Ruzzenente, B., Compton, A.G. et al. (2017) Biallelic mutations in MRPS34 lead to instability of the small mitoribosomal subunit and leigh syndrome. *Am. J. Hum. Genet.* **101**, 239–254 https://doi.org/10.1016/j.ajhg.2017.07.005
342 Miller, C., Saada, A., Shaul, N., Shabtai, N., Ben-Shalom, E., Shaag, A. et al. (2004) Defective mitochondrial translation caused by a ribosomal protein (MRPS16) mutation. *Ann. Neurol.* **56**, 734–738 https://doi.org/10.1002/ana.20282
343 Bugiardini, E., Mitchell, A.L., Rosa, I.D., Horning-Do, H.T., Pitmann, A.M., Poole, O.V. et al. (2019) MRPS25 mutations impair mitochondrial translation and cause encephalomyopathy. *Hum. Mol. Genet.* **28**, 2711–2719 https://doi.org/10.1093/hmg/ddz093
344 Gardeitchik, T., Mohamed, M., Ruzzenente, B., Karall, D., Guerrero-Castillo, S., Dalloyaux, D. et al. (2018) Bi-allelic mutations in the mitochondrial ribosomal protein MRPS2 cause sensorineural hearing loss, hypoglycemia, and multiple OXPHOS complex deficiencies. *Am. J. Hum. Genet.* **102**, 685–695 https://doi.org/10.1016/j.ajhg.2018.02.012
345 Menezes, M.J., Guo, Y., Zhang, J., Riley, L.G., Cooper, S.T., Thorburn, D.R. et al. (2015) Mutation in mitochondrial ribosomal protein S7 (MRPS7) causes congenital sensorineural deafness, progressive hepatic and renal failure and lactic acidemia. *Hum. Mol. Genet.* **24**, 2297–2307 https://doi.org/10.1093/hmg/ddu747
346 Santorelli, F.M., Tanji, K., Manta, P., Casali, C., Krishna, S., Hays, A.P. et al. (1999) Maternally inherited cardiomyopathy: an atypical presentation of the mtDNA 12S rRNA gene A1555G mutation. *Am. J. Hum. Genet.* **64**, 295–300 https://doi.org/10.1086/302188
347 Chatzispyrou, I.A., Alders, M., Guerrero-Castillo, S., Zapata Perez, R., Haagmans, M.A., Mouchiroud, L. et al. (2017) A homozygous missense mutation in ERAL1, encoding a mitochondrial rRNA chaperone, causes Perrault syndrome. *Hum. Mol. Genet.* **26**, 2541–2550 https://doi.org/10.1093/hmg/ddx152
348 Freckmann, M.L., Thorburn, D.R., Kirby, D.M., Kamath, K.R., Hammond, J., Dennett, X. et al. (1997) Mitochondrial electron transport chain defect presenting as hypoglycemia. *J. Pediatr.* **130**, 431–436 https://doi.org/10.1016/S0022-3476(97)70206-3
349 Saada, A., Shaag, A., Arnon, S., Dolfin, T., Miller, C., Fuchs-Telem, D. et al. (2007) Antenatal mitochondrial disease caused by mitochondrial ribosomal protein (MRPS22) mutation. *J. Med. Genet.* **44**, 784–786 https://doi.org/10.1136/jmg.2007.053116
350 Smits, P., Saada, A., Wortmann, S.B., Heister, A.J., Brink, M., Pfundt, R. et al. (2011) Mutation in mitochondrial ribosomal protein MRPS22 leads to Cornelia de Lange-like phenotype, brain abnormalities and hypertrophic cardiomyopathy. *Eur. J. Hum. Genet.* **19**, 394–399 https://doi.org/10.1038/ejhg.2010.214
351 Baertling, F., Haack, T.B., Rodenburg, R.J., Schaper, J., Seibt, A., Strom, T.M. et al. (2015) MRPS22 mutation causes fatal neonatal lactic acidosis with brain and heart abnormalities. *Neurogenetics* **16**, 237–240 https://doi.org/10.1007/s10048-015-0440-6
352 Cormier-Daire, V., Rustin, P., Rotig, A., Chretien, D., Le Merrer, M., Belli, D. et al. (1996) Craniofacial anomalies and malformations in respiratory chain deficiency. *Am. J. Med. Genet.* **66**, 457–463 https://doi.org/10.1002/(SICI)1096-8628(19961230)66:4<457::AID-AJMG15>3.0.CO;2-T
353 Pulman, J., Ruzzenente, B., Bianchi, L., Rio, M., Boddaert, N., Munnich, A. et al. (2019) Mutations in the MRPS28 gene encoding the small mitoribosomal subunit protein bS1m in a patient with intrauterine growth retardation, craniofacial dysmorphism and multisystemic involvement. *Hum. Mol. Genet.* **28**, 1445–1462 https://doi.org/10.1093/hmg/ddy441
354 Borna, N.N., Kishita, Y., Kohda, M., Lim, S.C., Shimura, M., Wu, Y. et al. (2019) Mitochondrial ribosomal protein PTCD3 mutations cause oxidative phosphorylation defects with Leigh syndrome. *Neurogenetics* **20**, 9–25 https://doi.org/10.1007/s10048-018-0561-9
355 Fung, S., Nishimura, T., Sasarman, F. and Shoubridge, E.A. (2013) The conserved interaction of C7orf30 with MRPL14 promotes biogenesis of the mitochondrial large ribosomal subunit and mitochondrial translation. *Mol. Biol. Cell* **24**, 184–193 https://doi.org/10.1091/mbc.e12-09-0651
356 Rorbach, J., Gammage, P.A. and Minczuk, M. (2012) C7orf30 is necessary for biogenesis of the large subunit of the mitochondrial ribosome. *Nucleic Acids Res.* **40**, 4097–4109 https://doi.org/10.1093/nar/gkr1282
357 Wanschers, B.F., Szklarczyk, R., Pajak, A., van den Brand, M.A., Gloerich, J., Rodenburg, R.J. et al. (2012) C7orf30 specifically associates with the large subunit of the mitochondrial ribosome and is involved in translation. *Nucleic Acids Res.* **40**, 4040–4051 https://doi.org/10.1093/nar/gkr1271
358 Brown, A., Rathore, S., Kimanius, D., Aibara, S., Bai, X.C., Rorbach, J. et al. (2017) Structures of the human mitochondrial ribosome in native states of assembly. *Nat. Struct. Mol. Biol.* **24**, 866–869 https://doi.org/10.1038/nsmb.3464
359 Maiti, P., Antonicka, H., Gingras, A.C., Shoubridge, E.A. and Barrientos, A. (2020) Human GTPBP5 (MTG2) fuels mitoribosome large subunit maturation by facilitating 16S rRNA methylation. *Nucleic Acids Res.* **48**, 7924–7943 https://doi.org/10.1093/nar/gkaa592
360 Kim, H.J. and Barrientos, A. (2018) MTG1 couples mitoribosome large subunit assembly with intersubunit bridge formation. *Nucleic Acids Res.* **46**, 8435–8453 https://doi.org/10.1093/nar/gky672
361 Lavdovskaia, E., Kolander, E., Steube, E., Mai, M.M., Urlaub, H. and Richter-Dennerlein, R. (2018) The human Obg protein GTPBP10 is involved in mitoribosomal biogenesis. *Nucleic Acids Res.* **46**, 8471–8482 https://doi.org/10.1093/nar/gky701
362 Maiti, P., Kim, H.J., Tu, Y.T. and Barrientos, A. (2018) Human GTPBP10 is required for mitoribosome maturation. *Nucleic Acids Res.* **46**, 11423–11437 https://doi.org/10.1093/nar/gky938
363 Spahr, H., Habermann, B., Gustafsson, C.M., Larsson, N.G. and Hallberg, B.M. (2012) Structure of the human MTERF4-NSUN4 protein complex that regulates mitochondrial ribosome biogenesis. *Proc. Natl. Acad. Sci. U.S.A.* **109**, 15253–15258 https://doi.org/10.1073/pnas.1210688109

© 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).

Biochemical Journal (2020) **477** 4085–4132
https://doi.org/10.1042/BCJ20190767

364 Yakubovskaya, E., Guja, K.E., Mejia, E., Castano, S., Hambardjieva, 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
365 Coulbault, L., Deslandes, B., Herlicoviez, 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
366 Liu, Z., Song, Y., Li, D., He, X., Li, S., Wu, B. et al. (2014) The novel mitochondrial 16S rRNA 2336T > C mutation is associated with hypertrophic cardiomyopathy. *J. Med. Genet.* **51**, 176–184 https://doi.org/10.1136/jmedgenet-2013-101818
367 Chalmers, R.M., Lamont, P.J., Nelson, I., Ellison, D.W., Thomas, N.H., Harding, A.E. et al. (1997) A mitochondrial DNA tRNA(Val) point mutation associated with adult-onset Leigh syndrome. *Neurology* **49**, 589–592 https://doi.org/10.1212/WNL.49.2.589
368 Tiranti, V., D'Agruma, L., Pareyson, D., Mora, M., Carrara, F., Zelante, L. et al. (1998) A novel mutation in the mitochondrial tRNA(Val) gene associated with a complex neurological presentation. *Ann. Neurol.* **43**, 98–101 https://doi.org/10.1002/ana.410430116
369 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
370 Arredondo, 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
371 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, a matter of quantity. *Mitochondrion* **15**, 34–39 https://doi.org/10.1016/j.mito.2014.03.010
372 Toyoshima, Y., Tanaka, Y. and Satomi, K. (2017) MELAS syndrome associated with a new mitochondrial tRNA-Val gene mutation (m.1616A > G). *BMJ Case Rep.* **2017**, bcr2017220934 https://doi.org/10.1136/bcr-2017-220934
373 Rorbach, J., Gao, F., Powell, C.A., D'Souza, A., Lightowlers, R.N., Minczuk, M. et al. (2016) Human mitochondrial ribosomes can switch their structural RNA composition. *Proc. Natl. Acad. Sci. U.S.A.* **113**, 12198–12201 https://doi.org/10.1073/pnas.1609338113
374 Gao, F., Wesolowska, M., Agami, R., Rooijers, K., Loayza-Puch, F., Lawless, C. et al. (2017) Using mitoribosomal profiling to investigate human mitochondrial translation. *Wellcome Open Res.* **2**, 116 https://doi.org/10.12688/wellcomeopenres.13119.1
375 Koripella, R.K., Sharma, M.R., Bhargava, K., Datta, P.P., Kaushal, P.S., Keshavan, P. et al. (2020) Structures of the human mitochondrial 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
376 Tsuboi, M., Morita, H., Nozaki, Y., Akama, K., Ueda, T., Ito, K. et al. (2009) EF-G2mt is an exclusive recycling factor in mammalian mitochondrial protein synthesis. *Mol. Cell* **35**, 502–510 https://doi.org/10.1016/j.molcel.2009.06.028
377 Nottia, M.D., Marchese, M., Verrigni, D., Mutti, C., Torraco, A., Oliva, R. et al. (2020) A homozygous MRPL24 mutation causes a complex movement disorder and affects the mitoribosome assembly. *Neurobiol. Dis.* **141**, 104880 https://doi.org/10.1016/j.nbd.2020.104880
378 Carroll, C.J., Isohanni, P., Poyhonen, R., Euro, L., Richter, U., Brilhante, V. et al. (2013) Whole-exome sequencing identifies a mutation in the mitochondrial ribosome protein MRPL44 to underlie mitochondrial infantile cardiomyopathy. *J. Med. Genet.* **50**, 151–159 https://doi.org/10.1136/jmedgenet-2012-101375
379 Distelmaier, F., Haack, T.B., Catarino, C.B., Gallenmuller, C., Rodenburg, R.J., Strom, T.M. et al. (2015) MRPL44 mutations cause a slowly progressive multisystem disease with childhood-onset hypertrophic cardiomyopathy. *Neurogenetics* **16**, 319–323 https://doi.org/10.1007/s10048-015-0444-2
380 Loeffen, J., Elpeleg, O., Smeitink, J., Smeets, R., Stockler-Ipsiroglu, S., Mandel, H. et al. (2001) Mutations in the complex I NDUFS2 gene of patients with cardiomyopathy and encephalomyopathy. *Ann. Neurol.* **49**, 195–201 https://doi.org/10.1002/1531-8249(20010201)49:2<195::AID-ANA39>3.0.CO;2-M
381 Ngu, L.H., Nijtmans, L.G., Distelmaier, F., Venselaar, H., van Emst-de Vries, S.E., van den Brand, M.A. et al. (2012) A catalytic defect in mitochondrial respiratory chain complex I due to a mutation in NDUFS2 in a patient with Leigh syndrome. *Biochim. Biophys. Acta* **1822**, 168–175 https://doi.org/10.1016/j.bbadis.2011.10.012
382 Benit, P., Slama, A., Cartault, F., Giurgea, I., Chretien, D., Lebon, S. et al. (2004) Mutant NDUFS3 subunit of mitochondrial complex I causes Leigh syndrome. *J. Med. Genet.* **41**, 14–17 https://doi.org/10.1136/jmg.2003.014316
383 Triepels, R.H., van den Heuvel, L.P., Loeffen, J.L., Buskens, C.A., Smeets, R.J., Rubio Gozalbo, M.E. et al. (1999) Leigh syndrome associated with a mutation in the NDUFS7 (PSST) nuclear encoded subunit of complex I. *Ann. Neurol.* **45**, 787–790 https://doi.org/10.1002/1531-8249(199906)45:6<787::AID-ANA13>3.0.CO;2-6
384 Lebon, S., Rodriguez, D., Bridoux, D., Zerrad, A., Rotig, A., Munnich, A. et al. (2007) A novel mutation in the human complex I NDUFS7 subunit associated with Leigh syndrome. *Mol. Genet. Metab.* **90**, 379–382 https://doi.org/10.1016/j.ymgme.2006.12.007
385 Loeffen, J., Smeitink, J., Triepels, R., Smeets, R., Schuelke, M., Sengers, R. et al. (1998) The first nuclear-encoded complex I mutation in a patient with Leigh syndrome. *Am. J. Hum. Genet.* **63**, 1598–1608 https://doi.org/10.1086/302154
386 Procaccio, V. and Wallace, D.C. (2004) Late-onset Leigh syndrome in a patient with mitochondrial complex I NDUFS8 mutations. *Neurology* **62**, 1899–1901 https://doi.org/10.1212/01.WNL.0000125251.56131.65
387 Marina, A.D., Schara, U., Pyle, A., Moller-Hartmann, C., Holinski-Feder, E., Abicht, A. et al. (2013) NDUFS8-related complex I deficiency extends phenotype from "PEO Plus" to Leigh Syndrome. *JIMD Rep.* **10**, 17–22 https://doi.org/10.1007/8904_2012_195
388 Baertling, F., Sanchez-Caballero, L., Timal, S., van den Brand, M.A., Ngu, L.H., Distelmaier, F. et al. (2017) Mutations in mitochondrial complex I assembly factor NDUFAF3 cause Leigh syndrome. *Mol. Genet. Metab.* **120**, 243–246 https://doi.org/10.1016/j.ymgme.2016.12.005
389 Ishiyama, A., Muramatsu, K., Uchino, S., Sakai, C., Matsushima, Y., Makioka, N. et al. (2018) NDUFAF3 variants that disrupt mitochondrial complex I assembly may associate with cavitating leukoencephalopathy. *Clin. Genet.* **93**, 1103–1106 https://doi.org/10.1111/cge.13215
390 Saada, A., Edvardson, S., Rapoport, M., Shaag, A., Amry, K., Miller, C. et al. (2008) C6ORF66 is an assembly factor of mitochondrial complex I. *Am. J. Hum. Genet.* **82**, 32–38 https://doi.org/10.1016/j.ajhg.2007.08.003
391 Gerards, M., Sluiter, W., van den Bosch, B.J., de Wit, L.E., Calis, C.M., Frentzen, M. et al. (2010) Defective complex I assembly due to C20orf7 mutations as a new cause of Leigh syndrome. *J. Med. Genet.* **47**, 507–512 https://doi.org/10.1136/jmg.2009.067553
392 Saada, A., Edvardson, S., Shaag, A., Chung, W.K., Segel, R., Miller, C. et al. (2012) Combined OXPHOS complex I and IV defect, due to mutated complex I assembly factor C20ORF7. *J. Inherit. Metab. Dis.* **35**, 125–131 https://doi.org/10.1007/s10545-011-9348-y

 © 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).

393 Simon, M.T., Eftekharian, S.S., Stover, A.E., Osborne, A.F., Braffman, B.H., Chang, R.C. et al. (2019) Novel mutations in the mitochondrial complex I assembly gene NDUFAF5 reveal heterogeneous phenotypes. *Mol. Genet. Metab.* **126**, 53–63 https://doi.org/10.1016/j.ymgme.2018.11.001
394 Yatsuka, Y., Kishita, Y., Formosa, L.E., Shimura, M., Nozaki, F., Fujii, T. et al. (2020) A homozygous variant in NDUFA8 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
395 Angebault, C., Charif, M., Guegen, N., Piro-Megy, C., de Camaret B, M., Procaccio, V. et al. (2015) Mutation in NDUFA13/GRIM19 leads to early onset hypotonia, dyskinesia and sensorial deficiencies, and mitochondrial complex I instability. *Hum. Mol. Genet.* **24**, 3948–3955 https://doi.org/10.1093/hmg/ddv133
396 Gonzalez-Quintana, A., Garcia-Consuegra, I., Belanger-Quintana, A., Serrano-Lorenzo, P., Lucia, A., Blazquez, A. et al. (2020) Novel NDUFA13 mutations associated with OXPHOS deficiency and leigh syndrome: a second family report. *Genes (Basel)* **11**, 855 https://doi.org/10.3390/genes11080855
397 Hartmannova, H., Piherova, L., Tauchmannova, K., Kidd, K., Acott, P.D., Crocker, J.F. et al. (2016) Acadian variant of Fanconi syndrome is caused by mitochondrial respiratory chain complex I deficiency due to a non-coding mutation in complex I assembly factor NDUFAF6. *Hum. Mol. Genet.* **25**, 4062–4079 https://doi.org/10.1093/hmg/ddw245
398 Baide-Mairena, H., Gaudo, P., Marti-Sanchez, L., Emperador, S., Sanchez-Montanez, A., Alonso-Luengo, O. et al. (2019) Mutations in the mitochondrial complex I assembly factor NDUFAF6 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
399 Pulkes, T., Liolitsa, D., Wills, 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.0000154471.33156.55
400 Hinttala, R., Smeets, R., Moilanen, J.S., Ugalde, C., Uusimaa, J., Smeitink, 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
401 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
402 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
403 Crimi, M., Papadimitriou, A., Galbiati, 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.0000117844.73436.68
404 Sarzi, E., Brown, M.D., Lebon, S., Chretien, D., Munnich, 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
405 Wang, K., Takahashi, Y., Gao, Z.L., Wang, G.X., Chen, X.W., Goto, J. et al. (2009) Mitochondrial ND3 as the novel causative gene for Leber hereditary optic neuropathy and dystonia. *Neurogenetics* **10**, 337–345 https://doi.org/10.1007/s10048-009-0194-0
406 Vodopivec, I., Cho, T.A., Rizzo, III, J.F., Frosch, M.P. and Sims, K.B. (2016) Mitochondrial encephalopathy and optic neuropathy due to m.10158 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
407 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 common mutations. *Hum. Mutat.* **6**, 311–325 https://doi.org/10.1002/humu.1380060405
408 Brown, M.D., Starikovskaya, 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.* **110**, 130–138 https://doi.org/10.1007/s00439-001-0660-8
409 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
410 Jun, A.S., 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
411 Ravn, 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 associated with severe MELAS syndrome. *Eur. J. Hum. Genet.* **9**, 805–809 https://doi.org/10.1038/sj.ejhg.5200712
412 Funalot, B., Reynier, P., Vighetto, A., Ranoux, D., Bonnefont, 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
413 Ugalde,, C., Triepels,, 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. *Ann. Neurol.* **54**, 665–669 https://doi.org/10.1002/ana.10734
414 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
415 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 ACAD9 mutations: report on nine patients. *Mol. Genet. Metab.* **118**, 185–189 https://doi.org/10.1016/j.ymgme.2016.05.005
416 Alston, C.L., Compton, A.G., Formosa, L.E., Strecker, V., Olahova, M., Haack, T.B. et al. (2016) Biallelic mutations in TMEM126B cause severe complex I deficiency with a variable clinical phenotype. *Am. J. Hum. Genet.* **99**, 217–227 https://doi.org/10.1016/j.ajhg.2016.05.021
417 Sanchez-Caballero, L., Ruzzenente, 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 deficiency. *Am. J. Hum. Genet.* **99**, 208–216 https://doi.org/10.1016/j.ajhg.2016.05.022
418 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
419 Isashiki, Y. and Nakagawa, M. (1991) Clinical correlation of mitochondrial DNA heteroplasmy and Leber's hereditary optic neuropathy. *Jpn. J. Ophthalmol.* **35**, 259–267 PMID: 1770665
420 Deschauer, M., Bamberg, C., Claus, D., Zierz, S., Turnbull, D.M. and Taylor, R.W. (2003) Late-onset encephalopathy associated with a C11777A mutation of mitochondrial DNA. *Neurology* **60**, 1357–1359 https://doi.org/10.1212/01.WNL.0000055869.99975.4B

© 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).

Biochemical Journal (2020) **477** 4085–4132
https://doi.org/10.1042/BCJ20190767

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 Apatean, D., Rakic, B., Brunel-Guitton, C., Hendson, 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.ymgmr.2018.12.006
423 Pulkes, T., Eunson, L., Patterson, V., Siddiqui, A., Wood, N.W., Nelson, I.P. et al. (1999) The mitochondrial DNA G13513A transition in ND5 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 ND5 mutation. *Eur. J. Hum. Genet.* **10**, 141–144 https://doi.org/10.1038/sj.ejhg.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**, 128–132 https://doi.org/10.1002/ana.10435
426 Alston, C.L., Morak, M., Reid, C., Hargreaves, I.P., Pope, S.A., Land, J.M. et al. (2010) A novel mitochondrial MTND5 frameshift mutation causing isolated complex I deficiency, renal failure and myopathy. *Neuromuscul. Disord.* **20**, 131–135 https://doi.org/10.1016/j.nmd.2009.10.010
427 Ng, Y.S., Lax, N.Z., Maddison, P., Alston, C.L., Blakely, E.L., Hepplewhite, P.D. et al. (2018) MT-ND5 mutation exhibits highly variable neurological manifestations at low mutant load. *EBioMedicine* **30**, 86–93 https://doi.org/10.1016/j.ebiom.2018.02.010
428 Andreu, A.L., Checcarelli, N., Iwata, S., Shanske, S. and DiMauro, S. (2000) A missense mutation in the mitochondrial cytochrome b gene in a revisited case with histiocytoid cardiomyopathy. *Pediatr. Res.* **48**, 311–314 https://doi.org/10.1203/00006450-200009000-00008
429 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/10.1016/j.ajhg.2008.05.002
430 Abdulhag, U.N., Soiferman, D., Schueler-Furman, O., Miller, C., Shaag, A., Elpeleg, O. et al. (2015) Mitochondrial complex IV deficiency, caused by mutated COX6B1, is associated with encephalomyopathy, hydrocephalus and cardiomyopathy. *Eur. J. Hum. Genet.* **23**, 159–164 https://doi.org/10.1038/ejhg.2014.85
431 Higuchi, Y., Okunushi, R., Hara, T., Hashiguchi, A., Yuan, J., Yoshimura, A. et al. (2018) Mutations in COA7 cause spinocerebellar ataxia with axonal neuropathy. *Brain* **141**, 1622–1636 https://doi.org/10.1093/brain/awy104
432 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.* **111**, 353–359 https://doi.org/10.1016/j.ymgme.2014.01.001
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 hypergonadotropic hypogonadism. *J. Neurol.* **263**, 2188–2195 https://doi.org/10.1007/s00415-016-8249-2
434 Prezant, T.R., Agapian, J.V., Bohlman, M.C., Bu, X., Oztas, S., Qiu, W.Q. et al. (1993) Mitochondrial ribosomal RNA mutation associated with both antibiotic-induced and non-syndromic deafness. *Nat. Genet.* **4**, 289–294 https://doi.org/10.1038/ng0793-289
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 aminoglycoside induced deafness. *Pharmacogenetics* **5**, 165–172 https://doi.org/10.1097/00008571-199506000-00005
436 Gao, Z., Chen, Y. and Guan, M.X. (2017) Mitochondrial DNA mutations associated with aminoglycoside induced ototoxicity. *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/10.1007/8904_2016_13
439 Serre, V., Rozanska, A., Beinat, M., Chretien, D., Boddaert, 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.bbadis.2013.04.014

© 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).