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Mitochondrial disorders caused by mutations in respiratory chain assembly factors

Francisca Diaz^{a,*}, Heike Kotarsky^b, Vineta Fellman^{b,c}, and Carlos T. Moraes^a

^aDepartment of Neurology, University of Miami Miller School of Medicine, Miami, Florida, USA

^bDepartment of Pediatrics, Clinical Sciences, Lund, Lund University, Lund, Sweden ^cDepartment of Pediatrics, University of Helsinki, Helsinki, Finland

Summary

Mitochondrial diseases involve the dysfunction of the oxidative phosphorylation (OXPHOS) system. This group of diseases presents with heterogeneous clinical symptoms affecting mainly organs with high energy demands. Defects in the multimeric complexes comprising the OXPHOS system have a dual genetic origin, mitochondrial or nuclear DNA. Although many nuclear DNA mutations involve genes coding for subunits of the respiratory complexes, the majority of mutations found to date affect factors that do not form part of the final complexes. These assembly factors or chaperones have multiple functions ranging from cofactor insertion to proper assembly/stability of the complexes. Although significant progress has been made in the last few years in the discovery of new assembly factors, the function of many remains elusive. Here, we describe assembly factors or chaperones that are required for respiratory chain complex assembly and their clinical relevance.

Keywords

Chaperones; Mitochondrial diseases; Newborn infant; Oxidative phosphorylation; Perinatal disorder; Respiratory chain deficiency

Introduction

Mitochondrial diseases are usually referred to as disorders with defects in the oxidative phosphorylation (OXPHOS) system. They can be caused by mutations in the nuclear or in the mitochondrial genome and therefore have distinct patterns of inheritance depending on the genetic origin. The incidence of mitochondrial DNA (mtDNA) mutations causing disease has been estimated to be 1 in 5000 children.¹ This large number is accompanied by the recent estimate that 1 in 200 people are carriers of pathogenic mtDNA mutations.²

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*Corresponding author. Addresses: 1420 NW 9th Ave., TSL Building, Room 228, Miami, FL 33136, USA. Tel.: +1 305 243 7489; fax: +1 305 243 6955. fdiaz1@med.miami.edu (F. Diaz).

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Conflict of interest statement

Nothing to declare.

Mitochondrial diseases, being heterogeneous in nature and affecting single or multiple organs, pose a challenge to develop effective treatments. In the last few years new strategies have emerged and some are currently being tested in clinical trials.^{3–4} In this review, we focus on mitochondrial diseases caused by mutations in the proteins with chaperone function that are essential for proper assembly and function but which do not form part of the final complex. Mutations causing disease in the neonatal period are summarized in Table 1.

Complex I

Complex I (CI), NADH:ubiquinone oxidoreductase or NADH dehydrogenase is the largest of the mitochondrial respiratory complexes comprising 45 subunits. Seven of its subunits are encoded by the mtDNA and the remaining by the nuclear DNA. CI catalyzes the transfer of electrons from NADH produced during the tricarboxylic acid cycle to coenzyme Q. Three-dimensional electron microscopy studies revealed that the structure of the complex resembles an ‘L’ with the presence of a peripheral hydrophilic arm (matrix arm) and a highly hydrophobic membrane arm.⁵ The assembly of this enzyme is very elaborate, and although many steps have been elucidated by studies in mutants of *Neurospora crassa* and by studies of patients with CI defects, the complete sequence of events remains unknown. Several models have been proposed for the assembly of the holoenzyme that differ in the details but agree in inasmuch as the complex is assembled in several modules that go on to form the peripheral and the membrane arms.^{6,7} Patients with mutations of nuclear origin leading to CI defects have severe manifestations during infancy and early childhood, frequently resulting in premature death.⁸ Numerous mutations in CI nuclear- and mtDNA-encoded subunits have been reported but only a fraction of the CI deficiency cases are caused by mutations in the structural subunits.⁹ CI defects are also caused by mutations in auxiliary proteins that do not form part of the final holoenzyme but which assist in the assembly process. Here we describe recently discovered CI chaperones and the clinical phenotypes associated with their mutations.

Complex I chaperones

NDUFAF1/CIA30—Studies in *N. crassa* led to the identification of two proteins associated with CI assembly intermediates CIA30 and CIA84 that were proposed to have chaperone function.¹⁰ The *CIA30* human homolog *NDUFAF1* gene located in chromosome 15q13.3 is ubiquitously expressed with slightly increased levels in heart, lung, kidney and liver.¹¹ Knockdown of *NDUFAF1* by RNA interference (RNAi) resulted in decreased levels of the fully assembled complex and activity in HEK293 cells. Although *NDUFAF1* was found to be associated with two assembly intermediates of 600 and 700 kDa its exact function has not been elucidated.¹²

The first patient described with a mutation in *NDUFAF1* had only 10% of control levels of this chaperone as well as a significant reduction of the levels of fully assembled CI. This patient was a compound heterozygote with c.1001A→C and c.1140A→G mutations predicted to cause T207P and K253R amino acid substitutions in highly conserved residues.¹³ This patient presented with failure to thrive, hypotonia, growth delay, lactic acidosis and cardiomyopathy after 11 months of age. At 3 years he was diagnosed with Wolff–Parkinson–White syndrome and subsequently had visual cortex dysfunction and pigmentary retinopathy at 11 years of age. He developed osteoporosis and kyphoscoliosis at age 16 years and presented mild-to-moderate intellectual disability. At the time of the report the patient was 20 years old.¹³

Ecsit—Tandem affinity purification experiments identified Ecsit (evolutionary conserved signaling intermediate in Toll pathway) as a binding partner of CIA30 in HEK293 cells.¹⁴ A portion of Ecsit, previously described as a cytosolic protein required for embryonic

development and inflammatory signaling, was located in the mitochondria. This mitochondrial isoform associated with CIA30 and it was found in assembly intermediates of about 460 and 830 kDa. Moreover, knockdown of Ecsit by RNAi caused a substantial reduction in the levels of NDUFAF1 and disturbed CI assembly, whereas knockdown of NDUFAF1 slightly altered Ecsit levels.¹⁴ Although Ecsit and NDUFAF1 are found in the same assembly intermediates and their function is unknown, previous results imply that the two proteins have independent functions and mitochondrial Ecsit might be required for NDUFAF1 stability.¹⁴

B17.2L/NDUFAF2—By whole genome comparison of fermentative and aerobic yeast, a putative CI assembly factor, B17.2L(NDUFAF2 or NDUFA12L),¹⁵ was identified. In *Yarrowia lipolytica* B17.2 is a structural subunit located in the matrix arm of complex I whereas its human paralogue B17.2L is a putative assembly factor. The first patient described was homozygous for the mutation (C182T) in B17.2L leading to a premature stop codon (R45X).¹⁵ This infant presented with a progressive leukoencephalopathy with vanishing white matter and cortical and vermian atrophy. At 12 months, the patient presented horizontal nystagmus, dysconjugated gaze and pale optic disc that eventually resulted in severe optic atrophy. She had myopathic facies, lethargy and acute ataxia. Brain lesions in the substantia nigra, mamillo and spinothalamic tracts were observed by magnetic resonance imaging (MRI) at 3 years of age but did not affect her intellect. She suffered a respiratory failure at 8 years, remaining comatose and immobile, dying at 13 years of age. Analysis of brain tissue revealed extensive degeneration of white matter accompanied by glia scarring. Muscle biopsy obtained at 3 years of age did not reveal any histological abnormality, albeit the biochemical analysis of OXPHOS enzymes revealed a moderate CI defect in muscle and a more severe defect in fibroblasts. The CI deficiency and the assembly defect was rescued when B17.2L was expressed in the patient's fibroblasts.¹⁵

B17.2L was associated mainly with a subassembly intermediate of 830 kDa in several CI patients, in particular in patients with mutations in NDUFV1 and NDUF54.^{15,16}

Two other patients homozygous for a codon change of methionine (ATG) to leucine (TTG) in position 1 were described.¹⁷ The mutation in the first methionine caused the complete absence of the chaperone and CI activity ranged from 24% to 36% of control in muscle mitochondria and 53% in fibroblast.¹⁷ Hypotonia, nystagmus and optic atrophy at 8 months of age were the initial findings in a boy who later had apneic spells and died at 21 months of age. MRI studies revealed lesions in the mamillothalamic and spinothalamic tracts, medial lemniscus and substantia nigra whereas cerebellum was only slightly affected and cortex and subcortical regions were spared. The second patient presented first symptoms at 20 months of age with apnea and ophthalmoplegia followed by myoclonic seizures and encountered a fatal apnea episode at 2 years of age. MRI studies revealed the same affected areas as in the first patient. Plasma and cerebrospinal fluid (CSF) lactate levels were normal in both patients.

More recently, Janssen et al.¹⁸ reported a patient with a chromosome 5 microdeletion that encompassed three genes: *NDUFAF2*, *ERCC8* and *ELOVL7*. *ERCC8* encodes the CS-A protein that is required for DNA repair whereas *ELOVL7* gene encodes an enzyme that participates in the synthesis of the very long chain fatty acid pathway. Fibroblasts from the patient with the microdeletion had 45% of control CI activity, and expression of *NDUFAF2* restored the activity to control levels. In the absence of this chaperone, fully assembled CI was not completely absent and 380 and 480 kDa intermediates were accumulated. Furthermore, some of the levels of CI subunits (NDUFS3, NDUF9 and NDUF6) as well as the chaperone NDUFAF1 were significantly reduced whereas the levels of NDUFAS2 were only slightly decreased.¹⁸

Thus, although the function of NDUFAF2/ B17.2L is still obscure, studies suggest that this chaperone might participate in late assembly steps, perhaps by stabilizing assembly intermediates.

C20orf7—A lethal neonatal CI defect was mapped to the *C20orf7* gene.¹⁹ The patient was homozygous for a missense mutation causing exchange of a conserved amino acid (L229P). The clinical findings were intrauterine growth restriction, ventricular septation, agenesis of the corpus callosum, lactic acidosis, and death by day 7.¹⁹

C20orf7 is a matrix protein associated with the inner mitochondrial membrane and its knockdown resulted in a decrease in CI activity. Fibroblasts from this patient showed CI assembly intermediates of ~400 kDa that contained ND1 but not ND2 subunit, indicating that this chaperone is involved in early assembly steps.¹⁹

Another investigation of members of a consanguineous family with 11 children revealed that three of them presented with a very similar Leigh syndrome (LS) and progressive spasticity with an onset at 3 years of age.²⁰ At 5 years, increased lactate in CSF and slight atrophy and hypodensity in caudate nuclei and putamen were observed. A year later, extrapyramidal movement disorder and delay of mental development were evident. At 23 years of age lesions in basal ganglia and brain stem were discovered. CI activity in both peripheral blood lymphocytes and muscle was decreased to 36–48% and 6–33% respectively in two of the patients. The cause of the disease was attributed to a mutation in the *C20orf7* gene although the patients also presented a mutation in the *CRLS1* (cardiolipin synthase 1) gene.²⁰

C6orf66/NDUFAF4—Five patients of a consanguineous family of Arab-Muslim origin with infantile encephalopathy and isolated CI deficiency were homozygous for a mutation in the *C6orf66* gene, later named *NDUFAF4*.²¹ The mutation was a T→C substitution at nucleotide 194 predicted to change a leucine for a proline at amino acid 65. The healthy family members were heterozygous for this mutation.²¹ The affected infants suffered metabolic acidosis soon after birth, and three of them died between 2 and 5 days of age. One had a severe cardiomyopathy. The other children survived for 18 months and 7 years of age (at the time of the report). They developed a severe encephalopathy with lactic acidosis in addition to kyphosis and spastic contractures. MRI studies revealed severe atrophy of white and gray matter as well as demyelination. CI deficiency was evident in both muscle and fibroblast (5.5–17% and 32–67% of controls respectively) and introduction of wild-type *C6orf66* into patients' fibroblasts restored CI defect.²¹ Although the exact function of NDUFAF4 remains obscure, an interdependence of NDUFAF4 (C6orf66) and NDUFAF3 (C3orf60) was discovered.²¹

C3orf60/NDUFAF3—Three families with different mutations in the *C3orf60* (*NDUFAF3*) gene were recently reported.²² A homozygous c.229 G→C mutation that caused a G77R change was found in three siblings with heterozygous parents. In the second family, the infant was homozygous for a c.365 G→C causing an R122P change. In the last family, the infant was a compound heterozygous with a c.2 T→C and a c.365 G→C that resulted in a disruption of the starting codon (M1T) and a R122T change respectively. The patients from the first family presented with lactic acidemia and increased muscle tone. Their brain MRI was normal as well as EEG and echocardiogram. They died at 3 months of age. The infant from the second family suffered from macrocephaly with wide anterior fontanelle and hypotonia. He also presented with elevated lactate and died aged 4 months. The third family patient suffered from myoclonic seizures, had diffuse brain leukomalacia in MRI, and died at 6 months of age. Muscle and fibroblast from the patients showed decreased CI activity. Analysis of CI assembly in the patient from the second family did not reveal any assembly

intermediates when probed for either ND1 or NDUF52. The patient's fibroblast CI defect was complemented when expressing NDUF3-GFP protein.²²

Experiments on RNAi of *NDUF3* in HeLa cells showed decreased CI protein and activity as well as a significant reduction in the levels of the assembly factor NDUF4. Likewise, interference of NDUF4 resulted in decreased levels of NDUF3 protein. NDUF3 and NDUF4 comigrate with the same CI assembly intermediates and interact with CI subunits (NDUF52, NDUF53, NDUF5 and NDUF8).²²

C8orf38—Using phylogenetic profiling, 19 other candidate genes were identified as CI-associated proteins.²³ All these candidate genes were investigated as causes of CI deficiency in two siblings presenting with lactic acidosis, ataxia, decreased muscle strength and rigidity. MRI revealed abnormalities consistent with LS. One sibling died at 34 months of age and the other was 22 months at the time of the study. The defect was associated with a mutation in the *C8orf38* gene (c.296A→G) in a conserved residue.²³ Further studies are required to validate the role of C8orf38 as a CI assembly factor, albeit knockdown of C8orf38 resulted in reduced levels and activity of CI.²³

Ind1/HuInd1—Studies in the *Yarrowia lipolytica* identified Ind1 as an iron–sulfur cluster binding protein. Deletion of the *Ind1* gene resulted in a marked decrease of CI levels and activity in the aerobic yeast.²⁴ Likewise, knockdown of the human version (*HULND1*) in HeLa cells showed that this protein is required for CI assembly and activity. Moreover, huInd1 appears to be specific for CI. Analysis of ⁵⁵Fe incorporation into the OXPHOS complexes showed a marked decrease in the iron content of CI but not in complex II or complex III in cells depleted of huInd1.²⁵ The knockdown of huInd1 resulted in an intermediate of about 450 kDa that does not contain subunits forming the peripheral arm of the complex (NDUF51, NDUFV1, NDUF13 or NDUF9) but contained one of the subunits of the membrane arm (NDUF6).²⁵

Taken together, these results suggest that huInd1 is involved in the delivery of iron–sulfur clusters to CI subunits and in the absence of this protein, the prosthetic group cannot be delivered and the subunits are unstable and degraded causing impairment in the assembly of the peripheral arm and accumulation of an assembly intermediate. To date, defects in this gene have not been found in patients with CI deficiency.

ACAD9—Using tandem affinity purification ACAD9 was pulled down as a binding candidate for NDUF3 and Ecsit.²⁶ ACAD9 belongs to the acyl-CoA dehydrogenase family and co-migrated with CI assembly intermediates of 500 and 800 kDa that also contained Ecsit and NDUF3. Knockdown of ACAD9 in HEK293 cells decreased the levels and activity of CI as well as the levels of the two other chaperones. Likewise, the levels of ACAD9 were decreased when knocking down Ecsit or NDUF3.²⁶

The first published ACAD9 patient was homozygous for the mutation c.1553 G→A that resulted in the change of a highly conserved residue (R518H). This infant suffered from hypertrophic cardiomyopathy at 8 months of age with elevated lactate in blood and CSF. Disease progressed and the patient was 18 years old at the time of the study, when exercise intolerance, mild hearing loss, and hypertrophic cardiomyopathy were noted. The second patient was a compound heterozygote affecting two highly conserved amino acids, and had moderate metabolic acidosis, anemia, progressive encephalopathy, and hypertrophic cardiomyopathy. The disease developed into multiple organ failure and death at 6 months of age.

Analysis of the levels of CI in both patients showed reduced levels of this complex as well as low levels of the chaperones Ecsit and NDUFAF1, albeit ACAD9 was only detected in patient 1. Expression of ACAD9 in the fibroblasts restored the defect.²⁶

Other ACAD9 (R532W change) patients with later onset of disease were reported from a double consanguineous family. Fatigue, vomiting, exercise intolerance, and lactic acidosis appeared at about 4 years age.²⁷ The muscles were predominantly affected and showed mitochondrial proliferation in the subsarcolemma. For the most part, brain appeared spared with the exception of a single stroke-like episode in two of the patients. Another patient with similar phenotype was a compound heterozygote (R127Q and R469W). The patients had a reduction of CI enzymatic activity but not of protein levels. Interestingly, these patients responded to riboflavin treatment which improved their CI deficiency.²⁷

Since none of the ACAD9 patients showed any evidence of β -oxidation, the function of the chaperone appears to be related to CI assembly. Haack et al. confirmed this by identifying other mutations in the ACAD9 gene in five new patients with CI deficiency.²⁸ The patients suffered from hypertrophic cardiomyopathy, encephalopathy and lactic acidosis with fatal outcome varying from 45 days to 12 years of age. CI activity ranged from 13% to 26% in muscle and from 32% to 38% in fibroblasts of control samples.²⁸

Other factors—There remain CI defects of unknown cause, suggesting the existence of other proteins involved in the assembly process. One of these is the apoptosis-inducing factor AIF. Knockdown of AIF resulted in a reduction of the levels of certain CI subunits (NDUFA9, NDUFB6 and NDUFS7) and the fully assembled complex in vitro in HeLa cells or in vivo in the Harlequin mouse suggesting a role as CI chaperone.²⁹ A mutation-causing deletion of an arginine (R201 Δ) in the N-terminal FAD binding domain of AIF has been identified in X-linked mitochondrial encephalopathy.³⁰ Analysis of patient fibroblasts revealed CIII and CIV deficiencies whereas CI was only slightly influenced; analysis in muscle revealed that CI, III and IV were affected. However, this was shown to be due to severe mitochondrial DNA deficiency.³⁰ Taken together, the role of AIF in mitochondrial function needs further investigation. Another newly discovered factor is FOXRED1. Mutations in this gene caused a severe CI deficiency in an infant leading to progressive encephalomyopathy. The CI defect from fibroblast derived from the patient was rescued by lentiviral expression of the protein. Silencing the gene expression decreased the steady state levels of CI in control fibroblasts. Although the exact function of FOXRED1 as a CI chaperone is unknown, its FAD-oxidoreductase domain suggests a functional role in electrons transfer reactions.⁸⁸

Complex II

Complex II (CII) or succinate dehydrogenase (SDH) is located in the inner membrane facing the mitochondrial matrix where it links the respiratory chain with the Krebs cycle. SDH catalyses the oxidation of succinate to fumarate, resulting in reduction of the FAD cofactor bound to SDH from which electrons are passed through three FeS centres to ubiquinone.³¹ CII is composed of four nuclear-encoded subunits (*SDHA–D*) of which subunits A and B form the enzyme complex whereas subunits C and D anchor the complex to the inner mitochondrial membrane.³² Mutations in either CII proteins or chaperones may cause LS (*SDAH*), infantile leukoencephalopathy (*SDAHF1*) or familial paraganglioma syndrome (*SDHB*, *C*, *D*, *SDHAF2*). Several proteins assisting assembly of CII have been described,³³ but only the two recently discovered factors *SDHAF1*³⁴ and *SDHAF2*³⁵ directly and specifically aid CII assembly.

Complex II chaperones

SDHAF1—SDHAF1 was first described in 2009 by Ghezzi et al. in two affected families with infantile leukoencephalopathy with severe CII deficiency.^{34,36} By genome-wide linkage analysis, two homozygous missense mutations were identified in an evolutionarily highly conserved gene coding for a mitochondrial matrix protein. Transfection of patient fibroblasts with wild-type *SDHAF1* resulted in full or partial recovery of structural and functional assembled CII.³⁴

SDHAF2—By investigation of uncharacterized but evolutionarily conserved mitochondrial proteins in yeast, Hao et al. identified a protein that co-purified with yeast SDH1 and named it SDHAF2 (SDH5 in yeast).³⁵ Deletion of yeast SDH5 resulted in loss of the SDH complex and activity, probably due to unstable assembly of SDH. Incorporation of the FAD cofactor was impaired in the mutant. A human mutation in *SDHAF2* was identified in a Dutch family with hereditary paraganglioma.^{35,37}

Other factors—In yeast, two additional factors indirectly modifying CII assembly have been described.

In respiratory-deficient yeast mutants, Flx1 was identified as FAD transporter necessary for FAD incorporation into CII.³⁸ Later a more general role of Flx1 on post-transcriptional expression of Sdh1 was suggested.^{33,35,39}

Tcm62 was initially shown to be required for yeast CII assembly⁴⁰ but later experiments indicate that it influences CII assembly more generally by affecting mitochondrial protein stability under stress⁴¹ or protein-folding capacity within mitochondria.⁴²

Complex III

Complex III (CIII, or cytochrome bc₁ complex; ubiquinol cytochrome *c* reductase) is located in the mitochondrial inner membrane where it facilitates electron transfer from reduced coenzyme Q to cytochrome *c*, a process coupled to simultaneous pumping of protons across the inner mitochondrial membrane. In mammals, CIII forms a homodimer that can also be found in association with CI and CIV,⁴³ forming supercomplexes that may be stabilized by cardiolipin.⁴⁴ Monomeric CIII is composed of three catalytic and eight structural subunits that are encoded by 10 nuclear and one mitochondrial genes. The respiratory subunits contain redox centres and include the nuclear-encoded cytochrome *c*1 (CYC1), the Rieske iron–sulfur protein (RISP, UQCRFS1) and the mitochondrial-encoded cytochrome *b* (MT-CYB). The nuclear-encoded structural proteins are the core proteins 1 and 2 (UQCRC1 and 2) and subunit 6 (UQCRH) involved in complex formation, subunits 7 (UQCRB) and 8 (UQCRQ) involved in ubiquinone binding, subunit 9 (UQCR10) that interacts with cytochrome *c*1 and subunit 10 that may function as RISP protein-binding factor. In mammals, assembly of CIII is assisted by BCS1L⁴⁵ and TTC19.⁴⁶

CIII deficiencies are rare, but they cause a broad spectrum of symptoms and display tissue specificity in humans.⁴⁷ They are caused by mutations in catalytic and structural subunits as well as in assembly factors. Mutations in the mitochondrial-encoded catalytic subunit, cytochrome *b*, have mainly been associated with myopathy;⁴⁸ however, cytochrome *b* mutations are also the cause of Leber's hereditary optic neuropathy (LHON),⁴⁹ encephalomyopathy,⁵⁰ encephalopathy⁵¹ cardiomyopathy⁴⁷ and severe neonatal polyvisceral failure.⁵² Structural genes as cause of CIII deficiency include UQCRB and UQCRQ. In one patient with hypoglycemia and lactic acidosis, re-arrangement of the UQCRB gene was identified.⁵³ In another patient suffering from severe psychomotor

retardation with mildly elevated lactate levels, a single missense mutation was identified in UQCRQ.⁵⁴

Complex III chaperones

BCS1L—CIII assembly has been extensively investigated in yeast where *bcs1* was identified as a factor necessary for the expression of functional CIII.⁵⁵ The protein Bcs1 acts as molecular chaperone assisting the incorporation of Rieske protein and Qcr10p into CIII.⁵⁶ Bcs1 shares 50% amino acid identity with its human ortholog, BCS1L.⁵⁷ In the BCS1L protein, three different domains, the N-terminal import domain, the BCS1L-specific domain and the C-terminal AAA ATPase domain can be identified. In humans, *BCS1L* mutations have been found in all three domains, thereby causing CIII deficiency, resulting in a variety of phenotypes. They range from the least severe Björnstad syndrome with neurosensory hearing loss and pili torti,^{58,59} a neurologic disease with muscle weakness and optic atrophy⁶⁰ to manifestations with encephalopathy, liver failure or tubulopathy, or combinations thereof.^{45,61–65} Manifestations with neonatal onset usually present with hepatopathy (V. Fellman and H. Kotarsky, in this issue), the most severe being the GRACILE (growth restriction, aminoaciduria, cholestasis, iron overload, lactic acidosis and early death) syndrome, caused by a homozygous missense mutation (S78G).⁶⁶ The phenotype is strikingly consistent,⁶⁷ presenting with severe fetal growth restriction, lactacidosis and early death, further described in this issue (V. Fellman). Different BCS1L mutations known to date are summarized in Figure 1.

TTC19—An additional CIII assembly factor has recently been identified in patients with CIII deficiency and a slowly progressive encephalopathy presenting with subtle motility changes, involuntary movements and regurgitations with an onset during the first months of life.⁴⁶ In these individuals, homozygous nonsense mutations were identified in the gene encoding tetratricopeptide 19 (TTC19) resulting in reduced expression of TTC19 mRNA and lack of TTC19 protein. TTC19 protein was localized to the inner mitochondrial membrane where it is part of two high molecular weight complexes of which at least one contains CIII intermediates. Accumulation of CIII assembly intermediates in affected patients suggests that TTC19 is important in early assembly. Knockout of TTC19 in *Drosophila melanogaster* results in low fertility and neurological abnormalities associated with CIII deficiency.

Complex IV

Complex IV (CIV) or cytochrome *c* oxidase is the terminal enzyme of the electron transport chain that transfers electrons from cytochrome *c* to oxygen while it translocates protons from the matrix into the intermembrane space. CIV is a homodimer of about 230 kDa constituted by 13 subunits. Three of its subunits form the catalytic core and are encoded by the mtDNA whereas the rest of the subunits are encoded by the nuclear genome. Due to its dual origin the assembly process of this complex is also very complicated and highly regulated. Most of what is known about CIV assembly has been elucidated from systematic studies in mutants from the yeast *Saccharomyces cerevisiae*. Studies in yeast have revealed the presence of about 40 assembly factors that aid numerous aspects of CIV biogenesis.⁶⁸ Of the large number of assembly factors found in yeast, only some have human homologs. Below we focus on those factors with clinical relevance.

Complex IV chaperones

Surf1—Mutations in the *Surf1* gene cause LS characterized by subacute neurodegeneration encompassing bilateral necrotic lesions in brain stem, thalamus and basal ganglia. These lesions consist of neuronal loss, vascularization and demyelination and cause an early onset

of symptoms typically including ataxia, nystagmus, dystonia, ophthalmoparesis and optic atrophy. Affected individuals die between 6 months to 12 years. The majority of the mutations reported in *Surf1* create premature termination codons whereas fewer mutations are missense mutations.⁶⁹ The latter mutations have been associated with milder phenotypes.⁷⁰ The CIV activity in patients with LS ranged from 10% to 25% of control values. One puzzling fact is that not all mutations in *Surf1* cause LS. The clinical symptoms of LS have also been associated with mutations in other genes located in the mtDNA, with *COX10*, and with certain defects in pyruvate dehydrogenase, CI and CV activities.⁷¹ The actual function of *Surf1* remains obscure but it participates in early assembly of CIV. Recent studies in bacteria point the function of two *Surf1* homologs *Surf1c* and *Surf1q* as heme *a* binding proteins.⁷²

LRPPRC—A less severe form of LS found exclusively in a region of Quebec (Charlevoix and Saguenay-Lac-St Jean) is the French-Canadian Leigh syndrome (FCLS) caused by mutations in the *LRPPRC* gene.⁷³ In FCLS death also occurs early in life, between 3 and 10 years and clinical presentations include mild psychomotor regression and lactic acidosis. The tissues with severe CIV deficiency in this disorder are brain and liver, whereas muscle, heart and kidney are relatively unaffected. The LRPPRC (leucine-rich pentatricopeptide repeat cassette) protein homolog in yeast (Pet309) is required for stability and translation of COX1 mRNA (CIV catalytic subunit 1 encoded by the mtDNA) by presumably binding to its 5'UTR. The exact function of the mammalian LRPPRC is still unknown since there are no significant 5'UTR in mitochondrial mRNA, suggesting that the protein acts through a different mechanism. The levels of this chaperone as well as the levels of most of the mitochondrial transcripts excluding the rRNA and tRNA were significantly decreased in LRPPRC patients.⁷⁴ This decrease of mitochondrial mRNA was not proportional and the levels of CIV transcripts were the most reduced. When decreasing the LRPPRC levels even further, a severe decrease in all mitochondrial transcripts was observed with a generalized defect in all mitochondrially encoded OXPHOS complexes. LRPPRC was found to interact with SLIRP and to bind mRNA. Although further studies are required, the authors propose that these two proteins are post-transcriptional regulators of mitochondrial genes.⁷⁴

TACO1—In a patient with late onset of LS, a mutation in the *CCD44* gene was identified resulting in impaired COX1 protein synthesis.⁷⁵ The gene was renamed *TACO1* since it encodes a protein that serves as 'translational activator of COX1'.⁷⁵ In other patients with late onset of LS and CIV deficiency, no mutations in *TACO1* were found, suggesting that mutations in this factor are rare or perhaps incompatible with life.⁷⁶

SCO1 and SCO2—Complex IV requires copper in two catalytic centers, Cu_A and Cu_B, located in COX2 and COX1 subunits respectively. Several assembly factors (COX17, SCO1, SCO2 and COX11) are implicated in the delivery of this metal.⁷⁷ Mutations in *SCO1* were described first in two neonate siblings suffering from severe metabolic acidosis and hepatic failure (microvesicular steatosis and hepatomegaly) and from metabolic acidosis and encephalopathy respectively. The first patient died at 2 months and the second at 5 days of age; they were compound heterozygous for a 2 bp deletion in nucleotide 363–364 resulting in frame shift and stop codon, and for a C→T transition at nucleotide 520 resulting in an amino acid change of a conserved residue (P174L). The severe COX defect (less than 1% of control values) was observed in liver and muscle tissues in the first patient.⁷⁸ Another *SCO1* case presented with hypertrophic cardiomyopathy, encephalopathy and hepatomegaly with a fatal outcome at 6 months. The patient was homozygous for the missense mutation c.394G→A resulting in a G132s change.⁷⁹

SCO2 mutations were first detected in three patients suffering from hypertrophic cardiomyopathy accompanied by encephalopathy manifested early in life.⁸⁰ The brain

lesions differed from the typical LS. SCO2 brain abnormalities included spongiform neurodegeneration (midbrain, pons and medulla) and microglia proliferation (thalamus). They were all compound heterozygotes with different combinations.⁸⁰ Since then, numerous cases of SCO2 mutations (at least 26 including deletions, insertions and premature stop codons) have been reported with the most common mutation being the E140K mutation. Interestingly, patients that were homozygous for the E140K mutation presented with a milder phenotype and delayed onset of disease (2–4 months of age versus neonate onset for compound heterozygous).⁸¹

COX10 and COX15—Another prosthetic group unique to CIV is heme *a*, which is associated with the COX1 subunit. COX10 and COX15 functions are related to biosynthesis of heme *a*. COX10, a farnesyl transferase, catalyzes the first step of the heme *a* biosynthesis by incorporating a farnesyl group and converting heme *b* into heme *o*. Subsequently, heme *o* is converted to heme *a* by the action of COX15 and a monooxygenase (not characterized in humans). Defects in this pathway result in clinical symptoms with early onset and fatal outcome from 2 months to 4 years of age. The first case of a *COX10* mutation was a newborn infant with severe lactic acidemia, hypotonia, ataxia and pyramidal symptoms in addition to leukodystrophy, and tubulopathy.⁸² A sibling presented with lactic acidosis after birth and died aged 5 days. Few other patients have been described suffering from anemia, sensorineural deafness and hypertrophic cardiomyopathy,⁸³ anemia and LS⁸³ and Leigh-like syndrome.^{84,85} A newborn infant with hypotonia, seizures, lactic acidosis and cardiomyopathy leading to death at 1 month of age was the first case reported with a *COX15* mutation.⁸³ Another infant had a similar but less dramatic clinical picture with hypotonia, motor regression, nystagmus, retinopathy, lactic acidosis and microcephaly by 7 months and LS by 1 year.⁸⁶ A third case with a very slowly progressing LS survived at least 16 years.⁸⁷ The CIV deficiency was 42% and 22% of control values in muscle and fibroblast, respectively, perhaps accounting for a milder phenotype.

Practice points

- Most individuals affected by complex I and complex IV deficiencies have an uneventful prenatal period and deficiencies are evident postnatally.
- Perinatal manifestations of BCS1L mutations present often with fetal growth restriction and lactacidosis.
- Neonatal genetic screening of complex I, complex III, and complex IV deficiencies should include genes coding for specific chaperones in addition to structural subunits.
- Early diagnosis of origin of OXPHOS defect could aid in treatment.
- Treatments for OXPHOS defects address solely the symptoms.

Research directions

- Further studies are required to elucidate of the complete sequence of assembly steps of complex I, complex III and complex IV and its regulation.
- A better understanding of the specific function of assembly chaperones and their interaction partners is required.
- Research focusing on understanding the genotype–clinical phenotype and the influence of genetic background and environmental factors is necessary for effective treatments.

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Figure 1.

BCS1L is encoded by eight exons with the start codon in exon 2 and the stop codon in exon 8. Mutations in the 5'UTR region causing complex III deficiency are shown. In the BCS1L protein three functional domains, the import domain, the BCS1L_N-specific domain and the AAA_ATPase domain can be identified. *BCS1L* mutations are indicated by their respective amino acid exchange. Mutations occur in all domains of the protein but cause a wide spectrum of symptoms ranging from mild complex III deficiency and Björnstad syndrome (shown above the BCS1L protein) to mutations causing mainly hepatopathy (bold, italic), mainly encephalopathy (underlined) or hepatopathy and encephalopathy (bold, italic and underlined) (shown below the BCS1L protein). *BCS1L* mutations are indicated by their respective amino acid exchange. Patients identified so far are either homozygous for mutations (P99L, S277N, S78G, T50A, G129R) or compound heterozygotes (R45C/R56ter, V327A/R56ter, G35R/184C, S78G/144Q, R73C/F368I, R155P/V353M).

Table 1

Mutations in assembly factors as cause of neonatal mitochondrial disorder

Assembly factor	Mutation	IUGR	Lactic acidosis	Leigh syndrome	Cardio myopathy	Liver disease	Other	Ref.
NDUFA2	ΔExon2		+	+	+			15
	R45X							
	ML1							
C20orf7	L229P	+	+	+				19–20
	I59F							
C3orf60	G77R		+				Weak muscle	22
NDUFAF3	R122P							
	R122T							
C6orf66	L65P		+		+			21
NDUFAF4								
ACAD9	Various		+	+	+			26–28
BCS1L	Various	+	+	+	+	+	Weak muscle	45,48,60–66
SURF1	Various		+	+				69
SCO1	P174L		+	+	+	+		78,79
	G132S							
	Stop							
SCO2	Various			+	+			80,81
COX10	P225L		+	+	+		Weak muscle	82,83,85
	T204K							
	T196K							
COX15	R217W		+		+			83
	S344P							
	H152X							

IUGR, intrauterine growth restriction.