Table 1. Overview of disorders of flavocoenzymes and flavoproteins associated with primary and secondary mitochondrial dysfunction

Disease name	Gene	Phenotype OMIM number	Primary clinical phenotype	Biochemical findings	Riboflavin responsiveness
Riboflavin transporter deficiency	*SLC52A2*	614707	Sensorimotor and cranial neuropathy Sensorineural hearing loss	Multiple acyl Co-A dehydrogenase defect (MADD) profile on acylcarnitine and ethylmalonic aciduria	Yes
	SLC52A3	211500	Sensorimotor and cranial neuropathy		
		211530	Sensorimotor and cranial neuropathy Sensorineural hearing loss		
FAD transporter deficiency	*SLC25A32*	616839	Neuromuscular e.g. exercise intolerance, ataxia, muscle weakness	MADD profile Complex II deficiency	Yes
FAD synthase deficiency	*FLAD1*	255100	Fatal infantile onset hypotonia, swallowing difficulties, respiratory insufficiency, cardiomyopathy Late onset lipid storage myopathy	MADD profile Combined respiratory chain deficiencies	Yes
Mitochondrial complex I deficiency, nuclear type 20	*ACAD9*	611126	Early onset and lethal hypertrophic cardiomyopathy, encephalopathy Later presentation with myopathy, e.g., exercise intolerance and muscle weakness	Complex I deficiency	Yes
Mitochondrial complex I deficiency, nuclear type 19	*FOXRED1*	618241	Leigh syndrome Encephalomyopathy	Complex I deficiency	Limited data (n = 2)
COQ10 deficiency-6	*COQ6*	614650	Steroid resistant nephrotic syndrome, sensorineural hearing loss	COQ10 deficiency	No data
Myopathy, mitochondrial progressive, with congenital cataract, hearing loss, and developmental delay	*GFER*	613076	Neuromuscular e.g. hypotonia, muscle weakness, psychomotor retardation, muscle weakness, Cataracts, hearing loss	Isolated Complex IV deficiency or combined deficiencies	No data
Auditory neuropathy and optic atrophy	*FDXR*	617717	Auditory neuropathy and optic atrophy, encephalopathy	Combined respiratory chain deficiencies	No data
Mitochondrial Complex I deficiency, nuclear type 4	*NDUFV1*	618225	Encephalopathy, motor delay Neurodevelopmental regression, Oculomotor impairment	Complex I deficiency	Limited data (n = 7), riboflavin administered with other supplements[94,97,98,101]
Mitochondrial Complex I deficiency, nuclear type 7	*NDUFV2*	618229	Hypertrophic cardiomyopathy Encephalopathy Leigh syndrome	Complex I deficiency	Limited data (n = 1), riboflavin administered with other supplements[113]
Mitochondrial Complex II deficiency	*SDHA*	252011	Leukoencephalopathy, Leigh syndrome, cardiomyopathy, Cancer susceptibility (gastrointestinal stromal tumours, paraganglioma/ pheochromocytoma, pituitary adenoma and renal carcinoma)	Complex II deficiency	No response (n = 2)[133]
Multiple acyl-coenzyme A dehydrogenase deficiency (MADD)	*ETFA, ETFB, ETFDH*	231680	Neonatal onset- with (type I) or without congenital anomalies (type II) and early onset encephalopathy and cardiomyopathy Late onset myopathy (type III)	Increase in short, medium and long chain acylcarnitines Characteristic MADD pattern in urinary organic acids	YES - established for late onset myopathy[138]
Dihydrolipoamide dehydrogenase (E3) deficiency	*DLD*	246900	Early onset encephalopathy, Primary liver involvement, Myopathy	Elevated lactate, pyruvate, alpha ketoglutarate, branched chain amino acids and alpha ketoacids	YES - established for myopathic form[146]

severely affected ACAD9-deficient patients could be attributed to a deficiency in brain FAO. To date, no patients with two *ACAD9* null mutations have been described, suggesting that the complete absence of the protein is lethal. Embryonic lethality was also proposed for the failure to generate homozygous knockout *ACAD9* mice[49].

Riboflavin supplementation resulted in alleviation of symptoms in 65% patients, and, most notably, improved survival was observed when commenced within the first year of life[51]. Riboflavin responsiveness may be related to its essential function as a precursor of FAD cofactor for ACAD enzyme activity and stability. It also increases ACAD9 protein levels and rescues Complex I assembly, while also functioning as a chemical chaperone by improving folding of *ACAD9* mutant proteins[49]. Schiff *et al.*[49] (2015) suggested that similar specific interventions used for VLCAD defects including avoidance of fasting, medium-chain triglycerides, or triheptanoin anaplerotic therapy could be beneficial in terms of long-term outcomes in patients.

FAD-dependent OXidoREDuctase deficiency (FOXRED1) (OMIM #618241)

FOXRED1 has been proposed to be a dual function protein. It plays a key role as an assembly factor for Complex I biogenesis, and, secondly, due to its oxidoreductase activity, it is hypothesized to participate in glycine metabolism which modulates glutathione biosynthesis, an antioxidant protecting the cells from ROS[53-55]. Co-immunoprecipitation experiments suggest that FOXRED1 facilitates Complex I assembly by associating with the 370-kDa subcomplex and two other FAD-dependent Complex I assembly factors, ACAD9 and probably AIFM1[55].

FOXRED1 mutations have been recognized as a cause of Complex I deficiency. To date, eight patients from six families have been described with variable clinical spectrum and severity[55-60]. Age of onset of clinical manifestations ranged from birth to early infancy with one prenatal onset of oligohydramnios, severe intrauterine growth retardation, and periventricular cysts[60]. The clinical manifestations included Leigh syndrome and infantile-onset encephalomyopathy with epilepsy, mild to severe psychomotor retardation, and hypotonia. Non-neurological features included congenital lactic acidosis, cardiomyopathy, hepatomegaly, kyphoscoliosis, optic atrophy, roving eye movements, strabismus, and distal renal tubular acidosis[55-58,60]. Brain neuroimaging (MRI) findings ranged from normal to delayed myelination and features consistent with Leigh syndrome[55-57]. Enzymatic studies supported Complex I deficiency in all patients. Additionally, a milder decrease in Complex II was observed in one patient[59]. The majority of mutations identified were missense. Riboflavin supplementation was reported in only two patients[57,60], of whom one had prenatal onset and died at three months of age[60]. Outcome was available in 6/8 patients: 4/6 are alive with median age of 17 years (age range: 10-22 years) and another died at eight years. In contrast to patients with other nuclear encoded Complex I defects who typically have a severe clinical presentation and associated early death, it has been suggested that pathogenic variants in the *FOXRED1* gene result in partial loss of function and are probably hypomorphic due to the longer survival of patients[61].

COQ6 deficiency (OMIM #614650)

Human Coenzyme Q10 monooxygenase 6 (COQ6) is a flavoprotein involved in the biosynthesis of Coenzyme Q10 which operates as a redox carrier by transferring electrons from respiratory chain Complexes I and II to Complex III. Coenzyme Q10 is also a potent antioxidant[62] and a cofactor of many mitochondrial dehydrogenases. It is required for pyrimidine nucleoside biosynthesis and has been implicated in the inhibition of apoptosis by preventing the collapse of the inner mitochondrial membrane[63]. Studies using human cell line lacking functional COQ6 showed impaired COQ synthesis, severe ATP deficiency, and increased production of reactive oxygen species[64].