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REVIEW ARTICLE

Recent topics: the diagnosis, molecular genesis, and treatment of mitochondrial diseases

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Abstract

Mitochondrial diseases are inherited metabolic diseases based on disorders of energy production. The expansion of exome analyses has led to the discovery of many pathogenic nuclear genes associated with these diseases, and research into the pathogenesis of metabolic diseases has progressed. In cases of Leigh syndrome, it is desirable to perform both biochemical and genetic analyses, and pathogenic gene mutations have been identified in over half of the cases analyzed this way. Tandem mass screening and organic acid analyses of urine can sometimes provide important information that leads to the identification of pathogenic genes. Our comprehensive gene analyses have led to the discovery of several novel genes for mitochondrial diseases. Indeed, we reported that *GTPBP3* and *QRSL1* are involved in mitochondrial DNA maturation. In 2017, as a result of international collaboration, we also identified that mutations in *ATAD3* and *C1QBP* cause mitochondrial disease. Given the varied pathogeneses, treatments for mitochondrial diseases should be specifically tailored to the mutated gene. Clinical trials of sodium pyruvate, 5-aminolevulinic acid with sodium ferrous citrate, and taurine as a treatment for mitochondrial disease have begun in Japan. Given that some mitochondrial diseases may respond well to certain treatments if the pathogenic gene can be identified, an early genetic diagnosis is crucial. Additionally, in Japan, prenatal diagnoses for mitochondrial diseases caused by nuclear genes have been achieved for genes shown to be pathogenic. Treatment and management approaches, including prenatal diagnoses, specifically tailored to the various phenotypes and pathologies of mitochondrial diseases are expected to become increasingly available.

Introduction

Mitochondria are intracellular organelles with a double membrane. Mitochondria play an essential role in cells, the biosynthesis of ATP via oxidative phosphorylation (OXPHOS). ATP is produced by the ATP synthase complex, which is driven by the proton motive force created by the respiratory chain complexes (complex I, III, and IV). If OXPHOS is impaired, organ damage will occur. This is referred to as mitochondrial respiratory chain disorder (MRCD), which is thought to occur at a frequency of 1 in 5000 births in Japan and Western countries [1, 2]. When considering mitochondrial diseases, we typically refer to classical types such as Leigh syndrome (LS) and mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS). However, many pathogenic nuclear genes have been discovered using next-generation sequencing in addition to biochemical studies such as enzymological analysis and Blue Native Polyacrylamide Gel Electrophoresis (BN-PAGE) western blotting. Therefore, many articles on mitochondrial disorders have been published. These papers have focused on cofactor metabolism, mitochondrial DNA (mtDNA), and/or nuclear DNA (nDNA) maturation metabolism, and the subunits and assembly factors of each complex. In the last 6 years, >100 novel genes associated with mitochondrial diseases have been identified, and approximately 300 causative genes have been identified in total [3, 4]. These studies have provided both a better understanding of the pathogenesis

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Primary role specific to OXPHOS biogenesis

OXPHOS Subunits
CI: MT-ND1, MT-ND2, MT-ND3, MT-ND4, MT-ND4L, MT-ND5, MT-ND6, NDUFA1, NDUFA2, NDUFA9, NDUFA10, NDUFA11, NDUFA12, NDUFA13, NDUFB3, NDUFB9, NDUFB10, NDUFB11, NDUFS1, NDUFS2, NDUFS3, NDUFS4, NDUFS6, NDUFS7, NDUFS8, NDUFV1, NDUFV2
CII: SDHA, SDHB, SDHD
CIII: MT-CYB, CYC1, UQCRB, UQCRC2, UQCRQ
CIV: MT-CO1, MT-CO2, COX4I1, COX4I2, COX5A, COX6A1, COX6B2, COX7B, COX8A, NDUFA4
CV: MT-ATP6, MT-ATP8, ATP5A1, ATP5E

Electron Carriers
CoQ: COQ2, **COQ4**, COQ5, COQ6, COQ7, COQ8A, COQ8B, COQ9, PDSS1, PDSS2
Cyt. c: CYCS, HCCS

mtDNA Homeostasis: DNA2, MGME1, POLG, POLG2, RNASEH1, TFAM, TWNK, **TOP3A**

Nucleotide Pools: ABAT, DGUOK, MPV17, RRM2B, SAMHD1, SUCLA2, SUCLG1, TK2, TYMP

mt-tRNA Biogenesis/Aminoacylation: MT-TA, MT-TC, MT-TD, MT-TE, MT-TF, MT-TG, MT-TH, MT-TI, MT-TK, MT-TL1, MT-TL2, MT-TM, MT-TN, MT-TP, MT-TQ, MT-TR, MT-TS1, MT-TS2, MT-TT, MT-TV, MT-TW, MT-TY, **GTPBP3**, MTFMT, MTO1, NSUN3, PUS1, **QRSL1**, TRIT1, TRMT5, TRMU, TRNT1, AARS2, CARS2, DARS2, EARS2, FARS2, GARS, HARS2, IARS2, KARS, LARS2, MARS2, NARS2, PARS2, RARS2, SARS2, TARS2, VARS2, WARS2, YARS2

Translation: C12orf65, GFM1, GFM2, RMND1, TACO1, TSFM, TUFM

Blue fonts indicate genes which we reported

OXPHOS Assembly Factors
CI: ACAD9, FOXRED1, NDUFAF1, NDUFAF2, NDUFAF3, NDUFAF4, NDUFAF5, NDUFAF6, NUBPL, TIMMDC1, TMEM126B
CII: SDHAF1
CIII: BCS1L, LYRM7, TTC19, UQCC2, UQCC3
CIV: COA3, COA5, COA6, COA7, COX10, COX14, COX15, COX20, PET100, PET117, SCO1, SCO2, SURF1
CV: ATPAF2, TMEM70

mtRNA Expression/Processing: ELAC2, FASTKD2, HSD17B10, LRPPRC, MRM2, MTPAP, PNPT1, TRMT10C

Mitoribosome Biogenesis: MT-RNR1, ERAL1, MRPL3, MRPL12, MRPL44, MRPS7, MRPS16, MRPS22, **MRPS23**, MRPS34

256 nuclear encoded
35 mtDNA encoded

Secondary impact on OXPHOS

Fe-S clusters: ABCB7, BOLA3, FDX1L, FDXR, FXN, GLRX5, IBA57, ISCA2, ISCU, LYRM4, NFS1, NFU1

Co-Factors: COASY, FLAD1, LIAS, LIPT1, LIPT2, PANK2, TPK1

Protein Quality Control: AFG3L2, CLPB, CLPP, HSPD1, LONP1, SPG7, YME1L1

Protein Import/Processing: AGK, AIFM1, DNAJC19, GFER, MIPEP, PMPCA, TIMM8A, TIMM50

Morphology: CHCHD10, C19orf70, DNM1L, GDAP1, MFF, MFN2, MSTO1, OPA1, SACS, SLC25A46, STAT2, TRAK1

Lipid Modification/Homeostasis: **ATAD3A**, PNPLA8, CHKB, SERAC1, PLA2G6, TAZ, **PNPLA4**

Metabolite Transport: SLC19A2, SLC19A3, SLC25A1, SLC25A3, SLC25A4, SLC25A12, SLC25A19, SLC25A24, **SLC25A26**, SLC25A32, SLC25A42, SLC39A8, MICU1, MICU2, MPC1

TCA cycle: ACO2, ALDH18A1, DLAT, DLD, FH, HAAO, IDH3A, IDH3B, KYNU, MDH2, MECR, NADK2, PDHA1, PDHB, PDHX, PDK3, PDP1, PPA2

Metabolism of Toxic Compounds: D2HGDH, ECHS1, ETHE1, HIBCH, L2HGDH, NAXE, TXN2

Apoptosis/Autophagy: HTRA2, VPS13C

Unclear Function: APOPT1, OPA3, CEP89, RTN4IP1, C19orf12, SFXN4, **C1QBP**, TMEM65, FBXL4, **IARS**

Fig. 1 Genes linked to disorders of mitochondrial energy generation (based on Frazier et al. [3]). Comprehensive gene analysis has revealed many causative genes, which can be categorized in based on their function

associated with each gene at the biochemical level, and have led to the development of specific treatments. In this review, we summarize the current understanding of mitochondrial disease and present new knowledge that is advancing the field.

Pathogenic genes in mitochondrial and nDNA

Various pathogenetic processes lead to a decrease in respiratory chain activity. Mitochondrial diseases can be grouped into six categories:

(1) Disorders of OXPHOS subunits and assembly factors.

(2) Defects of mitochondrial DNA, RNA, and protein synthesis.

(3) Defects in the substrate-generating upstream OXPHOS reactions.

(4) Defects in relevant cofactors.

(5) Defects in mitochondrial homeostasis.

(6) Production of inhibitors of the respiratory chain.

Almost all mitochondrial diseases can be assigned to the above categories, and deficiency of more than one respiratory chain enzyme common. Defects involving approximately 300 genes (Fig. 1) have been identified to date, making disorders of mitochondrial energy metabolism the most heterogeneous metabolic disease group. Approximately one-quarter of mitochondrial diseases diagnosed in childhood feature mtDNA abnormalities, whereas the remaining three-quarters are due to defects in nDNA [5].

Clinical diagnosis and specific tests for mitochondrial diseases

Schematics of the clinical diagnosis of mitochondrial diseases in Japanese children, and the affected respiratory chain complexes, are shown in Figs. 2a, b. Figure 2a shows a breakdown of the clinical diagnoses of mitochondrial

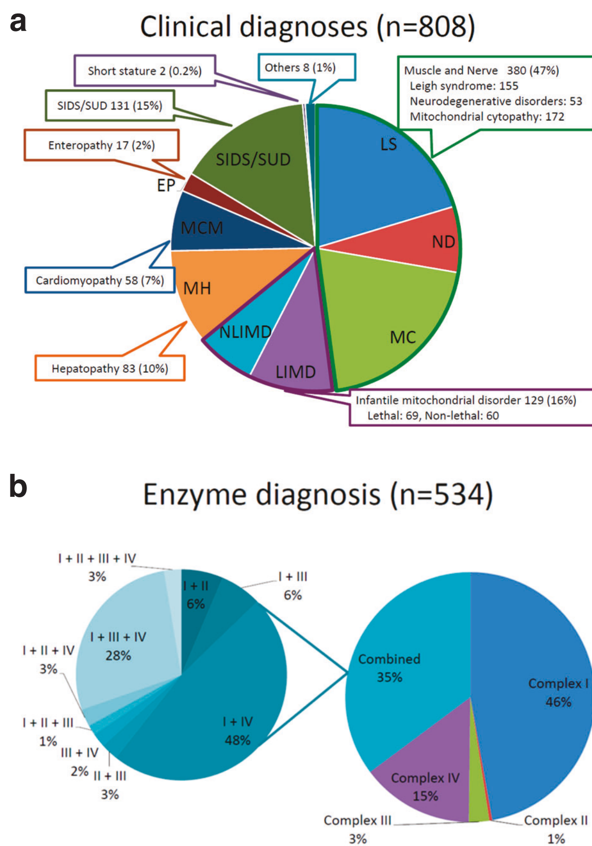

Fig. 2 **a** Breakdown of clinical diagnoses of mitochondrial disorders at our institute (as of June 2018). LS Leigh syndrome, ND neurodegenerative disorder, MC mitochondrial cytopathy, IMD infantile mitochondrial disease (lethal and non-lethal), MH mitochondrial hepatopathy, MCM mitochondrial cardiomyopathy, SIDS sudden infant death syndrome, SUD sudden unexpected death. **b** Breakdown of enzyme diagnosis in 534 Japanese patients with mitochondrial disorders. The most commonly observed defect is in complex I alone, whereas the second most common defect pattern is defects in multiple complexes

disorders in our institute as of June 2018. A total of 380 patients described nerve and muscle, including 155 patients with LS, 53 patients with neurodegenerative disorders for which no clear cause could be identified, and 172 patients with so-called “mitochondrial encephalomyopathy”. These 380 patients accounted for 47% of the total of 808 patients with mitochondrial disorders that presented at our institute during this period. Other forms of mitochondrial disorder accounted for roughly half of the cases, including 69 cases of lethal infantile mitochondrial disease. Together with non-lethal infantile mitochondrial disease, which follows the same course but in which patients survive beyond 1 year of age, the number reached 129, and was the most common clinical diagnosis. Lethal infantile mitochondrial disease encompasses hyperlactacidemia occurring in the neonatal period together with multiple organ failure. Most lethal infantile mitochondrial disease cases have poor outcomes, and most of these patients died with the cause unknown and no diagnosis established. Mitochondrial disorders showing single organ dysfunction, such as mitochondrial hepatopathy and cardiomyopathy, accounted for 10 and 7% of the cases, respectively.

Sudden infant death syndrome and sudden unexpected death accounted for 131 cases (16%). Some of these cases were categorized as LS, MC, and MCM, but in this category, it is difficult to identify the pathogenic gene.

In patients in whom the clinical phenotype is suggestive of mitochondrial disease, specific biochemical tests, pathological analyses, and gene analyses are available. Biochemical tests for mitochondrial disease include analyses of lactate, pyruvate, and amino acids (in serum or plasma), urine organic acid analysis, respiratory chain enzyme assays, and the oxygen consumption rate test. More recently, FGF21 and GDF15 are expected to be useful as specific biomarkers of mitochondrial disease [6].

Biochemical tests

Many patients with mitochondrial disease have a high lactate level. This is especially true in cases with a defective respiratory chain, where the ratio of lactate/pyruvate is elevated (>20). In contrast, in cases with defective pyruvate oxidation, such as pyruvate dehydrogenase complex deficiency, the lactate/pyruvate ratio is around 10. However, 10–20% of patients with mitochondrial disease have normal lactate levels. Reflecting high lactate levels, blood alanine levels are also elevated. Organic acid analysis in urine can provide significant information that can sometimes suggest which gene is affected in each particular case. Table 1 shows the relationship between organic acid analysis data and the expected causative gene in patients with mitochondrial diseases [7–13]. However, it should be noted that these organic acid analysis findings were not detected in all patients.

Respiratory chain complex enzyme activity

Measurement of mitochondrial respiratory chain (MRC) complex activity is significant for biochemical diagnosis, and is effective for predicting the causative gene in cases of suspected mitochondrial disease. For example, a selective defect in one respiratory chain complex can be indicative of a defect in a complex subunit or an assembly factor. Combined OXPHOS defects are also often identified in the diagnosis of mitochondrial disease [14]. At the Mitochondrial Disease Diagnosis Center in Chiba Children’s Hospital, combined complex deficiencies are the second leading cause of mitochondrial disease, and account for 35% of cases (Fig. 2b). However, combined complex deficiencies are often identified in enzyme assays as isolated respiratory

chain complex defects. In MELAS patients with the m.3243A>G mutation that affects mitochondrial tRNA$^{Leu (UUR)}$, isolated complex I (CI) deficiency is often found in enzyme assays of muscle. The level of cytochrome *c* oxidase (complex IV (CIV)) can also decrease together with complex I. In the same way, in patients with biallelic *MPV17* gene mutations, which cause hepatocerebral type mtDNA deletion syndrome, complex I, III, and IV (+V) deficiency is often found, but sometimes cases with CI deficiency alone are encountered.

Mayr et al. described the typical combinations of OXPHOS defects that are identified in patients [4], such as CI + CIV, CI + CIII + IV + V, CI + CII + CIII, CI + III/CII + III, CIII + CIV, or the involvement of all complexes (Fig. 3). Figure 2b shows the enzyme diagnosis in Japanese pediatric mitochondrial patients. CI deficiency is the most common defect followed by defects in multiple complexes, then CIV defects, such as the *SURF1* mutation associated with LS.

Table 1 Urine organic acid analysis with mitochondrial disease

•Mild methylmalonic aciduria
SUCLG1 defect, *SUCLA2* defect
•Ethylmalonic aciduria
ETHE1 defect
•3-Methylglutaconate
Barth syndrome (*TAZ* gene; cardiomyopathy, neutropenia)
MEGDEL syndrome (*SERAC1* gene; hearing loss, Leigh disease etc.)
•3-Methylglutaconate, 3-methylglutarate, 2-ethyl-3-hydroxypropionate etc.
mtDNA single deletion
ex. Pearson syndrome
•2,3-Dihydroxy-2-methylbutyrate and 3-methylglutaconate
ECHS1 deficiency
•3-Hydroxyisovalerate, 3-methylcrotonylglycine, methylcitrate,
3-Hydroxypropionate, 2-methyl-3-hydroxybutyrate
•(similar to multiple carboxylase deficiency)
Mutation in *MT-ATP6*(ex. 8993T>G)
Others; increasing the metabolite of TCA cycle

Measurement of oxygen consumption rate

Recently, microscale oxygraphy has been used to evaluate OXPHOS defects in basic and clinical studies. This approach is highly efficient for detecting mitochondrial respiratory defects in patients with genetically proven mitochondrial disease [15, 16]. Half of the cases in our LS cohort, with no apparent defect in MRC complex activities, showed a significant oxygen consumption rate decline. Although additional evidence needs to be accumulated, this finding suggests that microscale oxygraphy could be used as a screening tool to detect MRC defects, especially in cases in which each complex remains intact. If cellular oxygen consumption rate shows a significant reduction, genetic screening should be considered, even if MRC defects are not detected by enzyme assays of fibroblasts or peripheral organs.

Acylcarnitine analysis (tandem mass screening) and amino-acid analysis in cases of mitochondrial disease

In the acylcarnitine profile of patients with LS caused by m.8993T>G (MT-ATP6) mutation, low citrulline and elevated propionylcarnitine (C3) and 3-hydroxyisovalerylcarnitine (C5-OH) levels are often observed, along with fluctuating organic acids indicating multiple carboxylase deficiency. Persistent hypocitrullinemia is found in cases with MT-ATP6 defects and in other mitochondrial diseases including MELAS, mitochondrial neurogastrointestinal encephalopathy, and Pearson syndrome [12]. Low ATP production can lead to deficiencies in the delta-1-pyrroline-5-carboxylate synthetase (P5CS) and carbamoyl phosphate synthase I (CPSI) activities of enterocytes, with subsequent enterocyte dysfunction and impairment of the intestinal citrulline biosynthetic pathway [12, 17–23] (Fig. 4).

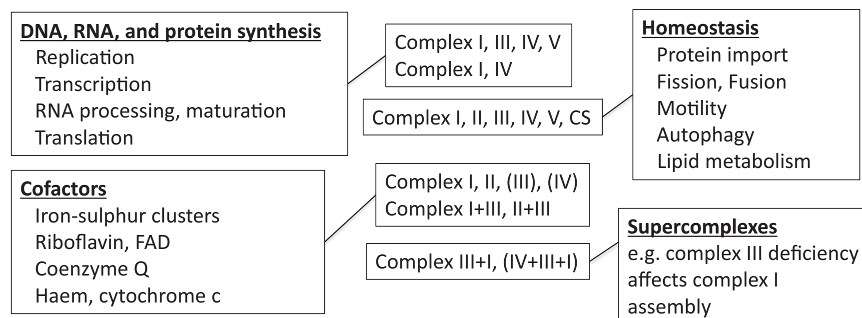

Fig. 3 Types of combined respiratory chain defects and their causes. Defects in complex I are the most common type, whereas defects in multiple complexes is the second most common. Considering the type of defect of multiple complexes along with the clinical phenotype, it is possible to predict the pathogenesis

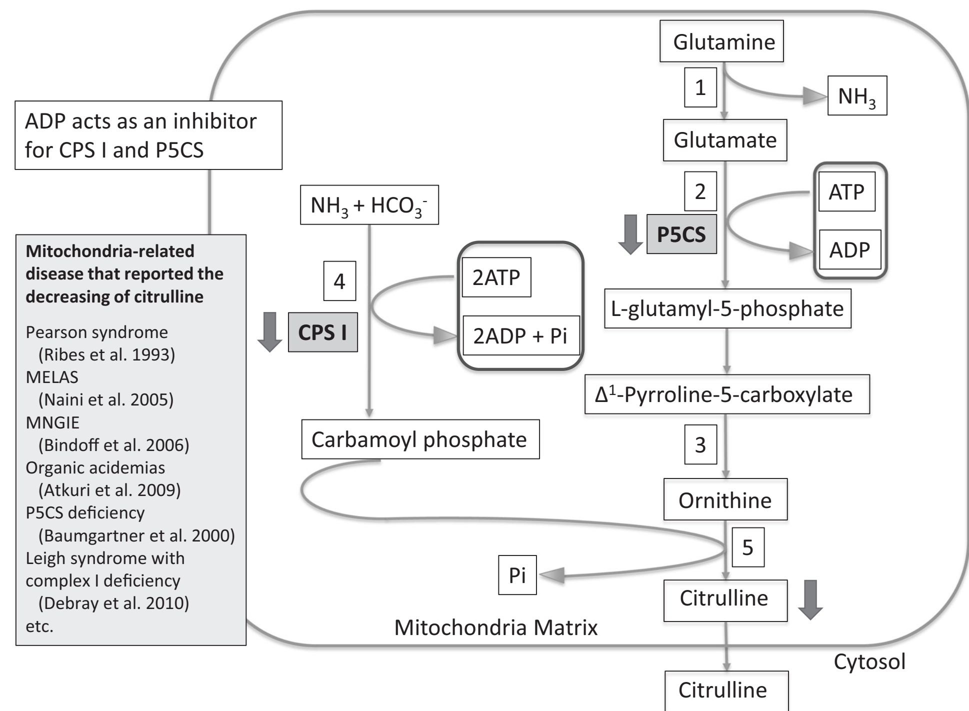

Fig. 4 Citrulline synthesis in mitochondrial enterocytes (based on Balasubramaniam [12]). 1. Glutaminase, 2. delta-1-pyrroline-5-carboxylate synthetase (P5CS), 3. ornithine-oxo-acid transaminase, 4. carbamoyl phosphate synthetase I (CPSI), 5. ornithine transcarbamylase (OTC)

Pathological tests

In respiratory chain dysfunction, ragged-red fibers, cytochrome *c* oxidase defects in muscle, and defects of some complexes in the liver are exhibited. Electron micrography often shows mitochondrial morphological abnormalities and increased mitochondrial numbers. However, these findings are not specific to mitochondrial disease, and particular attention is required to avoid over diagnosis. Immunohistochemical analysis of each respiratory complex is significant for enzymological evaluation.

Comprehensive genetic analysis

Approximately 300 mitochondrial disease causative genes have been identified. Therefore, comprehensive genomic analysis including both mtDNA and nDNA is needed to identify the gene mutations associated with mitochondrial disorders. Japanese data from a comprehensive gene analysis targeting MRCD were reported by Kohda et al. in 2016. This approach included whole-mtDNA and exome analyses using high-throughput sequencing and chromosomal aberration analyses using high-density oligonucleotide arrays. Using this approach, the causative gene was identified in 35% of MRCD cases [24]. Whole-exome sequence analyses, along with verification experiments, revealed *ECHS1* [25], *SLC25A26* [26], *MRPS23*, *QRSL1*, *PNPLA4* [24], *IARS* [27], and *TOP3A* [28] as novel causative genes of mitochondrial disorders, and many Japanese population-specific mutations were identified in our cohort.

In addition, our custom-designed gene panels (Agilent SureSelect), which cover >250 known nuclear genes, point mutations, and mtDNA deletions, are appropriate for analyzing cases with typical phenotypes of mitochondrial disease, such as MELAS, LS, and hepatopathy. In cases with these phenotypes, determination of the causative gene can be readily achieved using this gene panel could be used for rapid genetic diagnosis.

Through the expansion of whole-exome sequencing and the identification of pathogenic genes, the pathogenesis of each clinical phenotype has been revealed and connected to drug discovery research. Some groups of mitochondrial diseases, including cofactor metabolism (e.g., coenzyme Q_{10} deficiency) and the production of toxic metabolites (e.g., ECHS1 or HIBCH deficiency), have potentially become treatable (Table 2). Importantly, many of such treatable defects (e.g., *SLC19A3*, *ECHS1*, or *HIBCH* mutations) show clinical and neuroradiological similarities to classical mitochondrial disorders such as LS. Therefore, genetic tests have become more important in a clinical context to manage mitochondrial diseases.

Diagnosis and molecular basis of LS in Japan

Ogawa et al. reported data from biochemical and molecular analyses of 106 Japanese cases with LS [16]. Decreased respiratory chain enzyme activity, determined using muscle tissue or skin fibroblasts, was seen in about

Table 2 Inherited mitochondrial diseases with specific treatment options (based on Distelmaier [58])

Clinical syndrome	Affected gene	Therapeutic substance	Treatment response
Brown-Vialetto-VanLaere syndrome/Fazio-Londe disease	*SLC52A2*, *SLC52A3*, *SLC52A1*	Riboflavin (oral: 10–50 mg/kg/day)	Generally good
Biotin-thiamine-responsive basal ganglia disease	*SLC19A3*	Thiamine (oral: 10–20 mg/kg/day); Biotin (oral: 10–15 mg/kg/day)	Generally good
Biotinidase deficiency	*BTD*	Biotin (oral: 5–10 mg/kg/day)	Generally good
Holocarboxylase synthetase deficiency	*HLCS*	Biotin (oral: 10–20 mg/kg/day)	Variable but generally good
Thiamine pyrophosphokinase deficiency	*TPK1*	Thiamine (oral: ~20 mg/kg/day)	Variable (< 10 patients treated so far)
ACAD9 deficiency	*ACAD9*	Riboflavin (oral: 10–20 mg/kg/day)	Variable
Multiple acyl-CoA dehydrogenase deficiency	*ETFA, ETFB, ETFDH, SLC25A32, FLAD1*	Riboflavin (oral: ~10 mg/kg/day)	Generally good
Thiamine-responsive pyruvate dehydrogenase deficiency	*PDHA1*	Thiamine (oral: 30–40 mg/kg/day)	Variable
Coenzyme Q_{10} deficiency	*PDSS1, PDSS2*, COQ2, COQ4, COQ6, *COQ7, ADCK3, ADCK4, COQ9*	Coenzyme Q_{10} (oral: 10–30 mg/kg/day)	Highly variable depending on the underlying defect
Cytochrome *c* oxidase deficiency	*SCO2*, *COA6*	Copper–histidine (dose unclear; subcutaneous injections of up to 500 µg daily were suggested)	Unclear, only one SCO2 patient treated; only in vitro evidence for COA6
Molybdenum cofactor deficiency	*MOCS1, MOCS2, GPHN*	Cyclic pyranopterin monophosphate (intravenous: 80–320 µg/kg/day)	Generally good in MoCD type A patients
3-Hydroxyisobutyryl-CoA hydrolase deficiency	*HIBCH*	Valine-restricted diet	Unclear, only few patients treated
Enoyl-CoA hydratase deficiency	*ECHS1*	Valine-restricted diet	Unclear, only few patients treated so far
Thioredoxin 2 deficiency	*TXN2*	Antioxidant treatment (e.g., Idebenone up to 20 mg/kg/day)	Apparently good (only one patient reported)
Ethylmalonic encephalopathy	*ETHE1*	Metronidazole, *N*-acetyl cysteine as glutathione precursor, liver transplantation	Variable

The genes underlined in black were identified in Japanese patients

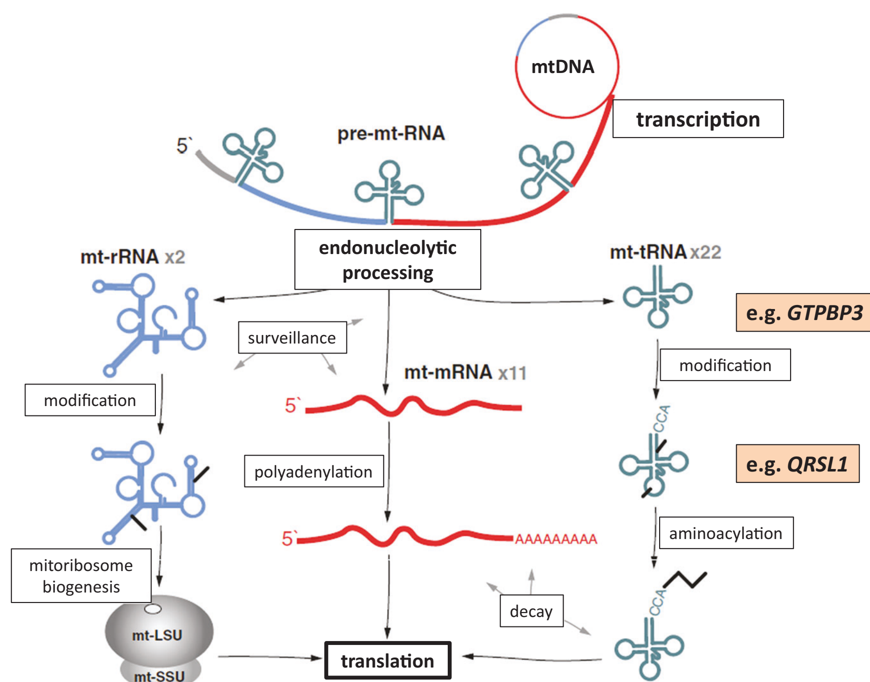

Fig. 5 Mitochondrial RNA metabolic pathway (based on Van Haute [30]). The generation of functional RNA molecules used for protein synthesis requires transcription, nucleolytic processing, post-transcriptional nucleotide modifications, polyadenylation of mt-mRNA, and mt-tRNA-aminoacylation. *QRSL1* is a mt-tRNA aminoacylation disorder. *GTPBP3* is the a tRNA modification disorder

60% of patients when either tissue was used, and in up to 70% of patients when both samples were used. Furthermore, microscale oxygraphy detected MRC impairment in 50% of cases with no enzyme activity defects. At present, the pathogenic gene mutation is identified in up to 90% of LS patients diagnosed by enzyme assay. Moreover, the pathogenic gene has been identified in about 60% of patients and mutations in *ECHS1*, *SURF1*, and *NDUFAF6* in nDNA were the most commonly observed.

Short-chain enoyl-CoA hydratase (ECHS1) is a multifunctional mitochondrial matrix enzyme involved in the oxidation of fatty acids and essential amino acids such as valine. ECHS1 deficiency was reported to involve a rare error of metabolism caused by biallelic *ECHS1* (OMIM 602292) mutations. The associated clinical presentation includes infantile-onset severe developmental delay, regression, seizures, cardiomyopathy, elevated lactate, and brain magnetic resonance imaging (MRI) abnormalities consistent with LS. The characteristic abnormal biochemical findings are secondary to dysfunction of valine metabolism. Therefore, a valine-restricted diet is considered as a treatment to avoid its toxic metabolites. Urine organic acid analysis by Gas Chromatography Mass Spectrometry (GC/MS) showed increased levels of 2,3-dihydroxy-2-methylbutyrate and 3-methylglutaconate, which are now considered significant markers of ECHS1 deficiency (not toxic markers of methacrylyl-CoA) [25, 29].

Identification of the novel causative genes of mitochondrial diseases

Mitochondrial diseases due to defects in mtRNA maturation

mtDNA is circular, double-stranded DNA, and contains 16,569 base pairs in humans. Mitochondrial RNA (mtRNA) maturation is necessary for the correct translation of mtDNA-encoded genes. Most of the mitochondrial translation process depends on nDNA-encoded proteins, so nuclear gene mutations can contribute to defects in mtRNA maturation. The pathway of mitochondrial RNA metabolism is shown in Fig. 5. mtDNA is transcribed into pre-mtRNA, which is followed by endonucleolytic processing. Then, 22 mitochondrial transfer RNAs are modified and aminoacylated for correct protein synthesis [30]. A number of nuclear genes associated with mtRNA maturation have been reported. We previously reported *GTPBP3*- and *QRSL1*-related mitochondrial diseases and have used them as examples below.

GTPBP3-related mitochondrial disease

GTPBP3 encodes the mitochondrial GTP-binding protein involved in catalyzing the formation of 5-taurinomethyluridine at the first (wobble) position in the anticodon of five mitochondrial tRNAs (mt-tRNAs). *GTPBP3* mutations inhibit taurine modification of mt-tRNA, leading to mitochondrial translation defects that result in severe organ dysfunction. *MTO1* (mitochondrial tRNA translation optimization 1), which is also associated with taurine modifications, has been described as a cause of mitochondrial diseases. Our international collaborative research results, reported in 2014, show that *GTPBP3* mutations cause severe infantile mitochondrial diseases [31]. m.3243A>G, the most common mtDNA mutation that can cause MELAS, is a point mutation in the mt-tRNA^{Leu} $^{(UUR)}$ gene and affects taurine modification. Although m.3243A>G and *GTPBP3* mutations can similarly impair mt-tRNA taurine modification, the *GTPBP3* mutation phenotype is a severe neonatal-onset mitochondrial disease characterized by severe lactic acidosis, acute encephalopathy, and hypertrophic cardiomyopathy.

QRSL1-related mitochondrial disease

Sibling cases of *QRSL1*-related mitochondrial disease present with tachypnea, lactic acidosis soon after birth, hypertrophic cardiomyopathy, hearing loss, and adrenal failure. Homozygous mutations in *QRSL1* [glutaminyl-tRNA synthase (glutamine-hydrolyzing)-like 1] are associated with MRC complex I, III, and IV defects (skeletal muscle, cardiac muscle, liver, and adrenal gland). Glutaminyl-tRNA synthetase catalyzes the attachment of glutamine to glutaminyl-tRNA. Recent genomic analyses revealed that this enzyme is only used in the eukaryotic cytoplasm and in a minority of bacteria. Mammalian mitochondrial genomes, the majority of bacterial genomes, and archaeal genomes do not encode the synthetase. Hence, in mammalian mitochondria and in the majority of bacteria and archaea, mitochondrial tRNA^{Gln} is first mis-aminoacylated with glutamate by a non-discriminating glutamyl tRNA synthase. Then, the glutamyl moiety on the Glu-tRNA^{Gln} is transamidated by GatCAB (glutamyl-tRNA^{Gln} amidotransferase) to synthesize Gln-tRNA^{Gln}. Human GatCAB is composed of three subunits, and *QRSL1* encodes human GatA. We demonstrated for the first time in 2016 that *QRSL1* mutations lead to a defect in human mitochondrial translation and are a cause of severe infantile mitochondrial disease [24]. Since then, *QRSL1*-related mitochondrial diseases have been reported in patients from the Netherlands, the United Kingdom, and the United States [32].

Novel causative genes reported in 2017

ATAD3 cluster deletion-related mitochondrial disease

We performed comprehensive genomic analyses of patients with severe neonatal-onset mitochondrial disease manifesting cerebellar dysfunction. Additionally, in 2017, *ATAD3* (ATPase family AAA domain-containing protein 3) gene cluster deletions were identified and reported in collaborative research performed in Japan, Australia, France, and the Netherlands [33]. Most species have a single *ATAD3* gene, but hominids have a cluster of three genes arranged in tandem: *ATAD3C*, *ATAD3B*, and *ATAD3C*. Biallelic genomic rearrangements and *ATAD3* cluster deletions were demonstrated in these cases. Japanese patients with *ATAD3* gene cluster deletions had congenital lactic acidosis, prominent cyanosis, progressive cardiac hypertrophy, and abnormal brain MRI findings, such as cerebral, cerebellar, and brain stem atrophy [26]. The accumulation of intracellular-free cholesterol in *ATAD3* mutant fibroblasts subjected to filipin staining was also observed. *ATAD3* is associated with cellular cholesterol homeostasis, mtDNA organization, adipogenesis, steroid and lipid biosynthesis, mitochondrial translation, and iron and heme homeostasis.

C1QBP-related mitochondrial disease

C1QBP (complement component 1 Q subcomponent-binding protein) was identified following comprehensive genomic analysis in patients primarily presenting with cardiomyopathy. We reported four cases from unrelated families with biallelic mutations in *C1QBP*, in collaborative research performed in Japan, Austria, the United Kingdom, and Germany [34]. Patients with *C1QBP* mutations had a wide range of age of onset, from neonatal to adult. Childhood- and adult-onset patients had peripheral neuropathy and progressive external ophthalmoplegia in addition to cardiomyopathy. *C1QBP* is a multifunctional, multicompartmental protein involved in mitochondrial respiration, inflammation, mitochondrial ribosome biogenesis, and apoptosis, among other things. Combined respiratory chain defects were observed in skeletal muscles and fibroblasts. In complementation assays using *C1qbp*$^{-/-}$ mouse fibroblasts, variants from each patient had significantly lower levels of C1QBP and mtDNA-encoded proteins (MT-CO1 and MT-CO3) than the wild type.

General, specific, and new treatment and management of mitochondrial diseases

The short-term objectives of treatments for mitochondrial diseases are to avoid acute deterioration such as stroke-like

episodes in MELAS, liver failure in mitochondrial hepatopathy, and multiple organ failure with lactic acidosis in neonatal-onset mitochondrial diseases. The long-term treatment objectives include prevention or mitigation of the progression of clinical symptoms by improving respiratory chain function, resulting in increased ATP production. However, very few clinical trials have investigated the efficacy of mitochondrial disease treatments. Therefore, there are few treatments available with sufficient evidence supporting their use. Furthermore, there are problems developing new biomarkers of mitochondrial diseases to evaluate the efficacy of treatments. Lactate, lactate/pyruvate ratio, and plasma alanine are not useful biomarkers for therapeutic effects because of their poor sensitivity and specificity. Thus, the development of biomarkers for therapeutic intervention in mitochondrial diseases is particularly important. Recently, it has been anticipated that GDF15, which has been reported to be a specific mitochondrial disease marker, can be applied in a clinical setting [6, 35, 36]. The treatments that are currently available or are under development are listed below:

Vitamins and cofactors to enhance mitochondrial function

Multiple vitamins (e.g., vitamins B, C, and E, and carnitine) and cofactors (coenzyme Q10), called "mitochondrial cocktails," have been widely used to treat mitochondrial diseases [37–39]. A Cochrane systematic review of mitochondrial therapies found no clear evidence supporting the use of vitamins and cofactors [40]. Most data supportive of the use of mitochondrial cocktails have come from case studies [41–44]. Since few adverse effects have been reported, the administration of mitochondrial cocktails is a reasonable approach, irrespective of whether the mitochondrial dysfunction is primary or secondary [45, 46].

Treatment for acute aggravation

Acute aggravation, including lactic acidosis and metabolic crisis, often occurs in situations with altered energy demands, such as febrile infections, longer periods of fasting, and dehydration [47]. The deterioration of the general and metabolic status tends to be refractory to standard treatment and may result in a rapidly fatal course [47–49]. The administration of oral and intravenous L-arginine, a precursor of nitric oxide, has been shown to improve clinical symptoms of stroke-like episodes in MELAS and to decrease the frequency and severity of the stroke [37, 50, 51]. Combination therapy with L-arginine and citrulline shows improved nitric oxide production and is expected to have a better therapeutic effect than L-arginine alone [52]. Catabolic state, induced by fasting, fever, and illness, should be avoided because of the increased production of toxic metabolites and reactive oxygen species by mitochondria [37]. Infusion therapy including dextrose should be performed with careful attention to the deterioration of lactic acidosis [53].

Nutrition therapy

There is little evidence regarding the efficacy of nutrition therapy for mitochondrial disease. However, the nutritional state of patients should be optimized because insufficient dietary intake leads to secondary mitochondrial dysfunction [54, 55]. A lipid-rich diet can contribute to the transfer of electrons and protons to complex II by promoting $FADH_2$ production. In patients with complex I deficiency, nutrition therapy might be effective [56, 57]. High-concentration glucose infusion and a carbohydrate-rich diet cause excessive nicotinamide adenine dinucleotide hydrate (NADH) accumulation, resulting in the deterioration of the redox balance. Therefore, from a biochemical perspective, a high-lipid and low-carbohydrate diet might be considered a treatment option. A valine-restricted diet has been conducted for ECHS1 deficiency to reduce toxic methacrylyl-CoA via the valine catabolic pathway [25, 29, 58]. The ketogenic diet is also used, and is effective, for patients with pyruvate dehydrogenase complex deficiency [59]. It is anticipated that further studies of nutrition therapy will be performed and that evidence on its efficacy will be accumulated.

Treatable mitochondrial diseases

Some mitochondrial diseases have specific treatments depending on the causative genetic defect (Table 2). Mitochondrial diseases associated with defects of cofactor metabolism account for the great majority of treatable diseases of this kind [58]. The identification of causative genes allows us to elucidate the pathophysiology, potentially leading to specific treatments. Therefore, it is important to confirm the genetic diagnosis as early as possible.

Development of new therapeutic options in Japan

Sodium pyruvate

In the cytoplasm of patients with mitochondrial diseases, excessive NADH is oxidized to NAD with a coupled reaction converting pyruvate to lactate. It has been suggested that sodium pyruvate can improve the redox state, leading increased ATP production via glycolysis [60, 61]. An investigator-initiated clinical trial of sodium pyruvate for MELAS/MELA and LS is ongoing in Japan.

EPI-743

EPI-743, a derivative of coenzyme Q10, can easily cross the blood–brain barrier and targets repletion of reduced intracellular glutathione [62, 63]. In open-label studies, EPI-743 modified disease progression in 13 children and 1 adult with genetically confirmed mitochondrial disease, improved clinical outcomes in children with LS, and arrested disease progression and reversed vision loss in patients with Leber hereditary optic neuropathy [62–65]. A medical company-initiated clinical trial of EPI-743 has been conducted on patients with LS in Japan [66].

5-Aminolevulinic acid and sodium ferrous citrate

Heme acts as a protein-bound prosthetic group in MRC complexes II, III, and IV, and cytochrome *c*. 5-Aminolevulinic acid (5-ALA) is a precursor of heme, which is generated by the insertion of ferrous iron into protoporphyrin IX. Therefore, 5-ALA combined with sodium ferrous citrate enhances heme production leading to the upregulation of complex levels [67, 68]. A clinical trial of 5-ALA with sodium ferrous citrate for infantile LS in patients under the age of 2 years has been performed, and the ranges of target age and form of mitochondrial diseases targeted using this approach will be expanded in 2018.

Taurine for MELAS with m.3243A>G mutation

Taurine supplementation is a specific treatment that is selected based on the particular pathophysiology of a case. Taurine can restore the taurine modification in MELAS patients with m.3243A>G mutation and promote the maturation of $tRNA^{Leu(UUR)}$. A physician-led, multicenter, open-label, 52-week phase III trial of taurine supplementation in MELAS cases has been completed, and promising results were obtained [69].

Prenatal diagnosis and mitochondrial replacement therapy

Mitochondrial diseases are caused by genetic mutations in both mtDNA and nDNA. Although prenatal testing for mtDNA-related mitochondrial diseases is difficult due to heteroplasmy and tissue specificity, nDNA-related mitochondrial diseases can be a focus of prenatal diagnosis. For mitochondrial diseases caused by nDNA mutations, such as LS, neonatal mitochondrial disease, and mtDNA depletion syndrome, prenatal diagnosis has been conducted if the causative gene has been identified [70].

Mitochondrial replacement therapy, which involves the transfer of the mother's oocyte nDNA to an enucleated donor oocyte with wild-type mtDNA, has been performed in the United Kingdom and the United States [71]. mtDNA-related diseases in which much higher levels of heteroplasmy develop, including LS with m.8993T>C/G mutation, are candidates for mitochondrial donation therapy. In Japan, there is currently no legislation pertaining to oocyte donation and germline genome editing. As such, there is a need for careful consideration of novel reproductive technologies, and the associated ethical implications in this country.

Creation of infrastructure for mitochondrial diseases-related medical practices: establishment of Mito-Network in Japan

To raise the quality of clinical practice for mitochondrial diseases, and with strong support from the Japanese Society of Mitochondrial Research and Medicine (J-Mit), we have established three pillars: registry systems, clinical guidelines, and diagnostic systems.

Establishment of clinical guidelines authorized by J-Mit

We published clinical guidelines authorized by J-Mit (http://www.j-mit.org/) involving seven types of mitochondrial phenotypes: infantile mitochondrial disease (congenital lactic acidosis), hepatopathy, cardiomyopathy, LS, MELAS, MERRF, and KSS/CPEO.

Construction of registry systems for mitochondrial diseases

We have established systems for registering cases of mitochondrial disease in neonates and children (J-MO Bank: http://mo-bank.com/index.html). In addition, for the purpose of sharing clinical, biochemical, and genetic data to identify causative genes and achieve drug discovery, we are planning to develop these systems to produce a global mitochondrial registry that is also accessible to researchers in Western countries. Moreover, we plan to construct an adult registry of mitochondrial diseases at the National Center of Neurology and Psychiatry (NCNP).

Establishment and development of specific diagnostic systems

To achieve accurate diagnosis of mitochondrial diseases in Japan, specific diagnostic systems including enzymological and pathological investigations, and comprehensive gene analyses are needed. We established an all-Japan collaboration system in each institution and provided links to the

above guidelines and the J-Mit homepage, which all doctors in Japan can easily access.

Conclusion and future prospects

More than a decade ago, many mitochondrial diseases could not be correctly diagnosed and their pathophysiology was unclear. However, in the last 10 years, research on gene discovery and the pathogenesis of mitochondrial disease has progressed dramatically in Japan. This has depended on the development of biochemical/enzymological analyses and comprehensive gene analyses. In light of the active roles played by the Japan Agency for Medical Research and Development (AMED) and certain drug companies in elucidating the mechanisms involved in mitochondrial diseases development, as well as drug discovery, optimism about the possibility of combating mitochondrial diseases is warranted. Mitochondrial diseases occurs at a frequency of 1 in 5000 births. However, when cases associated with a particular pathogenic gene account for only one or a few patients throughout Japan, which makes them considered ultra-rare diseases. Therefore, international collaboration is critically important for the advancement of many aspects of mitochondrial research, such as gene discovery, pathogenesis, drug discovery, and registry. As medical doctors, basic researchers, patients and family members, and drug company employees, we should unite in the pursuit of the goal of overcoming mitochondrial disease.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Skladal D, Halliday J, Thorburn DR. Minimum birth prevalence of mitochondrial respiratory chain disorders in children. Brain. 2003;126:1905–12.
2. Yamazaki T, Murayama K, Compton AG, Sugiana C, Harashima H, Amemiya S, et al. Molecular diagnosis of mitochondrial respiratory chain disorders in Japan: focusing on mitochondrial DNA depletion syndrome. Pediatr Int. 2014;56:180–7.
3. Frazie, AE, Thorburn DR, Compton AG. Mitochondrial energy generation disorders: genes, mechanisms and clues to pathology. J Biol Chem. 2017. https://doi.org/10.1074/jbc.R117.809194
4. Mayr JA, Haack TB, Freisinger P, Karall D, Makowski C, Koch J, et al. Spectrum of combined respiratory chain defects. J Inherit Metab Dis. 2015;38:629–40.
5. Ohtake A, Murayama K, Mori M, Harashima H, Yamazaki T, Tamaru S, et al. Diagnosis and molecular basis of mitochondrial respiratory chain disorders: exome sequencing for disease gene identification. Biochim Biophys Acta. 2014;1840:1355–9.
6. Gorman GS, Chinnery PF, DiMauro S, Hirano M, Koga Y, McFarland R, et al. Mitochondrial diseases. Nat Rev Dis Prim. 2016;2:16080.
7. Carrozzo R, Verrigni D, Rasmussen M, de Coo R, Amartino H, Bianchi M, et al. Succinate-CoA ligase deficiency due to mutations in SUCLA2 and SUCLG1: phenotype and genotype correlations in 71 patients. J Inherit Metab Dis. 2016;39:243–52.
8. Tiranti V, D'Adamo P, Briem E, Ferrari G, Mineri R, Lamantea E, et al. Ethylmalonic encephalopathy is caused by mutations in ETHE1, a gene encoding a mitochondrial matrix protein. Am J Hum Genet. 2004;74:239–52.
9. Wortmann SB, Kluijtmans LA, Engelke UF, Wevers RA, Morava E. The 3-methylglutaconic acidurias: what's new? J Inherit Metab Dis. 2012;35:13–22.
10. Semeraro M, Boenzi S, Carrozzo R, Diodato D, Martinelli D, Olivieri G, et al. The urinary organic acids profile in single large-scale mitochondrial DNA deletion disorders. Clin Chim Acta. 2018;481:156–60.
11. Huffnagel IC, Redeker EJW, Reneman L, Vaz FM, Ferdinandusse S, Poll-The BT. Mitochondrial encephalopathy and transient 3-methylglutaconic aciduria in ECHS1 deficiency: long-term follow-up. JIMD Rep. 2018;39:83–87.
12. Balasubramaniam S, Lewis B, Mock DM, Said HM, Tarailo-Graovac M, Mattman A, et al. Leigh-like syndrome due to homoplasmic m.8993T>G variant with hypocitrullinemia and unusual biochemical features suggestive of multiple carboxylase deficiency (MCD). JIMD Rep. 2017;33:99–107.
13. Larson AA, Balasubramaniam S, Christodoulou J, Burrage LC, Marom R, Graham BH, et al. Biochemical signatures mimicking multiple carboxylase deficiency in children with mutations in MT-ATP6. Mitochondrion 2018; e-pub ahead of print 4 January 2018; https://doi.org/10.1016/j.mito.2018.01.001
14. Honzik T, Tesarova M, Magner M, Mayr J, Jesina P, Vesela K, et al. Neonatal onset of mitochondrial disorders in 129 patients: clinical and laboratory characteristics and a new approach to diagnosis. J Inherit Metab Dis. 2012;35:749–59.
15. Invernizzi F, D'Amato I, Jensen PB, Ravaglia S, Zeviani M, Tiranti V. Microscale oxygraphy reveals OXPHOS impairment in MRC mutant cells. Mitochondrion. 2012;12:328–35.
16. Ogawa E, Shimura M, Fushimi T, Tajika M, Ichimoto K, Matsunaga A, et al. Clinical validity of biochemical and molecular analysis in diagnosing Leigh syndrome: a study of 106 Japanese patients. J Inherit Metab Dis. 2017;40:685–93.
17. Rabier D, Diry C, Rotig A, Rustin P, Heron B, Bardet J, et al. Persistent hypocitrullinaemia as a marker for mtDNA NARP T 8993 G mutation? J Inherit Metab Dis. 1998;21:216–9.
18. Ribes A, Riudor E, Valcarel R, Salva A, Castello F, Murillo S, et al. Pearson syndrome: altered tricarboxylic acid and urea-cycle metabolites, adrenal insufficiency and corneal opacities. J Inherit Metab Dis. 1993;16:537–40.
19. Naini A, Kaufmann P, Shanske S, Engelstad K, De Vivo DC, Schon EA. Hypocitrullinemia in patients with MELAS: an insight into the "MELAS paradox". J Neurol Sci. 2005;229-230:187–93.
20. Bindoff L. Mitochondrial gastroenterology. In: DiMauro S, Hirano M, Schon EA, editors. Mitochondrial medicine. Abingdon: Informa Healthcare; 2006, p. 143-59.
21. Atkuri KR, Cowan TM, Kwan T, Ng A, Herzenberg LA, Herzenberg LA, et al. Inherited disorders affecting mitochondrial

function are associated with glutathione deficiency and hypocitrullinemia. Proc Natl Acad Sci USA. 2009;106:3941–5.
22. Baumgartner MR, Hu CA, Almashanu S, Steel G, Obie C, Aral B, et al. Hyperammonemia with reduced ornithine, citrulline, arginine and proline: a new inborn error caused by a mutation in the gene encoding delta(1)-pyrroline-5-carboxylate synthase. Hum Mol Genet. 2000;9:2853–8.
23. Debray FG, Lambert M, Allard P, Mitchell GA. Low citrulline in Leigh disease: still a biomarker of maternally inherited Leigh syndrome. J Child Neurol. 2010;25:1000–2.
24. Kohda M, Tokuzawa Y, Kishita Y, Nyuzuki H, Moriyama Y, Mizuno Y, et al. A comprehensive genomic analysis reveals the genetic landscape of mitochondrial respiratory chain complex deficiencies. PLoS Genet. 2016;12:e1005679.
25. Haack TB, Jackson CB, Murayama K, Kremer LS, Schaller A, Kotzaeridou U, et al. Deficiency of ECHS1 causes mitochondrial encephalopathy with cardiac involvement. Ann Clin Transl Neurol. 2015;2:492–509.
26. Kishita Y, Pajak A, Bolar NA, Marobbio CM, Maffezzini C, Miniero DV, et al. Intra-mitochondrial methylation deficiency due to mutations in SLC25A26. Am J Hum Genet. 2015;97:761–8.
27. Kopajtich R, Murayama K, Janecke AR, Haack TB, Breuer M, Knisely AS, et al. Biallelic IARS mutations cause growth retardation with prenatal onset, intellectual disability, muscular hypotonia, and infantile hepatopathy. Am J Hum Genet. 2016;99:414–22.
28. Martin CA, Sarlos K, Logan CV, Thakur RS, Parry DA, Bizard AH, et al. Mutations in TOP3A cause a bloom syndrome-like disorder. Am J Hum Genet. 2018;103:221–31.
29. Peters H, Buck N, Wanders R, Ruiter J, Waterham H, Koster J, et al. ECHS1 mutations in Leigh disease: a new inborn error of metabolism affecting valine metabolism. Brain. 2014; 137:2903–8.
30. Van Haute L, Pearce SF, Powell CA, D'Souza AR, Nicholls TJ, Minczuk M. Mitochondrial transcript maturation and its disorders. J Inherit Metab Dis. 2015;38:655–80.
31. Kopajtich R, Nicholls TJ, Rorbach J, Metodiev MD, Freisinger P, Mandel H, et al. Mutations in GTPBP3 cause a mitochondrial translation defect associated with hypertrophic cardiomyopathy, lactic acidosis, and encephalopathy. Am J Hum Genet. 2014;95:708–20.
32. Kamps R, Szklarczyk R, Theunissen TE, Hellebrekers D, Sallevelt S, Boesten IB, et al. Genetic defects in mtDNA-encoded protein translation cause pediatric, mitochondrial cardiomyopathy with early-onset brain disease. Eur J Hum Genet. 2018;26:537–51.
33. Desai R, Frazier AE, Durigon R, Patel H, Jones AW, Dalla Rosa I, et al. ATAD3 gene cluster deletions cause cerebellar dysfunction associated with altered mitochondrial DNA and cholesterol metabolism. Brain. 2017;140:1595–610.
34. Feichtinger RG, Olahova M, Kishita Y, Garone C, Kremer LS, Yagi M, et al. Biallelic C1QBP mutations cause severe neonatal-, childhood-, or later-onset cardiomyopathy associated with combined respiratory-chain deficiencies. Am J Hum Genet. 2017;101:525–38.
35. Fujita Y, Ito M, Kojima T, Yatsuga S, Koga Y, Tanaka M. GDF15 is a novel biomarker to evaluate efficacy of pyruvate therapy for mitochondrial diseases. Mitochondrion. 2015;20:34–42.
36. Yatsuga S, Fujita Y, Ishii A, Fukumoto Y, Arahata H, Kakuma T, et al. Growth differentiation factor 15 as a useful biomarker for mitochondrial disorders. Ann Neurol. 2015;78:814–23.
37. Parikh S, Goldstein A, Koenig MK, Scaglia F, Enns GM, Saneto R, et al. Diagnosis and management of mitochondrial disease: a consensus statement from the Mitochondrial Medicine Society. Genet Med. 2015;17:689–701.
38. Kerr DS. Review of clinical trials for mitochondrial disorders: 1997-2012. Neurotherapeutics. 2013;10:307–19.
39. Kerr DS. Treatment of mitochondrial electron transport chain disorders: a review of clinical trials over the past decade. Mol Genet Metab. 2010;99:246–55.
40. Pfeffer G, Majamaa K, Turnbull DM, Thorburn D, Chinnery, PF. Treatment for mitochondrial disorders. Cochrane Database Syst Rev. 2012;Cd004426.
41. Rodriguez MC, MacDonald JR, Mahoney DJ, Parise G, Beal MF, Tarnopolsky MA. Beneficial effects of creatine, CoQ10, and lipoic acid in mitochondrial disorders. Muscle Nerve. 2007;35:235–42.
42. Glover EI, Martin J, Maher A, Thornhill RE, Moran GR, Tarnopolsky MA. A randomized trial of coenzyme Q10 in mitochondrial disorders. Muscle Nerve. 2010;42:739–48.
43. Marin SE, Mesterman R, Robinson B, Rodenburg RJ, Smeitink J, Tarnopolsky MA. Leigh syndrome associated with mitochondrial complex I deficiency due to novel mutations In NDUFV1 and NDUFS2. Gene. 2013;516:162–7.
44. Mermigkis C, Bouloukaki I, Mastorodemos V, Plaitakis A, Alogdianakis V, Siafakas N, et al. Medical treatment with thiamine, coenzyme Q, vitamins E and C, and carnitine improved obstructive sleep apnea in an adult case of Leigh disease. Sleep Breath. 2013;17:1129–35.
45. Pfeffer G, Horvath R, Klopstock T, Mootha VK, Suomalainen A, Koene S, et al. New treatments for mitochondrial disease-no time to drop our standards. Nat Rev Neurol. 2013;9:474–81.
46. Omata T, Fujii K, Takanashi J, Murayama K, Takayanagi M, Muta K, et al. Drugs indicated for mitochondrial dysfunction as treatments for acute encephalopathy with onset of febrile convulsive status epileptics. J Neurol Sci. 2016;360:57–60.
47. Danhauser K, Smeitink JA, Freisinger P, Sperl W, Sabir H, Hadzik B, et al. Treatment options for lactic acidosis and metabolic crisis in children with mitochondrial disease. J Inherit Metab Dis. 2015;38:467–75.
48. Koene S, Rodenburg RJ, van der Knaap MS, Willemsen MA, Sperl W, Laugel V, et al. Natural disease course and genotype-phenotype correlations in complex I deficiency caused by nuclear gene defects: what we learned from 130 cases. J Inherit Metab Dis. 2012;35:737–47.
49. Leary SC, Antonicka H, Sasarman F, Weraarpachai W, Cobine PA, Pan M, et al. Novel mutations in SCO1 as a cause of fatal infantile encephalopathy and lactic acidosis. Hum Mutat. 2013;34:1366–70.
50. Koga Y, Akita Y, Nishioka J, Yatsuga S, Povalko N, Tanabe Y, et al. L-arginine improves the symptoms of strokelike episodes in MELAS. Neurology. 2005;64:710–2.
51. Koga Y, Akita Y, Nishioka J, Yatsuga S, Povalko N, Katayama K, et al. MELAS and L-arginine therapy. Mitochondrion. 2007;7:133–9.
52. El-Hattab AW, Hsu JW, Emrick LT, Wong LJ, Craigen WJ, Jahoor F, et al. Restoration of impaired nitric oxide production in MELAS syndrome with citrulline and arginine supplementation. Mol Genet Metab. 2012;105:607–14.
53. Prietsch V, Lindner M, Zschocke J, Nyhan WL, Hoffmann GF. Emergency management of inherited metabolic diseases. J Inherit Metab Dis. 2002;25:531–46.
54. Morava E, Rodenburg R, van Essen HZ, De Vries M, Smeitink J. Dietary intervention and oxidative phosphorylation capacity. J Inherit Metab Dis. 2006;29:589.
55. Parikh S, Saneto R, Falk MJ, Anselm I, Cohen BH, Haas R, et al. A modern approach to the treatment of mitochondrial disease. Curr Treat Options Neurol. 2009;11:414–30.
56. Kaji S, Murayama K, Nagata I, Nagasaka H, Takayanagi M, Ohtake A, et al. Fluctuating liver functions in siblings with MPV17 mutations and possible improvement associated with dietary and pharmaceutical treatments targeting respiratory chain complex II. Mol Genet Metab. 2009;97:292–6.

57. Panetta J, Smith LJ, Boneh A. Effect of high-dose vitamins, coenzyme Q and high-fat diet in paediatric patients with mitochondrial diseases. J Inherit Metab Dis. 2004;27:487–98.
58. Distelmaier F, Haack TB, Wortmann SB, Mayr JA, Prokisch H. Treatable mitochondrial diseases: cofactor metabolism and beyond. Brain. 2017;140:e11.
59. Wexler ID, Hemalatha SG, McConnell J, Buist NR, Dahl HH, Berry SA, et al. Outcome of pyruvate dehydrogenase deficiency treated with ketogenic diets. Studies in patients with identical mutations. Neurology. 1997;49:1655–61.
60. Tanaka M, Nishigaki Y, Fuku N, Ibi T, Sahashi K, Koga Y. Therapeutic potential of pyruvate therapy for mitochondrial diseases. Mitochondrion. 2007;7:399–401.
61. Koga Y, Povalko N, Katayama K, Kakimoto N, Matsuishi T, Naito E, et al. Beneficial effect of pyruvate therapy on Leigh syndrome due to a novel mutation in PDH E1alpha gene. Brain Dev. 2012;34:87–91.
62. Martinelli D, Catteruccia M, Piemonte F, Pastore A, Tozzi G, Dionisi-Vici C, et al. EPI-743 reverses the progression of the pediatric mitochondrial disease–genetically defined Leigh Syndrome. Mol Genet Metab. 2012;107:383–8.
63. Enns GM, Kinsman SL, Perlman SL, Spicer KM, Abdenur JE, Cohen BH, et al. Initial experience in the treatment of inherited mitochondrial disease with EPI-743. Mol Genet Metab. 2012;105:91–102.
64. Sadun AA, Chicani CF, Ross-Cisneros FN, Barboni P, Thoolen M, Shrader WD, et al. Effect of EPI-743 on the clinical course of the mitochondrial disease Leber hereditary optic neuropathy. Arch Neurol. 2012;69:331–8.
65. Avula S, Parikh S, Demarest S, Kurz J, Gropman A. Treatment of mitochondrial disorders. Curr Treat Options Neurol. 2014;16:292.
66. Kouga T, Takagi M, Miyauchi A, Shimbo H, Iai M, Yamashita S, et al. Japanese Leigh syndrome case treated with EPI-743. Brain Dev. 2018;40:145–9.
67. Miura M, Ito K, Hayashi M, Nakajima M, Tanaka T, Ogura S. The effect of 5-aminolevulinic acid on cytochrome P450-mediated prodrug activation. PLoS ONE. 2015;10:e0131793.
68. Ogura S, Maruyama K, Hagiya Y, Sugiyama Y, Tsuchiya K, Takahashi K, et al. The effect of 5-aminolevulinic acid on cytochrome c oxidase activity in mouse liver. BMC Res Notes. 2011;4:66.
69. Ohsawa Y, Hagiwara H, Nishimatsu SI, Hirakawa A, Kamimura N, Ohtsubo H, et al. Taurine supplementation for prevention of stroke-like episodes in MELAS: a multicentre, open-label, 52-week phase III trial. J Neurol Neurosurg Psychiatry. 2018; e-pub ahead of print 17 April 2018; https://doi.org/10.1136/jnnp-2018-317964
70. Nesbitt V, Alston CL, Blakely EL, Fratter C, Feeney CL, Poulton J, et al. A national perspective on prenatal testing for mitochondrial disease. Eur J Hum Genet. 2014;22:1255–9.
71. Craven L, Murphy J, Turnbull DM, Taylor RW, Gorman GS, McFarland R. Scientific and ethical issues in mitochondrial donation. New Bioeth. 2018;24:57–73.