Chapter 30

Mitochondrial Epilepsies

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The Causal Disease

Definitions and Epidemiology of Mitochondrial Disease

Mitochondrial diseases are inherited conditions caused by gene defects that directly or indirectly affect the function of the oxidative phosphorylation (OXPHOS) system in the mitochondria [1]. The OXPHOS system comprises five multi-subunit enzyme complexes embedded in the inner mitochondrial membrane. Together, these enzyme complexes are responsible for generating the majority of cellular ATP (Figure 30.1). As a consequence, mitochondrial disorders predominantly affect organs with high energy demands, especially the brain, and epilepsy is a frequent manifestation. Mitochondrial disorders are estimated to affect at least 1 in 5000 births and may be caused by defects in mitochondrial DNA (37 maternally inherited genes located within the mitochondria themselves) or nuclear genes (>300 genes are currently linked to mitochondrial dysfunction).

Pathology, Physiology and Clinical Features of Mitochondrial Disease

Mitochondrial disease may be classified according to clinical syndrome, biochemical deficiency or class of gene defect. Mitochondrial diseases are frequently multisystem disorders (theoretically any organ may be affected in any combination) and epilepsy is rarely the sole (or even the first) clinical manifestation [2]. Clinical features frequently observed in mitochondrial disease are shown in Figure 30.2. Some patients have stereotyped manifestations fitting within a classical mitochondrial syndrome, others have nonspecific clinical features. Underlying disease mechanisms include defects of OXPHOS subunits or assembly factors (which may be associated with isolated deficiency of a single enzyme complex), and disorders of cofactor biosynthesis, mtDNA maintenance, and mitochondrial translation, import, membrane lipids and dynamics (all of which may be associated with multiple enzyme deficiencies). Downstream pathophysiological mechanisms are not completely understood and are not purely related to energetic failure. Impairment of other essential mitochondrial functions is also believed to contribute to pathogenesis of mitochondrial epilepsy, including alterations in calcium homeostasis, excitotoxicity, oxidative stress secondary to increased production of reactive oxygen species and depletion of cerebral folate, arginine, citrulline or NAD^+ [3].

Epilepsy in Mitochondrial Disease

Frequency of Epilepsy in Mitochondrial Disease

Seizures occur frequently in both paediatric and adult mitochondrial disease. Reported seizure frequency ranged from 23% in an adult cohort [4] to 35–61% in several paediatric cohorts [5–7].

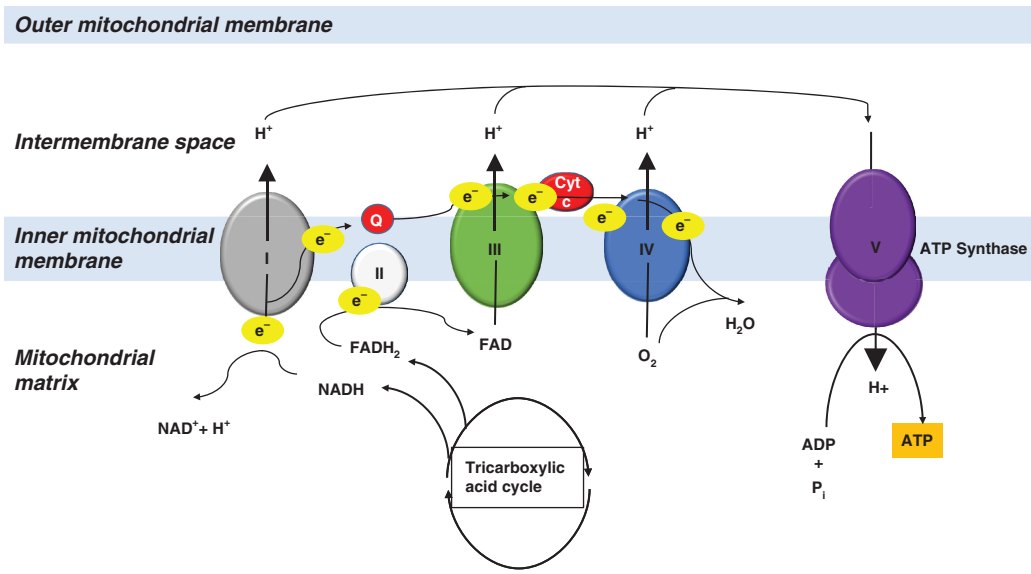

Figure 30.1 The mitochondrial oxidative phosphorylation system

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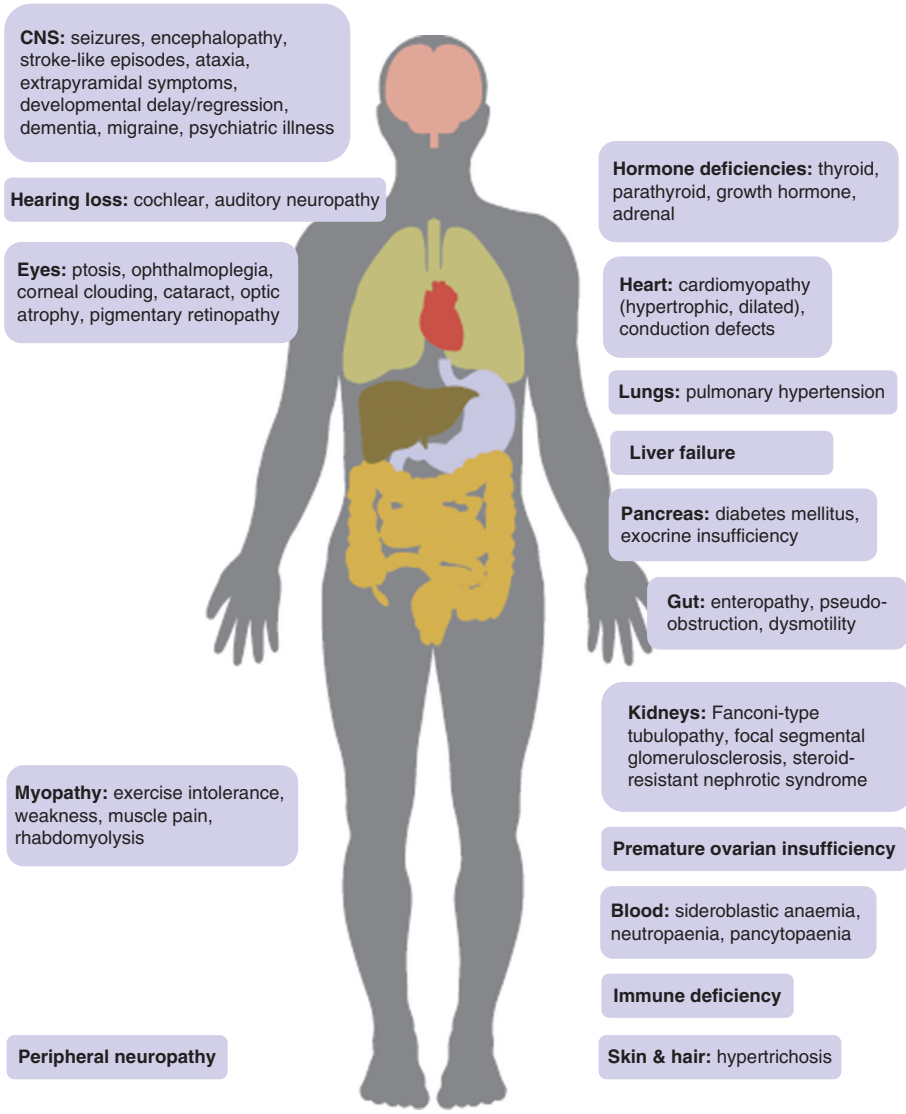

Figure 30.2 Clinical manifestations of mitochondrial disease

Epilepsy Phenotypes in Mitochondrial Disease

Seizure semiology has been reported in relatively few cohorts of mitochondrial epilepsy (Table 30.1). Epilepsy phenotypes frequently reported in mitochondrial diseases include neonatal refractory status and multiorgan failure, neonatal myoclonic epilepsy, infantile spasms, refractory/recurrent status epilepticus, epilepsia partialis continua and myoclonic epilepsy. Between 20 and 60% of patients have more than one seizure type [8, 9].

Mitochondrial Syndromic Epilepsies

Some of the classical mitochondrial syndromes most often associated with epilepsy are listed in Table 30.2 and described in the following section.

Epilepsy Associated with Mitochondrial DNA Mutations

MELAS

The syndrome of **m**itochondrial **e**ncephalomyopathy, **l**actic **a**cidosis and **s**troke-like episodes (MELAS) usually presents in the first decade of life with a stroke-like episode, often heralded by a migraine headache and/or focal seizure. Other clinical features include ataxia, sensorineural hearing loss, optic atrophy, cognitive decline, short stature, diabetes mellitus and cardiomyopathy. Eighty percent of cases with MELAS have the same maternally inherited mtDNA mutation, m.3243A>G in the *MT-TL1* gene encoding a transfer RNA (tRNA) for leucine. A review of 110 cases of MELAS revealed seizures in 96%, and myoclonus in 38% [12]. Seizures were the first symptom in 28% of cases. Sixty-two percent had generalised seizures, whilst focal seizures were present in 24%. It should be noted that the m.3243A>G mutation is associated with considerable clinical heterogeneity, and only 10% have the complete MELAS syndrome [13]. Thirty-four percent of patients with m.3243A>G had epilepsy in a large cohort [4]. Since m.3243A>G is present in 1 in 400 individuals (although most are oligosymptomatic or asymptomatic) this mutation makes a sizeable contribution to mitochondrial epilepsy.

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Table 30.1 Seizure semiology reported in mitochondrial disease cohorts

Study	Canafoglia *et al.* 2001 *Neurology* [10]	Lee *et al.* 2008 *Epilepsia* [11]	El Sabbagh *et al.* 2010 *Epilepsia* [9]	Chevallier *et al.* 2014 *Epilepsia* [8]
Size of cohort	*n* = 31	*n* = 48	*n* = 56	*n* = 165
Age range	Mixed (birth–44 years)	Paediatric (1–12 years)	Paediatric (birth–17 years)	Mixed (1–79 years)
Seizure semiology (% of cases)	*Juvenile/adult onset (n = 14)* Myoclonic epilepsy 43% Tonic–clonic seizures 21% Motor seizures 36% Somatosensory seizures 7% *Infantile onset (n = 17)* Partial epilepsy 65% Generalised epilepsy 24% West syndrome 12%	Partial seizures 17% Generalised epilepsy 29% Lennox–Gastaut 25% West syndrome 21% Ohtahara 4% Landau–Kleffner 4%	Myoclonic seizures 52% Infantile spasms 18% Focal seizures 23% Tonic seizures 9% Tonic–clonic seizures 9% Multiple seizure types ~60%	Generalised tonic–clonic 37% Complex partial 37% Myoclonus 22% Focal seizures 10% Infantile spasms 7% Atonic seizures 7% Generalised absence 2% Undefined 5% Status epilepticus 2% Epilepsia partialis continua 2% Nonconvulsive status epilepticus 3%

Table 30.2 Mitochondrial syndromes associated with epilepsy

Syndrome	Clinical features	Seizure types	Gene defects	Inheritance
MELAS	Mitochondrial encephalomyopathy, lactic acidosis, stroke-like episodes, migraine, sensorineural hearing loss, cognitive decline, diabetes, cardiomyopathy	Focal seizures (motor, visual), myoclonic seizures, status epilepticus	*MT-TL1* (80%), other mtDNA point mutations	Maternal
MERRF	Myoclonic epilepsy with ragged-red fibres, ataxia, sensorineural hearing loss, cognitive decline, multiple lipomata	Progressive myoclonus, GTCS	*MT-TK* (80%), other mtDNA point mutations	Maternal
NARP	Neurogenic muscle weakness, ataxia, retinitis pigmentosa, sensorineural hearing loss, cognitive decline	GTCS	*MT-ATP6*	Maternal
Leigh	Subacute necrotising encephalomyelopathy: stepwise neurodevelopmental regression, hypotonia, dystonia, ataxia, ophthalmoparesis, nystagmus, optic atrophy	Multiple seizure types including infantile spasms and other generalised seizures	>89 genes (mtDNA and nuclear)	Maternal, AR, X-linked
Alpers	Neurodevelopmental delay/ regression, intractable epilepsy, +/– liver failure	Refractory seizures, often focal initially (may be explosive onset) with subsequent generalisation, EPC, frequent status epilepticus	*POLG, FARS2, NARS2, PARS2*	AR
MEMSA	Myoclonic epilepsy, myopathy, sensory ataxia	Focal clonic or myoclonic seizures, EPC, GTCS, status epilepticus	*POLG*	AR

EPC epilepsia partialis continua; GTCS generalised tonic–clonic seizures

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MERRF

Myoclonic **e**pilepsy with **r**agged-**r**ed **f**ibres (MERRF) is also caused by a maternally inherited mtDNA point mutation, m.8344A>G in the *MT-TK* gene encoding the tRNA for lysine. In an Italian cohort of 34 adults with MERRF, 35% had generalised epilepsy, 24% had myoclonus and 24% had cerebellar ataxia [14]. Other clinical features include myopathy, sensorineural hearing loss, pigmentary retinopathy, cognitive decline and multiple symmetrical lipomatosis. Rarely, patients with MERRF may have stroke-like episodes, and these may be preceded by status epilepticus.

NARP

The maternally inherited disorder **n**europathy, **a**taxia and **r**etinitis **p**igmentosa (NARP) is caused by point mutations in the *MT-ATP6* gene encoding a subunit of complex V. Individuals with the same mutations at high load (>95%) have maternally inherited Leigh syndrome (see below) and frequently present with seizures, often infantile spasms. Seizures are less frequently observed in adults with NARP but generalised tonic–clonic seizures have been reported, associated with generalized spike and wave (GSW) discharges on EEG [15].

Single Mitochondrial Deletion Disorders

Large-scale deletions of mtDNA are a relatively frequent cause of mitochondrial disease and are associated with a broad spectrum of phenotypes, ranging in severity from the infantile onset Pearson marrow pancreas syndrome to Kearns-Sayre syndrome to isolated progressive external ophthalmoplegia starting in adult life [16]. Although seizures may occur in Kearns–Sayre syndrome, they were uncommon in a large paediatric cohort [17].

Epilepsy Associated with Nuclear Gene Defects

Alpers–Huttenlocher syndrome

The Alpers–Huttenlocher syndrome was originally described as a clinical triad of intractable epilepsy, neurodevelopmental regression and liver failure with characteristic neuropathological features. Focal lesions typically affect the occipital and parietal cortex (especially the striate cortex) and are characterised by neuronal loss, vacuolation of the neuropil and astrocytosis [18]. However since the genetic basis of this disorder was identified as recessive mutations in *POLG*, encoding the catalytic subunit of DNA polymerase gamma (the only DNA polymerase in the mitochondrion), it is increasingly recognised that many affected individuals have seizures and developmental regression without hepatic involvement [19]. The liver failure may be precipitated by sodium valproate treatment (for this reason sodium valproate therapy is typically avoided in mitochondrial epilepsies) but may also occur in the absence of valproate exposure. Alpers–Huttenlocher syndrome is characterised by relentless disease progression and often leads to death in early childhood [19].

MEGDEL

The relatively recently reported disorder MEGDEL (3-**me**thyl-**g**lutaconic aciduria with **d**eafness, **e**ncephalopathy, **L**eigh-like) is caused by a recessive defect of lipid remodelling at the mitochondrial associated membranes and is frequently associated with seizures. Typically there is neonatal onset of feeding problems, failure to thrive, hypotonia and hypoglycaemia, often associated with hepatic dysfunction. By age 2 years progressive deafness, dystonia, spasticity and developmental regression are apparent. Seizures may start in the neonatal period or later in childhood [20].

Epilepsy Associated with either Mitochondrial or Nuclear Gene Defects

Leigh Syndrome

Leigh syndrome is the most frequent presentation of mitochondrial disease and is also traditionally a neuropathological diagnosis, namely focal bilaterally symmetrical subacute necrotic lesions characterised by demyelination, vacuolation, gliosis and capillary proliferation with relative preservation of neurons [21, 22]. However, Leigh syndrome is increasingly diagnosed on the basis of MRI features (bilateral symmetrical signal abnormality involving the basal ganglia and/or brainstem) in association with elevated lactate levels in blood and/or CSF and compatible clinical features [22]. The syndrome is characterised by stepwise neurological deterioration leading to early death, often in childhood. There is extreme genetic heterogeneity, with defects of >89 mitochondrial and nuclear genes linked to Leigh syndrome to date [23]. Thirty-nine percent of a multinational cohort of 130 children with Leigh syndrome had seizures, including 22% with generalised seizures, 14% with focal seizures and 6% with epileptic spasms [24].

Nonsyndromic Mitochondrial Epilepsies

Many patients with mitochondrial disease do not present with classical syndromic phenotypes and may have variable multisystem features. Furthermore, an unknown proportion of patients may have pure epilepsy presentations, especially infantile epileptic encephalopathies, since mitochondrial disease may not be suspected in the absence of multisystem manifestations.

OXPHOS subunit and assembly factor deficiencies

Epilepsy has been reported in association with mutations of subunits and assembly factors of all five OXPHOS complexes (Table 30.3). Isolated complex I deficiency accounts for ~25–30% of childhood mitochondrial disease and typical clinical presentations include Leigh syndrome and leukoencephalopathies, frequently with seizures [25]. Complex II deficiency is extremely rare but also presents with Leigh syndrome or a leukoencephalopathy with seizures, for example secondary to mutations in the SDHA or SDHB subunits or the SDHAF1 assembly factor. Isolated complex III deficiency is also relatively infrequent, but seizures have been reported in patients with mutations in the mitochondrial subunit cytochrome *b*, one nuclear subunit (UQCRC2) and four assembly factors (BCS1L, TTC19, HCCS and UQCC2). Isolated complex IV (cytochrome oxidase, COX) deficiency is observed in ~25% cases of childhood onset mitochondrial disease and is genetically extremely heterogeneous, with reported causes including mutations in COX subunits and assembly factors and defects of

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Table 30.3 Genetic defects underlying the mitochondrial epilepsies

Disease mechanism	Biochemical defect	Gene defects and modes of inheritance
OXPHOS subunit defects	Complex I	**Mat:** *MT-ND1 MT-ND3 MT-ND4 MT-ND5 MT-ND6*
		AR: *NDUFV1 NDUFS2 NDUFS3 NDUFS4 NDUFS6 NDUFS7 NDUFS8 NDUFA2 NDUFA10 NDUFA11 NDUFA13*
		XL: *NDUFA1 NDUFB11*
	Complex II	**AR:** *SDHA SDHD*
	Complex III	**Mat:** *MT-CYB*
		AR: *UQCRC2*
	Complex IV	**Mat:** *MT-CO1 MT-CO2*
		AR: *COX6B1 COX8A NDUFA4*
	Complex V	**Mat:** *MT-ATP6*
		AR: *ATP5A1*
OXPHOS assembly factor defects	Complex I	**AR:** *NDUFAF1 NDUFAF2 NDUFAF3 NDUFAF4 NDUFAF5 NDUFAF6 FOXRED1 ACAD9 NUBPL C17orf89*
	Complex II	**AR:** *SDHAF1*
	Complex III	**AR:** *BCS1L HCCS UQCC2 TTC19*
	Complex IV	**AR:** *SURF1 FASTKD2 SCO1 SCO2 COX10 COX15 PET100*
	Complex V	**AR:** *ATPAF2 TMEM70*
mtDNA maintenance defects	Multiple RC enzymes (but can be isolated deficiency or normal enzyme activities)	**AR:** *POLG TWNK SUCLA2 SUCLG1 RRM2B TYMP DGUOK TK2 MPV17 FBXL4 ABAT*
Mitochondrial translation defects	Multiple RC enzymes (but can be isolated deficiency or normal enzyme activities)	**Mat:** *MT-TC MT-TD MT-TF MT-TH MT-TI MT-TK MT-TL1 MT-TN MT-TP MT-TQ MT-TS1 MT-TS2 MT-TT MT-TV MT-TW*
		AR: *CARS2 EARS2 FARS2 NARS2 PARS2 RARS2 VARS2 KARS QARS MRPS22 MTFMT MTO1 GTPBP3 PNPT1 TRIT1 TRNT1 LRPPRC TFSM GFM1 RMND1*
		XL: *HSD17B10*
Pyruvate dehydrogenase deficiency	Pyruvate dehydrogenase	**AR:** *PDHB DLD PDHX*
		XL: *PDHA1*
Defects of cofactor biosynthesis and transport	Coenzyme Q_{10}	**AR:** *PDSS2 COQ2 COQ4 COQ6 COQ9 COQ8A COQ8B*
	Lipoic acid	**AR:** *LIAS*
	Iron sulphur clusters	**AR:** *NFU1 BOLA3 NFS1*
	Thiamine	**AR:** *SLC19A3 SLC25A19 TPK1*
	NADP	**AR:** *NADK*
	Manganese	**AR:** *SLC39A8*
Mitochondrial import	Multiple RC enzymes or normal enzyme activities	**AR:** *SLC25A1 SLC25A12 SLC25A22 DNAJC19 TIMM50 AIFM1*
Defects of mitochondrial lipid membranes and dynamics	Multiple RC enzymes or normal enzyme activities	**AR:** *SERAC1 AGK DNM1L MFF STAT2 SLC25A46*
Defects of mitochondrial quality control	Multiple RC enzymes or normal enzyme activities	**AR:** *CLPB AFG3L2 HSPD1 HTRA2 MIPEP SACS*
Respiratory chain toxicity	Multiple RC enzymes, isolated complex IV deficiency or normal enzyme activities	**AR:** *ETHE1 HIBCH ECHS1 NAXE*
Other mechanisms	Multiple RC enzymes, isolated complex IV deficiency or normal enzyme activities	**AR:** *APOPT1 TXN2 PPA2*

AR autosomal recessive; Mat maternal inheritance; NADP nicotinamide adenine dinucleotide phosphate; XL X-linked.

mtDNA maintenance or mitochondrial translation. Furthermore, COX deficiency may sometimes be a secondary phenomenon with a non-mitochondrial underlying cause. Mutations of the maternally inherited complex V subunit ATP6 have been associated with maternally inherited Leigh syndrome, and affected individuals may present with infantile spasms. Few mutations have been described in nuclear encoded subunits of complex V, but patients with mutations in the ATP5A1 subunit were reported to have seizures, as were patients with mutations in the assembly factors ATPAF2 and TMEM70.

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Mitochondrial DNA Depletion Syndromes

Patients with the mtDNA depletion syndrome (MDDS) have a severe quantitative defect of mtDNA. In the most severely affected cases this may be <5–10% of levels observed in tissues from age-matched healthy control subjects [26]. The underlying problem is an inability to maintain the mtDNA, either because of a defect in the replication machinery, or because of impaired nucleoside supply for mtDNA synthesis. Phenotypically, MDDS can be divided into myopathic, encephalomyopathic and hepatocerebral subgroups. By far the most frequent cause of MDDS is mutation of *POLG*. The Alpers–Huttenlocher syndrome (see above) is one form of hepatocerebral MDDS caused by recessive *POLG* mutations, but recessive *POLG* mutations can also cause other epilepsy syndromes in older children and adolescents, which are collectively known as **m**yoclonic **e**pilepsy, **m**yopathy, **s**ensory **a**taxia (MEMSA). Other genetic causes of MDDS associated with seizures include mutations of *TWNK* encoding the twinkle helicase, *RRM2B* encoding a subunit of the ribonucleotide reductase, *DGUOK, TK2, SUCLA2, SUCLG1, TYMP* and *MPV17* encoding proteins implicated in mitochondrial deoxynucleotide homeostasis, *ABAT* encoding GABA transaminase, and *FBXL4* encoding a protein whose precise function remains unknown [27].

Disorders of Mitochondrial Translation

In recent years there has been an explosion of reports of defects of mitochondrial translation, particularly mutations of the tRNA synthetases required for aminoacylation of the 22 mitochondrial tRNA molecules prior to their attachment to the mitochondrial ribosome for protein synthesis. Mutations in *RARS2* are associated with intractable seizures and developmental stasis from birth, typically with initial severe lactic acidosis (which later resolves) and pontocerebellar hypoplasia on neuro-imaging, whilst mutations in *FARS2, NARS2* and *PARS2* have been reported to cause an Alpers-like phenotype [28]. Other disorders of mitochondrial translation associated with seizures include several defects of mitochondrial tRNA modification and mutations in the mitochondrial translation factors GFM1 and TSFM (Table 30.3).

Disorders of Cofactor Biosynthesis

Another rapidly growing group of mitochondrial epilepsies are disorders of cofactor biosynthesis, particularly coenzyme Q_{10} (CoQ_{10}) and iron–sulphur (Fe–S) clusters. CoQ_{10} is a lipophilic electron carrier transferring electrons from fatty acid β-oxidation and OXPHOS complexes I and II to complex III, and also acts as a potent intramitochondrial antioxidant. Eight defects of CoQ_{10} biosynthesis have now been reported and seven of these are characterised by epilepsy associated with the following clinical features: infantile multisystem encephalomyopathies (*PDSS2, COQ2, COQ4* and *COQ9*), ataxia (*COQ8A*) or steroid-resistant nephrotic syndrome (*COQ6, COQ8B*) [29]. Fe–S clusters are required for the function of several mitochondrial enzymes, including respiratory chain enzyme complexes I–III, the Krebs cycle enzyme aconitase and the electron transfer flavoprotein dehydrogenase. Several disorders of Fe–S cluster biosynthesis have been reported [30], associated with epilepsy in some cases (*NFU1, BOLA3, LIAS, NFS1*). Defects of supply of other cofactors (including thiamine, NADP, copper and manganese) to the respiratory chain have also been linked to epilepsy (see Table 30.3 for a comprehensive list).

Disorders of Mitochondrial Import

Mitochondria have complex mechanisms for importing hundreds of solutes and proteins. Mutations in several solute transporters have been linked to epilepsy, including the citrate transporter (*SLC25A1*) and two mitochondrial glutamate carriers (*SLC25A12* and *SLC25A22*). Mutations in *SLC25A22* in particular have been reported in several families associated with severe epilepsy syndromes, including early myoclonic epilepsy with burst suppression and migrating partial seizures of infancy. Defects of mitochondrial protein importers associated with epilepsy include mutations in *TIMM50* and *DNAJC19*.

Disorders of Mitochondrial Membrane Lipids and Dynamics

Mitochondria are dynamic organelles, constantly undergoing fission and fusion events. Several defects of mitochondrial dynamics have been reported, associated with epilepsy in some cases. For example, several infants with mutations in *DNM1L*, encoding the major mitochondrial fission protein, had intractable seizures. More recently mutations in the mitochondrial fission factor (MFF) and the JAK-STAT cytokine STAT2 have also been reported to cause epilepsy [31].

Other Disease Mechanisms

Relatively new disease mechanisms for mitochondrial disease recognised more recently include disorders of mitochondrial quality control (e.g. *CLPB, AFG3L2, HSPD1, HTRA2, MIPEP* and *SACS* mutations) and toxic damage to the respiratory chain, for example in ethylmalonic encephalopathy and HIBCH and ECHS1 deficiencies (two disorders of valine degradation) [32, 33].

Secondary Mitochondrial Epilepsies

Various disorders with prominent epilepsy have been reported to cause secondary respiratory chain dysfunction, for example GM3 synthase deficiency, infantile epileptic encephalopathy caused by *STXBP1* mutations and Dravet syndrome associated with *SCN1A* mutations [34–38].

Diagnostic Tests for Mitochondrial Disease

Mitochondrial diagnostics is currently in a state of flux, as the field moves from muscle biopsy as a first stage diagnostic test to NGS as the frontline test. However, even when NGS approaches are being utilised, it is important to take a multidisciplinary approach to mitochondrial diagnostics, encompassing multisystem clinical, imaging, neurophysiological, and biochemical (and, where available, histological) information as well as genetic data.

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EEG

The most frequent EEG abnormality reported in mitochondrial disease is nonspecific slowing. EEGs were systematically performed in 109 subjects in one cohort and were abnormal in 61% of these [8]. The most frequent abnormalities were combined epileptiform discharges and slowing (33%), epileptiform discharges (22%), generalised slowing (7%) and focal slowing (3%). In this study 39% of epileptic discharges were focal, and multifocal, generalised or both in the remaining 61%. EEG findings are particularly useful in suggesting the possibility of *POLG* mutations. In Alpers syndrome the initial EEG may reveal unilateral occipital rhythmic high-amplitude delta with superimposed (poly)spikes (RHADS), although later in the disease EEG changes may be generalised and nonspecific [39]. Patients with other *POLG* epileptic syndromes such as MEMSA also have predominantly occipital changes, typically occipital slow wave and epileptic activity early in the disease course [40].

Imaging

Characteristic neuro-imaging changes are observed in some mitochondrial syndromes. For example, patients with Leigh syndrome typically have bilateral symmetrical T2 hyperintense lesions in the basal ganglia and/or brainstem, and those with MELAS have stroke-like lesions in parieto-occipital regions. Other patients may have a leukoencephalopathy (e.g. those with mutations of subunits of complexes I and II) or pontocerebellar hypoplasia (*RARS2*). However, in many cases with mitochondrial epilepsy neuro-imaging appearances may be nonspecific, such as cerebral or cerebellar atrophy, or even normal.

Metabolic Investigations

Various metabolites may be elevated in mitochondrial disease, including lactate, the amino acid alanine, fibroblast growth factor 21 (FGF21) and growth and differentiation factor 15 (GDF15) [41, 42]. However none has universally increased levels in mitochondrial disease, making these metabolites unreliable as screening tests.

Muscle Biopsy

Traditionally, diagnosis of mitochondrial disease rested on biopsy of skeletal muscle (or occasionally another affected tissue such as liver or heart). An open or large bore needle biopsy is required to provide sufficient tissue for the necessary analyses: histochemical, ultrastructural, biochemical and genetic. The ragged-red fibre (characterised by subsarcolemmal proliferation of mitochondria visualised by the modified Gomori trichrome stain), is traditionally viewed as the morphological hallmark of mitochondrial disease, but is mainly a feature of adult mitochondrial disease associated with defective mtDNA (including point mutations and single or multiple deletions). Ragged-red fibres are rarely seen in the paediatric population, but may be a feature of MDDS or disease caused by mtDNA point mutations, including MELAS and MERRF. Electron microscopy may reveal ultrastructural abnormalities of the mitochondria, such as whorled cristae or crystalline inclusions, but features may be nonspecific. Biochemical investigation of biopsied muscle may include spectrophotometric assays of individual OXPHOS complexes, interrogation of global mitochondrial function using polarography, or assessment of OXPHOS complex assembly using blue native gel electrophoresis. Genetic investigation of mtDNA in muscle may include screening for rearrangements, quantitation of mtDNA copy number, and full sequencing of the mitochondrial genome.

Genetic Testing

Single gene testing is appropriate when clinical, imaging or EEG data point to a specific syndromic diagnosis, such as MELAS or Alpers syndrome (test for m.3243A>G or *POLG* mutations respectively). However, since >300 genes have now been linked to mitochondrial disease, and >140 of these may be associated with seizures (Table 30.3), single gene testing is frequently an inefficient diagnostic strategy, and NGS is increasingly being employed as a first-line genetic diagnostic test [43]. NGS may be of a candidate gene panel, such as genes implicated in epileptic encephalopathies or the mitochondrial proteome, or of the whole exome or even whole genome sequencing.

Assessment of Multisystem Involvement

Screening for multisystem involvement, which is a typical finding in infantile and childhood onset mitochondrial disease, is important since it may help to identify a specific syndromic diagnosis. Furthermore, knowledge of multisystem disease manifestations allows appropriate supportive therapy to be provided.

Principles of Management

Management of Mitochondrial Disease

Curative therapies for mitochondrial disease are lacking, but many supportive therapies are available for the multisystem manifestations of mitochondrial disorders, for example brow suspension surgery for severe ptosis, hearing aids, replacement of deficient hormones, gastrostomy feeding and pacing for cardiac conduction defects [44]. Other treatments include correction of electrolyte imbalances in patients with renal tubulopathies, CoQ_{10} replacement for disorders of CoQ_{10} biosynthesis, folinic acid supplementation in cases with cerebral folate deficiency, and L-arginine or L-citrulline supplementation in MELAS syndrome or if plasma levels are low [45]. The role of cofactor and antioxidant supplementation is controversial and currently there is no supportive evidence for these treatments [46].

Antiepileptic Drugs

Mitochondrial epilepsy is frequently refractory to drug therapy, and the overwhelming majority of affected individuals

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receive two or more AEDs [9, 47]. Randomised controlled trials have not been performed to determine which AEDs are most efficacious in mitochondrial disease. However, the clinical and genetic heterogeneity of mitochondrial epilepsy, with many underlying disease mechanisms, would likely hamper the design and interpretation of such trials. Combinations of a sodium channel blocker such as lamotrigine and a benzodiazepine (e.g. clobazam), together with levetiracetam or topiramate as needed, appear to be relatively efficacious, particularly in *POLG*-related epilepsy, the most common mitochondrial epilepsy [47, 48]. Other therapies which have been reported in *POLG* disease include magnesium infusion, L-carnitine supplementation and folinic acid [47], but the anecdotal nature of these reports and the unpredictable natural history of mitochondrial disease make it difficult to ascertain whether these agents are truly efficacious. Sodium valproate is absolutely contraindicated in patients with *POLG* mutations, because of the risk of inducing liver failure [39], but may be well tolerated and effective in controlling seizures in patients with other types of mitochondrial epilepsy. However, most specialists would prefer to use another AED in patients with mitochondrial disease, and only use valproate if other AEDs have failed.

Experimental Approaches

The ketogenic diet (a high fat, low carbohydrate diet) has been suggested as a treatment for mitochondrial epilepsy, but so far experience is limited to case reports and small case series [2]. Although a formal clinical trial would help to determine whether the ketogenic diet is efficacious, a modified Atkins diet was poorly tolerated in a small series of patients with mitochondrial myopathy, causing muscle pain and elevation of creatine kinase [49]. This may therefore not be an acceptable treatment for mitochondrial disease. Another option could be supplementation with the fatty acid decanoic acid, which is thought to be the active component of the ketogenic diet and appears to stimulate mitochondrial biogenesis in cell models [50, 51], but clinical trials have yet to be performed. Other experimental approaches currently being explored on a research basis include the development of novel antioxidants and agents to stimulate mitochondrial biogenesis, nucleoside supplementation for some forms of MDDS, and gene therapy for both mtDNA and nuclear gene encoded mitochondrial disorders [45, 52].

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