Mitochondrial disease and epilepsy

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PUBLICATION DATA
Accepted for publication 21st October 2011.
Published online 28th January 2012.

ABBREVIATIONS

AED	Antiepileptic drug
COX	Cytochrome oxidase
MDDS	mtDNA depletion syndrome
MELAS	Mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes
MEMSA	Myoclonus, epilepsy, myopathy, sensory ataxia
MERRF	Myoclonic epilepsy with ragged red fibres
MILS	Maternally inherited Leigh syndrome
MIRAS	Mitochondrial recessive ataxia syndrome
NARP	Neurogenic muscle weakness, ataxia, retinitis pigmentosa
mtDNA	Mitochondrial DNA
OXPHOS	Oxidative phosphorylation
SNHL	Sensorineural hearing loss

Mitochondrial respiratory chain disorders are relatively common inborn errors of energy metabolism, with a combined prevalence of one in 5000. These disorders typically affect tissues with high energy requirements, and cerebral involvement occurs frequently in childhood, often manifesting in seizures. Mitochondrial diseases are genetically heterogeneous; to date, mutations have been reported in all 37 mitochondrially encoded genes and more than 80 nuclear genes. The major genetic causes of mitochondrial epilepsy are mitochondrial DNA mutations (including those typically associated with the mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes [MELAS] and myoclonic epilepsy with ragged red fibres [MERRF] syndromes); mutations in *POLG* (classically associated with Alpers syndrome but also presenting as the mitochondrial recessive ataxia syndrome [MIRAS], spinocerebellar ataxia with epilepsy [SCAE], and myoclonus, epilepsy, myopathy, sensory ataxia [MEMSA] syndromes in older individuals) and other disorders of mitochondrial DNA maintenance; complex I deficiency; disorders of coenzyme Q_{10} biosynthesis; and disorders of mitochondrial translation such as *RARS2* mutations. It is not clear why some genetic defects, but not others, are particularly associated with seizures. Epilepsy may be the presenting feature of mitochondrial disease but is often part of a multisystem clinical presentation. Mitochondrial epilepsy may be very difficult to manage, and is often a poor prognostic feature. At present there are no curative treatments for mitochondrial disease. Individuals with mitochondrial epilepsy are frequently prescribed multiple anticonvulsants, and the role of vitamins and other nutritional supplements and the ketogenic diet remain unproven.

Mitochondria are extremely dynamic subcellular organelles with a multitude of functions. The best known of these functions is ATP generation by the oxidative phosphorylation (OXPHOS) system, but mitochondria also have important roles in intracellular calcium homeostasis, generation of reactive oxygen species, regulation of apoptosis (programmed cell death), and cell-specific functions, such as neurotransmitter synthesis in neuronal cells. The term 'mitochondrial disease' refers to any disorder affecting the respiratory chain and OXPHOS system, a series of five multisubunit enzyme complexes (complexes I–V) embedded in the inner mitochondrial membrane.[1] Mitochondrial disorders are common, with an estimated birth prevalence of one in 5000,[2] although recently we have demonstrated that one in 500 children has a pathogenic mitochondrial DNA (mtDNA) mutation.[3] Mitochondria are unique amongst cellular organelles in that they contain their own genetic material, the small (~16.6kb), circular mtDNA molecule. This small genome is exclusively maternally inherited and is present inside the mitochondria of cells in multiple copies. The 37 mitochondrial genes encode 13 proteins (all components of the mitochondrial respiratory chain/OXPHOS system) and 24 RNA molecules necessary for the intramitochondrial synthesis of these 13 proteins. Correct coordinated expression of the 13 proteins encoded by the mitochondrial genome is essential for efficient mitochondrial energy production (Fig. 1), and also requires the contribution of many of the ~1500 nuclear-encoded proteins that constitute the mitochondrial proteome.[4]

This review discusses the clinical epilepsy phenotypes observed in mitochondrial disease together with the biochemical classification, molecular genetics, and management of mitochondrial epilepsies.

EPILEPSY PHENOTYPES, CLINICAL RECOGNITION, AND DIAGNOSIS OF MITOCHONDRIAL DISEASE

The exact prevalence of mitochondrial epilepsy is not known, but seizures have been reported to occur in ~35 to 60% of individuals with biochemically confirmed mitochondrial disease.[5,6] In another study, one-third of individuals with refractory seizures were found to have biochemical evidence of

mitochondrial dysfunction.[7] Few reports have systematically examined epilepsy phenotypes in the context of mitochondrial disease.[6,8,9] In one series of 48 individuals with epilepsy and confirmed mitochondrial respiratory chain defects, two had Otahara syndrome, 10 had West syndrome, 12 had Lennox–Gastaut syndrome, two had Landau–Kleffner syndrome, 14 had generalized epilepsy, and eight had partial epilepsy.[9] Another study, of 56 children with mitochondrial disease and seizures, classified the types of epilepsy observed into six groups according to age at onset and major seizure type: (1) neonatal refractory status and multiorgan failure; (2) neonatal myoclonic epilepsy; (3) infantile spasms; (4) refractory/recurrent status epilepticus; (5) epilepsia partialis continua; and (6) myoclonic epilepsy.[8] Although seizures may be the presenting symptom of mitochondrial disease,[10] the first seizures were preceded by other symptoms in more than 80% of cases in this series.[8] Initial symptoms may include failure to thrive, developmental delay, ataxia, and evidence of multiorgan involvement. El Sabbagh et al.[8] reported that 60% of their cases had several seizure types, emphasizing the complexity of this group of disorders.

Clinical recognition of mitochondrial epilepsy is difficult. Explosive onset of focal epilepsy, epilepsia partialis continua, or status epilepticus should arouse suspicion of Alpers syndrome, particularly in cases in which predominant occipital epileptiform discharges are observed on the electroencephalogram.[11] Electroencephalographic changes are not specific in other mitochondrial syndromes. Elevation of lactate in blood and/or cerebrospinal fluid may be a clue, but normal values do not exclude mitochondrial disease. Elevated alanine in the plasma amino acid profile is suggestive of persistent lactic acidosis. Involvement of other organs, such as sensorineural hearing loss (SNHL), pigmentary retinopathy, cardiomyopathy or cardiac conduction defects, diabetes mellitus, liver disease, and renal tubulopathy, may also be useful pointers to an underlying mitochondrial disorder.

What this paper adds

- This review provides a comprehensive list of the nuclear gene causes of mitochondrial epilepsy.
- An overview of the therapeutic options available to treat mitochondrial epilepsy is provided.

There is no single criterion standard diagnostic test for mitochondrial disease. Coordinated investigation across a range of testing modalities, including neuroimaging, metabolite profiling, and histological, biochemical, and genetic analysis of muscle (or sometimes liver) biopsy, is often necessary in order to make a definitive diagnosis. Muscle histology may be normal, or may reveal ragged red fibres and cytochrome oxidase (COX)-negative fibres. Both of these changes point to a defect of mtDNA, which may be a deletion or point mutation, or quantitative defect (mtDNA depletion). In very specific cases, such as MELAS (mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes) or Alpers syndromes, it may be possible to make a genetic diagnosis (mtDNA mutation m.3243A>G or *POLG* mutations respectively) in DNA extracted from blood without the need for tissue biopsy. In most cases, however, genetic investigations are directed by the biochemical findings in the muscle biopsy.

THE GENETIC BASIS OF MITOCHONDRIAL EPILEPSY

Mitochondrial diseases may be classified by the clinical phenotype or by the biochemical defect identified in the skeletal

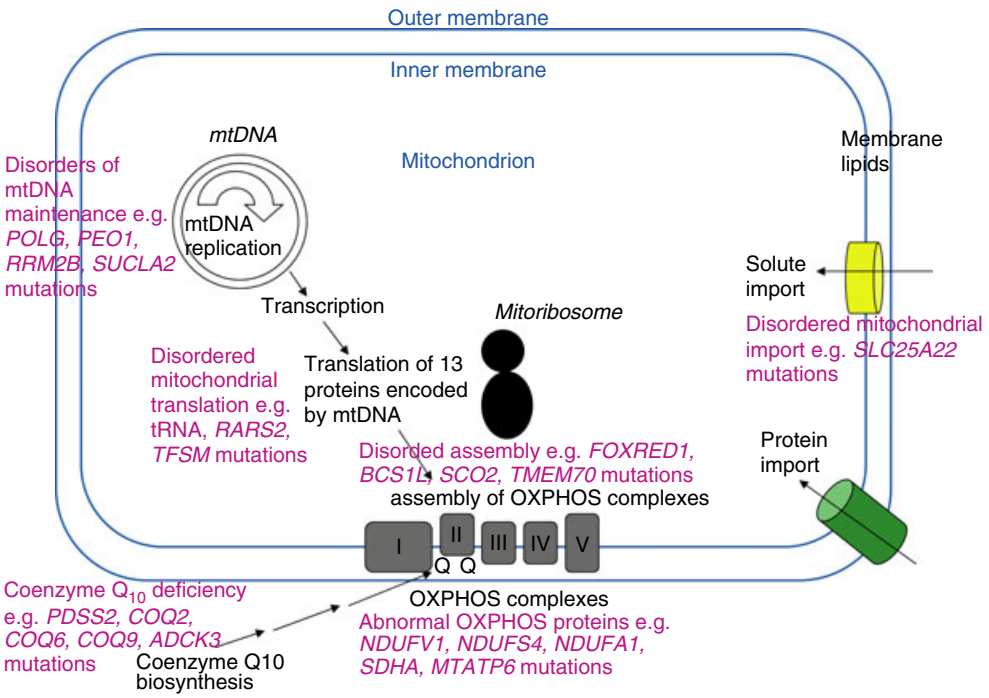

Figure 1: Molecular mechanisms leading to mitochondrial epilepsy. mtDNA, mitochondrial DNA; OXPHOS, oxidative phosphorylation.

muscle (or another affected tissue). This may be an isolated deficiency of a single respiratory chain complex (most commonly complex I or complex IV) or multiple defects affecting several enzyme complexes. Another classification system is according to the underlying genetic defect. A genetic classification system is preferable for a number of reasons. The same biochemical defect may be caused by many different genetic defects, for example mutations in more than 30 genes have been associated with isolated complex I deficiency. Furthermore, normal respiratory chain activities have been observed in individuals with genetically proven mitochondrial disease. However, at present, genetic classifications are inevitably incomplete since the responsible mutation is identified in only 20 to 25% of childhood cases using routine diagnostic tests, although in specialized research laboratories focusing on highly selected subgroups of individuals and using the latest next-generation sequencing technologies the diagnostic rate may approach 50%.[12] This review will take a combined biochemical and genetic approach to the classification of mitochondrial epilepsy (Fig. 1 and Table I).

Isolated complex I deficiency

Complex I deficiency is the most commonly identified biochemical defect in most centres, accounting for 25 to 30% of all mitochondrial disease presenting in childhood. The relative proportion of complex I deficiency in mitochondrial epilepsy may be even higher.[9] Mutations have been reported in all seven mtDNA-encoded subunits of complex I, presenting with seizures in many cases, for example in individuals with mutations in the ND5 subunit, which appear to be particularly associated with Leigh syndrome (subacute necrotizing encephalomyelopathy) or MELAS syndrome.[13,14] Mutations in mitochondrial transfer RNA (tRNA) genes and large-scale rearrangements of the mtDNA may also lead to isolated complex I deficiency, and overall changes in mtDNA account for 20 to 25% of cases of complex I deficiency. Mutations have been reported in 13 of the 38 nuclear-encoded subunits of the enzyme in another 20 to 25% of individuals with complex I deficiency. Of these nuclear subunits, epilepsy has been associated with mutations in NDUFV1, NDUFS4, NDUFS8, and NDUFA1.[15–18] The remaining ~50% of complex I deficiency is believed to be caused by mutations in proteins needed for assembly and/or proper functioning of the enzyme. Nine assembly genes have so far been associated with complex I deficiency, including three new genes reported in the last few months of 2010.[12,19,20] Epilepsy is a feature of mutations in five of the nine known complex I assembly factors: NDUFAF2, NDUFAF3/C3orf60, NDUFAF4/C6orf66, C8orf38, and FOXRED1.[4,19,21–23] Myoclonic epilepsy appears to be particularly associated with complex I subunit mutations, both mtDNA and nuclear encoded, and has also been reported in some of the complex I assembly defects (Table I). Myoclonic seizures were apparently responsive to antiepileptic drugs (AEDs) in two individuals with nuclear-encoded complex I deficiency,[18,19] which is in marked contrast to the seizures associated with other mitochondrial diseases, in particular *POLG* mutation-related epilepsy, which is typically resistant to multiple AEDs.

Isolated complex II deficiency

Complex II deficiency is a rare disorder and usually presents as Leigh syndrome caused by mutations in the SDHA subunit, although recently two assembly factors have been identified. Epilepsy appears to be unusual in complex II deficiency, but may still occur; for example, a 9-year-old female with Leigh syndrome caused by *SDHA* mutations had focal and generalized seizures.[24]

Isolated complex III deficiency

Complex III deficiency is also rare. Mutations have been reported in cytochrome *b*, the only mtDNA-encoded subunit of the enzyme, and in two of the 10 nuclear-encoded subunits. Only one complex III assembly factor, BCS1L, has been characterized in humans, and mutations in the *BCS1L* gene are associated with a broad spectrum of disease, ranging from the severe neonatal-onset growth retardation, aminoaciduria, cholestasis, iron overload, lactic acidosis, and early death (GRACILE) syndrome to the milder Björnstad syndrome (congenital SNHL with pili torti). Seizures have occasionally been reported in children with *BCS1L* mutations, but are not recognized to be a major feature of complex III deficiency. Seizures were documented in two unrelated individuals with *BCS1L* mutations characterized by a progressive encephalopathy with early-onset developmental delay and spastic quadriplegia, associated with lactic acidosis, cerebral atrophy, and basal ganglia changes on magnetic resonance imaging.[25] One of these two individuals also had SNHL and brittle hair reminiscent of Björnstad syndrome, indicating the clinical continuum of *BCS1L* mutation-related disease.

Isolated complex IV deficiency

Complex IV (COX) deficiency is a relatively common cause of mitochondrial disease, representing ~25% of childhood-onset cases. Mutations affecting subunits of this enzyme are relatively rare, and are only occasionally associated with epilepsy.[26] As with complex I deficiency, most individuals are thought to have mutations in genes encoding assembly factors of the enzyme. One of the most frequent presentations of COX deficiency is Leigh syndrome, which in approximately 50% of cases is caused by mutations in the gene coding for the SURF1 assembly factor.[27,28] Although seizures occur in approximately 40% of individuals with Leigh syndrome,[29,30] they appear to be a rare feature of SURF1 deficiency.[28] Mutations in the genes coding for seven other assembly factors for COX have been linked to human disease,[31] only occasionally associated with seizures (Table I). For example, focal and gelastic seizures were noted in two siblings with developmental delay, hemiplegia, and asymmetrical brain atrophy caused by mutations in *FASTKD2*, which encodes a protein of unknown function that may be involved in apoptosis.[32] Epilepsy has also been reported in some individuals with hypertrophic cardiomyopathy and encephalopathy caused by mutations in the SCO2 assembly factor, which is part of the molecular system responsible for inserting copper prosthetic groups into the COX holoenzyme. Seizures were documented in seven of 10 individuals with *SCO2* mutations in one series,

Table I: Nuclear genes reported to cause mitochondrial epilepsy

Gene	Mode of inheritance	Biochemical defect	Epilepsy phenotype	Other clinical features	References
NDUFV1	AR	Complex I	Myoclonic epilepsy	Hypotonia, spasticity, developmental regression, vomiting, strabismus, macrocystic leukodystrophy	Schuelke et al.[15]
NDUFS4	AR	Complex I	Not specified	Leigh-like encephalopathy	van den Heuvel et al.[16]
NDUFS8	AR	Complex I	Erratic seizures	Leigh syndrome, HCM	Loeffen et al.[17]
NDUFA1	X-linked	Complex I	Myoclonic epilepsy (bursts of polyspike and wave)	Leigh syndrome	Fernandez-Moreira et al.[18]
NDUFAF2	AR	Complex I	Myoclonic epilepsy	Encephalopathy, characteristic MRI brain appearances	Barghuti et al.[21]
NDUFAF3	AR	Complex I	Myoclonic epilepsy (burst suppression)	Fatal infantile LA	Saada et al.[22]
NDUFAF4	AR	Complex I	Generalized tonic–clonic convulsions	Infantile encephalomyopathy, dystonia, optic atrophy, deafness, LA; HCM in one case	Saada et al.[23]
C8orf38	AR	Complex I	Focal seizures	Leigh syndrome	Pagliarini et al.[4]
FOXRED1	AR	Complex I	Myoclonic and generalized epilepsy	Infantile onset encephalomyopathy; Leigh syndrome	Calvo et al.[12]; Fassone et al.[19]
SDHA	AR	Complex II	Focal and generalized seizures	Leigh syndrome	Horvath et al.[24]
BCS1L	AR	Complex III	Not specified	Progressive encephalopathy, LA and basal ganglia lesions; SNHL and brittle hair	Fernandez-Vizarra et al.[25]
SURF1	AR	Complex IV	Not specified	Leigh syndrome	Sue et al.[28]
FASTKD2	AR	Complex IV	Focal and gelastic seizures	Encephalomyopathy (developmental delay, hemiplegia, asymmetrical brain atrophy)	Ghezzi et al.[32]
SCO2	AR	Complex IV	Not specified	HCM, encephalomyopathy	Jaksch et al.[33]
COX10	AR	Complex IV	Status epilepticus	Encephalomyopathy (hypotonia, weakness, ataxia, ptosis, leukodystrophy)	Valnot et al.[34]
COX15	AR	Complex IV	Not specified	HCM, hypotonia, LA	Antonicka et al.[35]
ATP12	AR	Complex V	Not specified	Dysmorphic features, LA, cerebral atrophy, corpus callosal dysgenesis	De Meirleir et al.[38]
TMEM70	AR	Complex V	Generalized seizures	HCM, IUGR, cataracts, hypertonia, LA, hyperammonaemia	Spiegel et al.[39]
POLG	AR	Multiple RC enzymes	Myoclonus, focal and generalized seizures, EPC (characteristic EEG changes)	Alpers syndrome; hepatocerebral MDDS; SCAE; MIRAS; MEMSA	Wolf et al.[11]; Saneto and Naviaux[44]
PEO1	AR	Multiple RC enzymes	Myoclonus, EPC, status epilepticus	IOSCA; hepatocerebral MDDS	Hakonen et al.[45]; Sarzi et al.[47]
SUCLA2	AR	Multiple RC enzymes	Generalized seizures	Encephalomyopathic MDDS, SNHL, methylmalonic aciduria	Elpeleg et al.[49]
RRM2B	AR	Multiple RC enzymes	Tonic–clonic seizures, status epilepticus	MDDS with hypotonia, respiratory distress, proximal renal tubulopathy, diarrhoea, LA	Bourdon et al.[48]; Kollberg et al.[91]
PDSS2	AR	Coenzyme Q_{10}	Focal seizures with secondary generalization	SRNS, Leigh syndrome	Lopez et al.[61]
COQ2	AR	Coenzyme Q_{10}	Not specified	SRNS with progressive kidney disease, encephalomyopathy; multiorgan failure	Quinzii et al.[62]; Mollet et al.[63]; Salviati et al.[92]
COQ6	AR	Coenzyme Q_{10}	Not specified	SRNS with progressive kidney disease, SNHL, ataxia	Heeringa et al.[65]
COQ9	AR	Coenzyme Q_{10}	Generalized seizures	Profound developmental delay, LA, HCM, renal tubulopathy	Duncan et al.[64]; Rahman et al.[93]
ADCK3 (CABC1)	AR	Coenzyme Q_{10}	Generalized tonic–clonic seizures, EPC	Ataxia, cerebellar atrophy	Mollet et al.[66]

Table I: Continued

Gene	Mode of inheritance	Biochemical defect	Epilepsy phenotype	Other clinical features	References
RARS2	AR	Multiple RC enzymes (but can be normal)	Intractable myoclonic and generalized seizures	Profound developmental delay; pontocerebellar hypoplasia	Edvardson et al.[55]; Rankin et al.[56]; Glamuzina et al.[57]
TFSM	AR	Multiple RC enzymes	Generalized seizures	Encephalomyopathy, muscle weakness, hypotonia and rhabdomyolysis	Smeitink et al.[58]
AIFM1	X-linked	Multiple RC enzymes	Generalized seizures	Encephalomyopathy, axonal sensorimotor neuropathy	Ghezzi et al.[94]
SLC25A22	AR	Normal in fibroblasts	Neonatal myoclonic and focal seizures (burst suppression)	Early infantile-onset epileptic encephalopathy with burst suppression (Otahara syndrome)	Molinari et al.[68,69]

AR, autosomal recessive; HCM, hypertrophic cardiomyopathy; MRI, magnetic resonance imaging; LA, lactic acidosis; SNHL, sensorineural hearing loss; RC, respiratory chain; IUGR, intrauterine growth restriction; MDDS, mitochondrial DNA depletion syndrome; SCAE, spinocerebellar ataxia with epilepsy; MIRAS, mitochondrial recessive ataxia syndrome; MEMSA, myoclonus, epilepsy, myopathy, sensory ataxia; IOSCA, infantile onset spinocerebellar ataxia; SRNS, steroid-resistant nephrotic syndrome.

suggesting that seizures may be a relatively frequent occurrence in this condition.[33] Initial presentation was with cardiorespiratory symptoms related to hypertrophic cardiomyopathy; seizures occurred subsequently. Seizures have also occasionally been reported in individuals with mutations in the *COX10* and *COX15* genes, which are necessary for the biosynthesis of the haem prosthetic group of COX.[34,35]

Isolated complex V deficiency

Activity of the mitochondrial ATP synthase (complex V) can only be reliably assayed in fresh tissue. This assay is offered in only a few specialized centres, and so complex V deficiency is probably underdiagnosed. Maternally inherited Leigh syndrome (MILS) is caused relatively frequently by mutations in the mtDNA-encoded ATP6 subunit of complex V, in particular the m.8993T>G mutation, and may be associated with seizures.[36] The m.8993T>G mutation has been linked to heterogeneous clinical phenotypes, which are broadly related to the mutant load. Mutation loads of more than 90% are associated with MILS, whereas levels of around 70 to 90% may present as NARP (neurogenic muscle weakness, ataxia, retinitis pigmentosa) syndrome.[37] An extensive clinical and molecular survey of Leigh syndrome in Australia suggested that the m.8993T>G mutation is a relatively common cause of Leigh syndrome with seizures.[29] Heterogeneous seizure types, including myoclonic and generalized tonic–clonic seizures, may occur in both NARP and MILS. Infantile spasms may be the presenting feature of MILS and are usually responsive to anticonvulsant medications. Other clinical features include hypotonia, spasticity, dystonia, ataxia, movement disorders, and peripheral neuropathy. The mutation load is usually high in blood in both MILS and NARP, so molecular testing in blood is the preferred diagnostic method for these conditions. MILS is usually rapidly progressive, with death from respiratory failure in early childhood. NARP is associated with a more indolent course with survival well into adult life. Nuclear-encoded defects of complex V have so far been associated primarily with hypertrophic cardiomyopathy phenotypes, although seizures have occasionally been reported (Table I).[38,39]

Multiple respiratory chain defects

Approximately 25% of children with mitochondrial disease present with multiple OXPHOS defects, involving two or more of the enzyme complexes. Combined OXPHOS deficiencies may occur for a number of reasons, including disorders of mtDNA maintenance, disorders of mitochondrial translation, and defective biosynthesis of coenzyme Q_{10} (Table I).

Disorders of mitochondrial DNA maintenance

The mtDNA depletion syndrome (MDDS) is characterized by a severe quantitative reduction in the mtDNA copy number. Residual mtDNA levels in affected tissues may be as low as 1 to 2% of those observed in comparison healthy individuals.[40] MDDS is most frequently caused by mutations in the *POLG* gene, encoding the catalytic subunit of the DNA

polymerase gamma, the only polymerase able to replicate mtDNA. Among our cohort of 26 individuals with MDDS who had residual mtDNA less than 35% of healthy comparison values, we found *POLG* mutations in 19 cases, many of whom experienced seizures[11,41] (S. Rahman and J-W. Taanman, unpublished data). The clinical spectrum of hepatocerebral MDDS caused by *POLG* mutations overlaps that of the Alpers syndrome of progressive neuronal degeneration with epilepsy. Individuals with Alpers syndrome typically present with focal clonic and complex focal seizures. Epilepsia partialis continua is also frequently seen. The initial electroencephalogram may be characteristic, with unilateral occipital rhythmic high-amplitude slow activity and superimposed (poly)spikes, although subsequently discharges tend to generalize.[11] Other clinical features in Alpers syndrome include global developmental delay and regression, progressive microcephaly, cortical visual impairment with abnormal visual-evoked potentials, and evidence of liver disease such as elevated hepatic transaminases. Magnetic resonance brain scans may be normal or show non-specific changes such as progressive cerebral atrophy. Alpers syndrome was initially diagnosed neuropathologically; histological features include spongiosis, neuronal loss, and astrocytosis affecting the cerebral cortex, particularly the calcarine cortex, which explains the visual loss in this condition.[42] Liver histology in Alpers syndrome may reveal steatosis, hepatocyte loss, bile duct proliferation, and fibrosis, frequently with frank cirrhosis.[42] More than 150 mutations have been reported in the *POLG* gene, but a handful of these have been particularly associated with Alpers syndrome. The two most prevalent mutations are A467T and W748S, which are present at a frequency of approximately 1% in some white populations.[43] A rapid molecular diagnosis of Alpers syndrome may be achieved in some cases by screening for selected 'common' mutations in DNA extracted from blood, and progressing to full *POLG* sequence analysis if clinically indicated.

Older individuals with *POLG* mutations may have multiple mtDNA deletions rather than depletion, but seizures can still be the predominant clinical problem. Various acronyms have been coined for the adolescent/adult-onset *POLG* mutation disorders associated with myoclonus/epilepsy, including SCAE, MIRAS, and MEMSA.[44] Individuals with milder or heterozygous *POLG* mutations may present with progressive external ophthalmoplegia without cerebral involvement.[43]

Eight other genes have also been reported to cause MDDS,[40] and seizures are often associated with mutations in these genes. In particular, recessive *PEO1* (*C10orf2*) mutations affecting the Twinkle helicase involved in mtDNA replication can cause a severe epileptic encephalopathy.[45] This was initially described as infantile-onset spinocerebellar ataxia, a Finnish heritage disease characterized by severe neurodegeneration with ataxia, hypotonia, athetoid movement disorder, and SNHL in addition to severe epilepsy,[46] but recessive *PEO1* mutations have subsequently been reported in other ethnic groups.[47] The clinical phenotype associated with *PEO1* mutations overlaps that of Alpers syndrome caused by *POLG* mutations. Seizures are also a feature of MDDS caused by mutations in *RRM2B*, encoding the p53R2 subunit of ribonucleotide reductase, and *SUCLA2*, encoding the beta subunit of the Krebs cycle enzyme succinyl-CoA synthase, both of which are required to maintain nucleotide pools for mtDNA synthesis.[48,49]

Disorders of mitochondrial translation

Disorders affecting the intramitochondrial synthesis of the 13 mtDNA-encoded OXPHOS proteins are an increasingly recognized cause of mitochondrial disease. The most frequent disorders of mitochondrial translation affect the mitochondrial tRNA genes, either as point mutations such as m.3243A>G and m.8344A>G, causing the MELAS and MERRF syndromes respectively,[50,51] or by large-scale deletion involving several tRNA genes, as in the Pearson marrow pancreas and Kearns–Sayre syndromes. MERRF syndrome is one of the more frequent causes of progressive myoclonic epilepsy,[52] and has also been associated with other mitochondrial tRNA mutations.[53] Myoclonus and focal and generalized seizures may all occur in MERRF. Other clinical features include myopathy, ataxia, peripheral neuropathy, hearing loss, dementia, and multiple lipomas. Most cases have the common point mutation m.8344A>G in the *MTTK* gene, but MERRF has also been linked to other mtDNA point mutations (http://www.mitomap.org). MELAS syndrome has been associated with several mtDNA point mutations, although approximately 80% of cases have the common m.3243A>G mutation in the *MTTL1* gene (http://www.mitomap.org). Focal seizures may be the presenting feature,[13] but generalized epilepsy also occurs. Other clinical features of the MELAS syndrome include migraine, recurrent vomiting, and stroke-like episodes which may cause cortical blindness, hemiparesis, or hemianopia.

More recently, nuclear-encoded defects of mtDNA translation have been reported,[54] and these may also be associated with epilepsy. Mutations of RARS2, an enzyme required for aminoacylaton of the mitochondrial tRNA for arginine, appear to be particularly associated with intractable epilepsy.[55] Although multiple respiratory chain defects were present in the index case of the first reported family, one sibling had isolated complex I deficiency while another had isolated COX deficiency.[55] Furthermore, respiratory chain activities were normal in an unrelated case.[56] Severe lactic acidosis and pontocerebellar hypoplasia are present at birth, and the clinical course is of progressive neurological decline with severe intractable epilepsy and profound developmental delay. *RARS2* mutations should be considered in any child with pontocerebellar hypoplasia and initial lactic acidosis, regardless of the muscle respiratory chain activities.[57] Epilepsy has also been reported in an individual with mutations in *TFSM* encoding the mitochondrial translation elongation factor EFTs.[58]

Coenzyme Q_{10} deficiency

Defective biosynthesis of the lipophilic electron carrier coenzyme Q_{10} (CoQ_{10}) typically presents with combined deficiency of complexes I + III or II + III[59] and is frequently associated with seizures. The first phenotype reported to be

caused by CoQ_{10} deficiency was of recurrent rhabdomyolysis associated with an encephalopathy with prominent seizures.[60] The molecular cause of this type of CoQ_{10} deficiency remains unknown, but seizures are also a feature of other phenotypes of CoQ_{10} deficiency in which the genetic basis has recently been defined. For example, a multisystem disorder of infancy characterized by encephalomyopathy and renal involvement has recently been linked to deficiencies of the CoQ_{10} biosynthetic enzymes encoded by *COQ2*, *PDSS2*, and *COQ9*.[61–64] Individuals with *COQ6* mutations present with isolated steroid-resistant nephrotic syndrome, which may be associated with seizures, ataxia, and SNHL.[65] In other individuals, mutations in the kinase ADCK3 cause CoQ_{10} deficiency with cerebellar ataxia, generalized seizures, and epilepsia partialis continua.[66,67] Although rare, it is important to recognize disorders of CoQ_{10} biosynthesis, since at present they represent the most readily treatable subgroup of mitochondrial disease. To this end, we have recently published a diagnostic algorithm to aid in the clinical diagnosis of CoQ_{10} deficiency.[59]

Other mitochondrial disorders associated with epilepsy

Other relatively recently described mitochondrial diseases include disorders of mitochondrial dynamics (fission and fusion) and of solute transport across the inner mitochondrial membrane. Mutations have been reported in the mitochondrial glutamate carrier (encoded by the *SLC25A22* gene) in some infants with neonatal or early infantile-onset epileptic encephalopathy with burst suppression (Otahara syndrome).[68,69] All individuals reported so far have had a homogeneous phenotype, with myoclonic and focal seizures from the first days of life, associated with microcephaly, hypotonia, and profound developmental delay. Electroencephalography demonstrated burst suppression, and the electroretinogram and visual-evoked responses were also abnormal. Magnetic resonance brain imaging showed hypoplasia of the cerebellum and corpus callosum, with abnormal gyration of temporoparietal regions and hypomyelination of the temporal poles. Respiratory chain activities were normal in cultured skin fibroblasts from one case. Muscle respiratory chain activities have not been reported, but it is possible that they would also be normal as *SLC25A22* appears to be expressed exclusively in the brain.[68]

PATHOGENESIS OF MITOCHONDRIAL EPILEPSY

The pathomechanisms leading to epilepsy in mitochondrial disorders are not clear. Evidence from mouse models has shown that specific inhibitors of the respiratory chain may induce seizures. Subcutaneous injection of potassium cyanide (KCN, inhibits COX) resulted in dose-dependent tonic seizures in treated mice, whilst 3-nitropopionate (inhibitor of complex II) induced clonic seizures, again in a dose-dependent manner.[70,71] Energy failure undoubtedly plays a role but does not explain the phenotypic variability of mitochondrial epilepsy, nor why epilepsy is not a feature of all mitochondrial disorders. Other aspects of mitochondrial dysfunction, such as reactive oxygen species production, abnormal calcium handling, and increased apoptosis, are also likely to contribute to seizure generation. Brain lipid peroxidation levels were increased in the KCN mouse model, supporting a role for reactive oxygen species formation in the pathogenesis of seizures in these animals.[70] There is some evidence that seizures themselves can trigger mitochondrial dysfunction,[72] implicating a vicious spiral in the aetiology of mitochondrial epilepsy (Fig. 2). It is also possible that mitochondrial epilepsy may be precipitated by an autoimmune response. For example, a male who presented with acute encephalopathy and pathogenic *POLG* mutations had evidence of acute disseminated encephalomyelitis on histological examination of a brain biopsy.[73] Finally, in occasional individuals with mitochondrial disease, seizures may be secondary to electrolyte disturbances arising from severe renal tubulopathy.

MANAGEMENT OF MITOCHONDRIAL EPILEPSY

Mitochondrial epilepsy can be very difficult to manage. It is important to identify and treat disorders of CoQ_{10} biosynthesis, since these remain the only readily treatable causes of mitochondrial epilepsy.[59] Recognition and treatment of electrolyte disturbances caused by renal tubulopathy is also critical. In all other individuals, symptomatic treatment remains the mainstay of management. The choice of AED depends on the seizure type. Sodium valproate is well known to be a broad-spectrum AED effective against many types of partial and generalized seizures. However, there is evidence that valproate may worsen mitochondrial disease symptoms in both mtDNA and nuclear-encoded mitochondrial diseases. For example, case reports have documented worsening of seizures and triggering of stroke-like episodes in MELAS syndrome and disease progression in individuals with other mtDNA mutations and with COX deficiency.[74–77] Individuals at greatest risk of valproate-related toxicity are those with *POLG* mutations, in whom the drug may trigger fulminant and fatal hepatic failure.[11,78,79] For this reason, it is advisable to avoid valproate in individuals in whom there is a strong suspicion of mitochondrial disease, and especially in cases where pathogenic *POLG* mutations have been identified. If valproate needs to be considered, for example because of lack of seizure control using alternative AEDs, then there is some evidence that prophylactic co-treatment with L-carnitine may ameliorate the

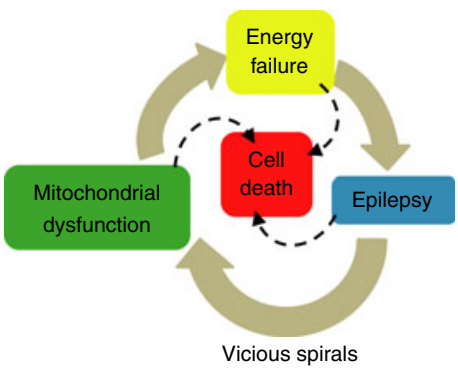

Figure 2: Pathogenesis of mitochondrial epilepsy.

adverse effects of valproate.[80] Levetiracetam is the first choice for myoclonus in MERRF syndrome,[81] while lamotrigine may exert a neuroprotective effect.[82] However, there is no single AED that is effective in all cases of mitochondrial epilepsy, and it is important to tailor therapy to the individual. In many cases, multiple AEDs are needed to achieve seizure control, and in some individuals (particularly those with Alpers syndrome) it may not be possible to control seizures in the terminal stages of the disease.

Vigilant monitoring for and treatment of multisystem disease manifestations is essential. Interventions which may be required include hearing aids, insulin, thyroxine, and drugs for cardiomyopathy. Studies in Japanese individuals with the m.3243A>G mutation have suggested that arginine may reduce the severity and frequency of stroke-like episodes in individuals with MELAS syndrome.[83] There is some anecdotal evidence that seizures in the Kearns–Sayre syndrome may be related to white matter lesions associated with cerebral folate (5-methyltetrahydrofolate) deficiency, and that folinic acid supplementation may lead to symptomatic benefit in these individuals.[84] The role of other vitamins and nutritional supplements is not clear, and so far has not been supported by clinical trials.[85] However, the prognosis for mitochondrial epilepsy is extremely poor, and there is clearly an urgent need for novel treatments for these individuals. A fatal outcome was observed in 45% (22/56) of a French cohort, and 50% of these individuals died within 9 months of epilepsy onset.[8]

The ketogenic diet is a high-fat, low-carbohydrate diet that aims to stimulate fatty acid utilization by mitochondrial beta-oxidation, with subsequent formation of ketone bodies to provide an alternative energy source for brain and other tissues. Ketone bodies are metabolized to acetyl-CoA, which feeds into the Krebs cycle and thence to the respiratory chain/OXPHOS system to generate ATP, and may at least partially bypass complex I. Preliminary preclinical studies have suggested that ketogenic diet may be beneficial in a subgroup of mitochondrial disease, namely those with mtDNA deletions. A study in which cultured cells (transmitochondrial cybrids) harbouring a heteroplasmic mtDNA deletion were grown in a ketone-rich culture medium devoid of glucose demonstrated that the ketogenic diet favoured wild-type over mutant mtDNA, leading to a reduction in the proportion of deleted mtDNA and functional rescue of the respiratory chain defect in these cells.[86] The authors proposed that ketogenic diet treatment might be beneficial for individuals with heteroplasmic mtDNA deletions. A more recent study investigated the effects of ketogenic diet in the 'deletor' mutant mouse.[87] This is a transgenic mouse harbouring a mutation in the Twinkle helicase, leading to the accumulation of multiple mtDNA deletions and a late-onset myopathy.[88] This study showed reduction in some features of mitochondrial disease in the mice, particularly with presymptomatic initiation of ketogenic diet.

A number of case reports have described ketogenic diet in the treatment of children with mitochondrial disease,[89] but few studies have examined the effects of ketogenic diet in a more systematic way. One retrospective study used ketogenic diet in 24 children with respiratory chain defects and epilepsy and reported that 50% became seizure free on ketogenic diet.[9] However, duration of follow-up and long-term outcome for these individuals were not reported. Another retrospective study followed 14 individuals with confirmed respiratory chain defects treated with a classical ketogenic diet for at least 6 months.[90] Eight individuals appeared to benefit from ketogenic diet in terms of cessation or dramatic reduction of seizures. Significant side effects were seen, with one individual experiencing persistent metabolic acidosis and two developing recurrent symptomatic hypoglycaemia that resulted, in one, in the ketogenic diet being withdrawn and, in the other, prednisolone treatment to maintain blood glucose. Furthermore, two individuals died as a result of disease progression despite treatment, including one who became seizure free on the ketogenic diet. In a third study, some individuals benefited from a ketogenic diet, but no dramatic improvements were observed.[8] Overall, there is a suggestion that, although seizure frequency may be reduced on a ketogenic diet, the diet does not appear to influence the relentlessly progressive course of mitochondrial disease in many individuals. It is not clear whether some subgroups of mitochondrial disease may respond better than others to a ketogenic diet. Formal clinical trials are needed, with the aim of determining which individuals are likely to benefit from ketogenic diet.

CONCLUSION

In summary, seizures occur frequently in mitochondrial disease. They may be the presenting feature but are often part of a multisystem presentation. Mitochondrial epilepsies are biochemically and genetically heterogeneous, but some of the more common causes are mtDNA mutations and mutations in *POLG*. A rapidly increasing number of nuclear gene defects have been linked to mitochondrial epilepsy (Table I). The pathogenesis of mitochondrial epilepsy remains poorly understood, contributing to the immense difficulties in treating this condition. Epilepsy is a poor prognostic sign in mitochondrial disease, and there is an urgent need for formal clinical trials of candidate treatments, including the ketogenic diet and novel therapeutic agents.

ACKNOWLEDGEMENTS

SR receives funding from Great Ormond Street Hospital Children's Charity, Ataxia UK, the Child Health Research Appeal Trust, the Children's Liver Disease Foundation, Climb, and the Medical Research Council. An earlier version of this article was published in the Sheffield Children's Hospital Metabolic Disease Newsletter.

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