Review article

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Myoclonus epilepsy in mitochondrial disorders

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ABSTRACT – Mitochondrial disorders is a group of clinical entities associated with abnormalities of the mitochondrial respiratory chain (MRC), which carries out the oxidative phosphorylation (OXPHOS) of ADP into ATP. As the MRC is the result of genetic complementation between two separate genomes, nuclear and mitochondrial, OXPHOS failure can derive from mutations in either nuclear-encoded, or mitochondrial-encoded, genes. Epilepsy is a relatively common feature of mitochondrial disease, especially in early-onset encephalopathies of infants and children. However, the two most common entities associated with epilepsy include MERRF, for Myoclonic Epilepsy with Ragged Red Fibers, and AHS, or Alpers-Huttenlocher syndrome, also known as hepatopathic poliodystrophy. Whilst MERRF is a maternally inherited condition caused by mtDNA mutations, particularly the 8344A>G substitution in the gene encoding mt-tRNA^{Lys}, AHS is typically caused by recessive mutations in *POLG*, encoding the catalytic subunit of polymerase gamma, the only mtDNA polymerase in humans. AHS is the most severe, early-onset, invariably fatal syndrome within a disease spectrum, which also include other epileptogenic entities, all due to *POLG* mutations and including Spino-cerebellar Ataxia and Epilepsy (SCAE). This review reports the main clinical, neuroimaging, biochemical, and molecular features of epilepsy-related mitochondrial syndrome, particularly MERRF and AHS.

Key words: MERRF, MELAS, Alpers-Huttenlocher syndrome, hepatopathic poliodystrophy, mitochondrial DNA, oxidative phosphorylation, mitochondrial respiratory chain, progressive myoclonus epilepsies

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The term 'mitochondrial disorders' is, to a large extent, applied to the clinical syndromes associated with abnormalities of the common final pathway of mitochondrial energy metabolism, *i.e.* oxidative phosphorylation (OXPHOS). OXPHOS takes place in the inner mitochondrial membrane involving five enzymatic complexes which form the mitochondrial respiratory chain (MRC). From a genetic perspective, the MRC is unique since it is encoded by two complementary separate genetic systems: the nuclear and the mitochondrial genomes. Because of this dual genetic control, OXPHOS disorders may be due to mutations in mitochondrial deoxyribonucleic acid (mtDNA) or nuclear DNA genes encoding either structural components of the MRC complexes or factors controlling their expression, assembly, function and turnover (Smeitink *et al*., 2001). Mitochondria contain the only extra-nuclear

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source of DNA in animal cells (Nass, 1966). MtDNA is a circular, double stranded, 16,569 base-pair molecule of DNA which encodes 37 genes, including 13 polypeptides essential for the formation and function of four of the five MRC complexes, namely complex I, III, IV and V, two ribosomal RNAs (12S and 16S rRNA), and 22 transfer RNAs (tRNA) (Anderson *et al*., 1981). All other OXPHOS-related proteins, including most of the MRC subunits, MRC assembly factors, factors necessary for mtDNA maintenance and expression, etc., are synthesized in the cytosol and are specifically targeted, sorted and imported to their final mitochondrial location (Mokranjac & Neupert, 2005). The mitochondrial genome has unique features that distinguish it from the nuclear genome; for instance, in sexuate organisms, mtDNA is strictly maternally inherited and present in several hundred to several thousand copies within a single cell, the number varying amongst different cell types, mostly based on the energy demand of each tissue and organ (Taylor & Turnbull, 2005). The mtDNA genes have no introns, hardly any non-coding intervening regions, and for most cases the termination codons are completed by post-transcriptional polyadenylation (Anderson *et al*., 1981). The genetic code of mtDNA differs between many species, including humans, such that genomes between species may be reciprocally untranslatable; this partly explains why mtDNA is translated *in situ* by protein synthesis machinery that is completely independent from that operating in the cytosol for the translation of nuclear genes. In human mtDNA, the displacement loop (D-loop) is the only major non-coding region of the molecule which is formed by the displacement of the two DNA strands by a third DNA strand, the so-called 7S DNA.

An important contribution to the elucidation of the molecular basis of mitochondrial disorders has come from the discovery of an impressive, ever-expanding number of pathogenic mutations in mtDNA. In cases in which a mtDNA mutation is not found, mitochondrial disease is defined by the detection of a specific biochemical defect in OXPHOS, or the observation of typical morphological clues, or a combination of the two. In many instances, pathogenic mtDNA mutations can coexist alongside non-mutated mtDNA in the same cell, tissue and organism, a condition known as heteroplasmy (Hayashi *et al*., 1991; Larsson & Clayton, 1995). The percentage of pathogenic heteroplasmy dictates the phenotype, according to a 'threshold effect', *i.e*. a critical amount below which mutations do not manifest any clinical or biochemical phenotype. This threshold level varies from tissue to tissue and depends on the intrinsic pathogenicity of each mutation but, in general, ranges from 50-60 per cent (DiMauro, *et al*., 1985; Rosing *et al*., 1985; Mita *et al*., 1990; Hayashi *et al*., 1991; Moraes *et al*., 1992; Shoubridge, 1994; Traff *et al*., 1995; Parikh *et al*., 2008) for the most severe mutations, to more than 90 percent for the mildest mutations. A paradigmatic example is the m.8993T>G mutation associated with NARP (neuropathy, ataxia, retinitis pigmentosa) syndrome (DiMauro, *et al*., 1985). The relationship between the mutation load and clinical severity was first documented by Tatuch *et al*., who showed that around 70 percent heteroplasmy in skeletal muscle results in adult-onset of a slowly progressive syndrome corresponding to the acronymic features of NARP, whereas higher degrees of heteroplasmy (around 90 percent) cause severe, early-onset, maternally inherited Leigh syndrome (MILS) (Rosing *et al*., 1985).

MtDNA disease has an extremely variable phenotype and can present at any age (Traff *et al*., 1995). The clinical features usually affect tissues characterized by high metabolic demand, such as the central nervous system, the skeletal muscle, or the heart. However, other tissues are frequently involved, such as the β cells in the pancreas (leading to diabetes), the hair cells of the cochlea (causing deafness), or the renal tubules (leading to kidney dysfunction).

While epilepsy is a recurrent manifestation of mitochondrial disease, its exact prevalence is not known. In contrast, 35-60 per cent of individuals with refractory seizures display biochemical evidence of mitochondrial dysfunction (Parikh *et al*., 2008). A few studies have systematically examined the epileptic manifestations of mitochondrial disease (Khurana *et al*., 2008; Lee *et al*., 2008; El Sabbagh *et al*., 2010). Although seizures may be the presenting symptom at onset (Hayashi *et al*., 1991; Canafoglia *et al*., 2001), in more than 80 percent of cases, the first seizure is preceded by some other symptoms (El Sabbagh *et al*., 2010), including, for example, failure to thrive, developmental delay, ataxia, or evidence of multi-organ involvement. In children with respiratory chain disorders, different seizure types can occur in as many as 60 percent of cases (El Sabbagh *et al*., 2010). Whilst clinical identification of mitochondrial epilepsy may be difficult, one of the most common forms is myoclonic epilepsy, either as typical MERRF syndrome (myoclonic epilepsy with ragged red fibres) or within the context of other, complex epileptic manifestations.

Clinical manifestations

In 1921, Ramsay Hunt described six patients with a disorder characterized by ataxia, myoclonus, and epilepsy, which he called '*dyssynergia cerebellaris myoclonica*' (Hunt, 1921). Over 50 years then passed before Tsairis *et al*. linked this entity to mitochondrial abnormalities in the skeletal muscle in one family, hallmarked by the presence of ragged-red fibres

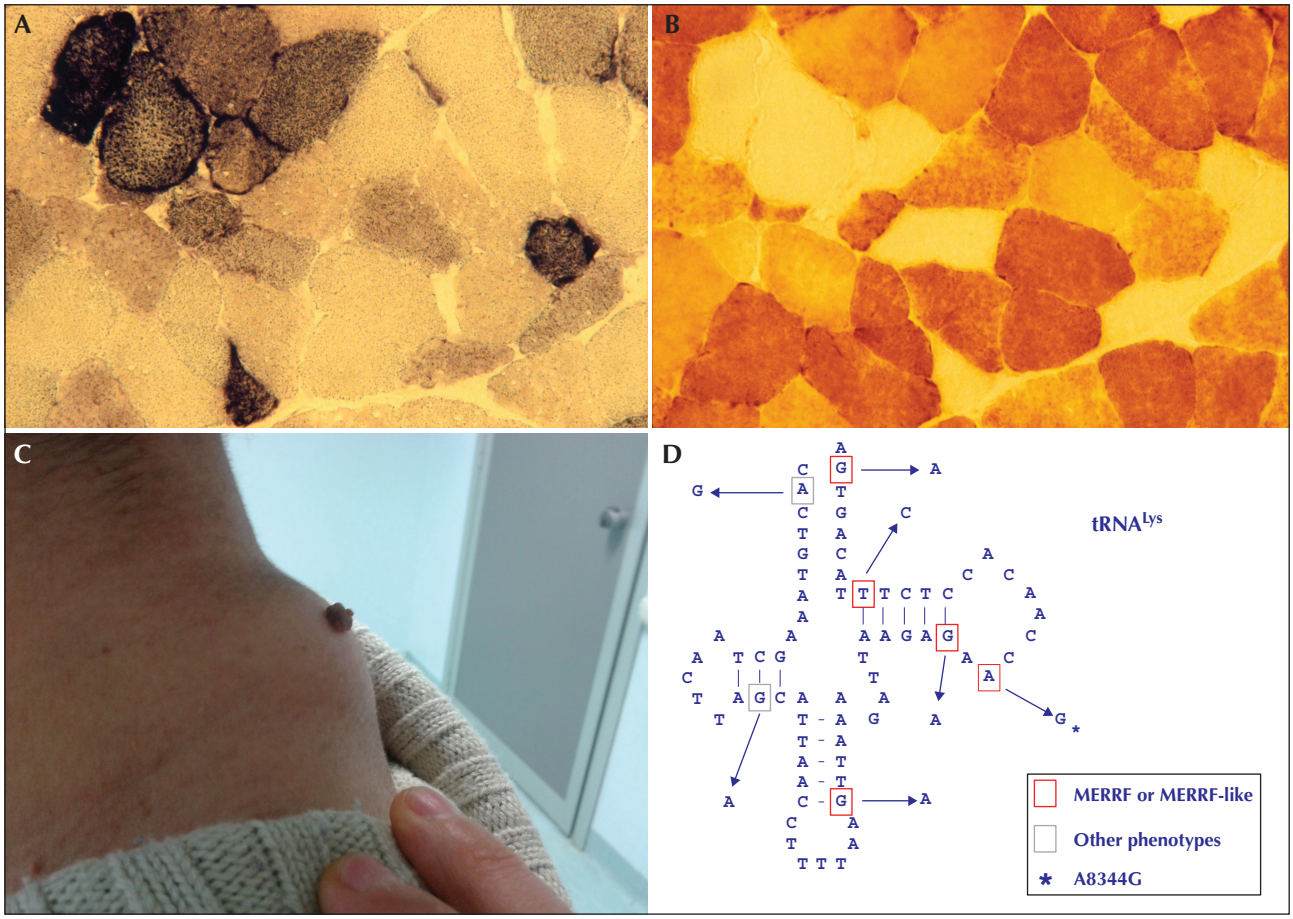

Figure 1. Muscle biopsy of a MERRF patient presenting with COX deficient (A) and intense SDH-positive (B) RRF. (C) Neck lipoma in a 67-year-old MERRF patient. (D) Lys tRNA highlighting the most frequent mutations associated with MERRF or MERF-Like syndrome.

(Tsairis *et al*., 1973). This family was described in great detail, leading to the term 'classic MERRF' in 1989 (Lombes *et al*., 1989). MERRF was one of the three major, multisystem syndromes first classified as 'mitochondrial encephalomyopathy' (DiMauro *et al*., 1985). MERRF has two other historical distinctions:

– it was the first well-defined human disease in which maternal inheritance was clearly demonstrated, thus suggesting a mitochondrial DNA defect (mtDNA) (Rosing *et al*., 1985);

– the first mitochondrial encephalomyopathy in which a molecular mtDNA defect was actually identified.

It is also one of the most common and clinically better defined mitochondrial syndromes. MERRF is, in fact, a multi-system disorder, hallmarked by myoclonus, episodes of generalized epilepsy, progressive ataxia, and ragged-red fibres (RRF) with partial deficiency of cytochrome c oxidase (COX) (19-20-21) (*figure 1 A, B*). Although the onset is usually in childhood, early development is normal and adult onset is not uncommon. Besides the defining criteria, common clinical manifestations include hearing loss, peripheral neuropathy, cognitive decay and eventually dementia, short stature, exercise intolerance, and optic atrophy and ataxia. Less common clinical signs (seen in < 50 percent of the patients) include cardiomyopathy, pigmentary retinopathy, pyramidal signs, ophthalmoparesis, and the appearance of multiple lipomas, particularly in the neck and upper trunk (*figure 1C*). As is usually the case with mitochondrial encephalomyopathies, maternal family members may be symptomatic, oligosymptomatic, or asymptomatic. While several heteroplasmic point mutations, mostly affecting the gene encoding mt-tRNA^{Lys}, are responsible for MERRF, by far the most frequent MERRF mutation is the m.8344A>G substitution in the T-Ψ-C loop of mt-tRNA^{Lys} (*figure 1D*). A few unusual clinical presentations, characterized by overlapping symptoms between MERRF and MELAS (mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes), have been reported either as isolated cases or, more often, in pedigrees in which typical MERRF patients were also present. Several studies have reported in detail the wide spectrum of clinical

presentations associated with the m.8334A>G mutation (Berkovic *et al*., 1991; Hammans *et al*., 1993; Silvestri *et al*., 1993; Howell *et al*., 1996; Mancuso *et al*., 2013), but certain features and variants deserve special attention. For instance, peripheral neuropathy is not uncommon in MERRF, being usually sensory-motor and contributing to the onset and progression of gait ataxia. At least one case has been reported to be characterized by predominant motor symptoms, thus phenocopying Charcot-Marie-Tooth disease (Howell *et al*., 1996).

In 1975, long before the molecular defects of MERRF became known, Karl Ekbom (Ekbom, 1975) described multiple lipomas in association with hereditary ataxia, photomyoclonus and skeletal deformities in a family in which the 8344A>G mutation was later documented (Berkovic *et al*., 1991; Traff *et al*., 1995). These tumours, varying in size from small subcutaneous nodules to disfiguring masses, are usually located in the nape of the neck and the shoulder area. They have been reported in numerous patients with the 8344A>G mutation (Larsson *et al*., 1992; Holme *et al*., 1993; Calabresi *et al*., 1994; Naumann *et al*., 1995; Austin *et al*., 1998). Maternal inheritance was evident in a large family (Rosing *et al*., 1985) which was again confirmed to harbour the m.8344A>G substitution in the mt-tRNALys gene (Shoffner *et al*., 1990). Not only was this the first molecular defect to be reported for a mitochondrial encephalomyopathy, but also the first to be identified for a specific form of epilepsy. The m.8344A>G mutation is present in about 90 percent of MERRF patients. Two additional mutations have been associated with MERRF, both affecting the mt-tRNA^{Lys} gene. The first mutation, m.8356T>C, was discovered simultaneously in an American family with typical MERRF (Silvestri *et al*., 1992), and in an Italian family in which typical MERRF symptoms coexisted with stroke-like episodes and migraines, thus justifying the definition as a "MERRF/MELAS" overlap syndrome (Zeviani *et al*., 1993). A common clinical feature of both families was hyperthyroidism, which is rather unusual in mitochondrial diseases and may, therefore, be related to this specific mutation. A third family with the same mutation was later reported in Japan; the proband had typical MERRF, but a maternal aunt had stroke-like episodes, another example of MERRF/MELAS overlap (Sano *et al*., 1996).

The second mutation, m.8363G>A, was first identified in two unrelated American families with maternally inherited cardiomyopathy, which was severe enough to cause early death in several members of one family (Santorelli *et al*., 1996). Although cardiomyopathy dominated the clinical picture, additional signs included encephalomyopathy, neurosensory hearing loss, progressive external ophthalmoparesis, intellectual disability, limb weakness, and peripheral neuropathy, variably affecting members of both families. Interestingly, cerebellar symptoms were frequent, including ataxia, dysmetria, slurred speech, and gait instability. Interestingly, one proband had 'horse collar' lipomas. The same mutation was later identified in two unrelated Japanese patients with typical MERRF, one of whom also presented cardiomyopathy (Ozawa *et al*., 1997). Recently a new mutation, m.3291T>C, has been associated with MERRF/MELAS syndrome in a 19-year-old Chinese man (Liu *et al*., 2014).

The m.8344A>G mutation is virtually always heteroplasmic, and the mutation threshold for typical MERRF is usually high or very high (*i.e*. affecting about 60-90 per cent of total mtDNA), which suggests that this mutation is relatively benign (Shoffner *et al*., 1990). As mentioned earlier, the three mutations associated with MERRF all affect highly conserved nucleotides in the mt-tRNA^{Lys} gene. The m.8344A>G and the m.8356T>C mutations are located in the T-Ψ-C loop, while the m.8363G>A mutation is located in the aminoacyl acceptor stem of the putative cloverleaf (secondary) structure of the mt-tRNA^{Lys} transcript (Santorelli *et al*., 1996). A single patient with typical MERRF symptoms, *i.e*. myoclonus, seizures, ataxia, and RRF, but, in addition, peripheral neuropathy, dementia, and neuroradiological evidence of mild cerebral and severe cerebellar atrophy, had no mutation in the tRNA^{Lys} gene, but rather multiple mtDNA deletions in muscle, suggesting impairment in a nuclear gene product controlling mtDNA integrity, such as POLG.

Laboratory tests

Patients with typical MERRF have elevated blood lactate and pyruvate at rest, both increasing abnormally upon moderate exercise. CSF protein levels are often increased, but rarely exceed 100 mg%. Electromyography and nerve conduction studies are usually compatible with a predominantly myopathic pattern, except when motor peripheral neuropathy is clearly present. Electroencephalography is typically characterized by generalized spike and wave discharges with background slowing, but focal epileptiform discharges may also be seen (*figure 2*).

A CT or MRI scan may show brain atrophy and basal ganglia calcifications. Phosphorus magnetic resonance spectroscopy of the gastrocnemius muscle in eight patients (only three of whom showed signs of myopathy) revealed mitochondrial dysfunction in all, as evidenced by increased relative intracellular inorganic phosphate (Pi) concentration and decreased phosphocreatine to Pi ratio (Rahman, 2012). However, no mitochondrial dysfunction was seen in the brain using the same technique.

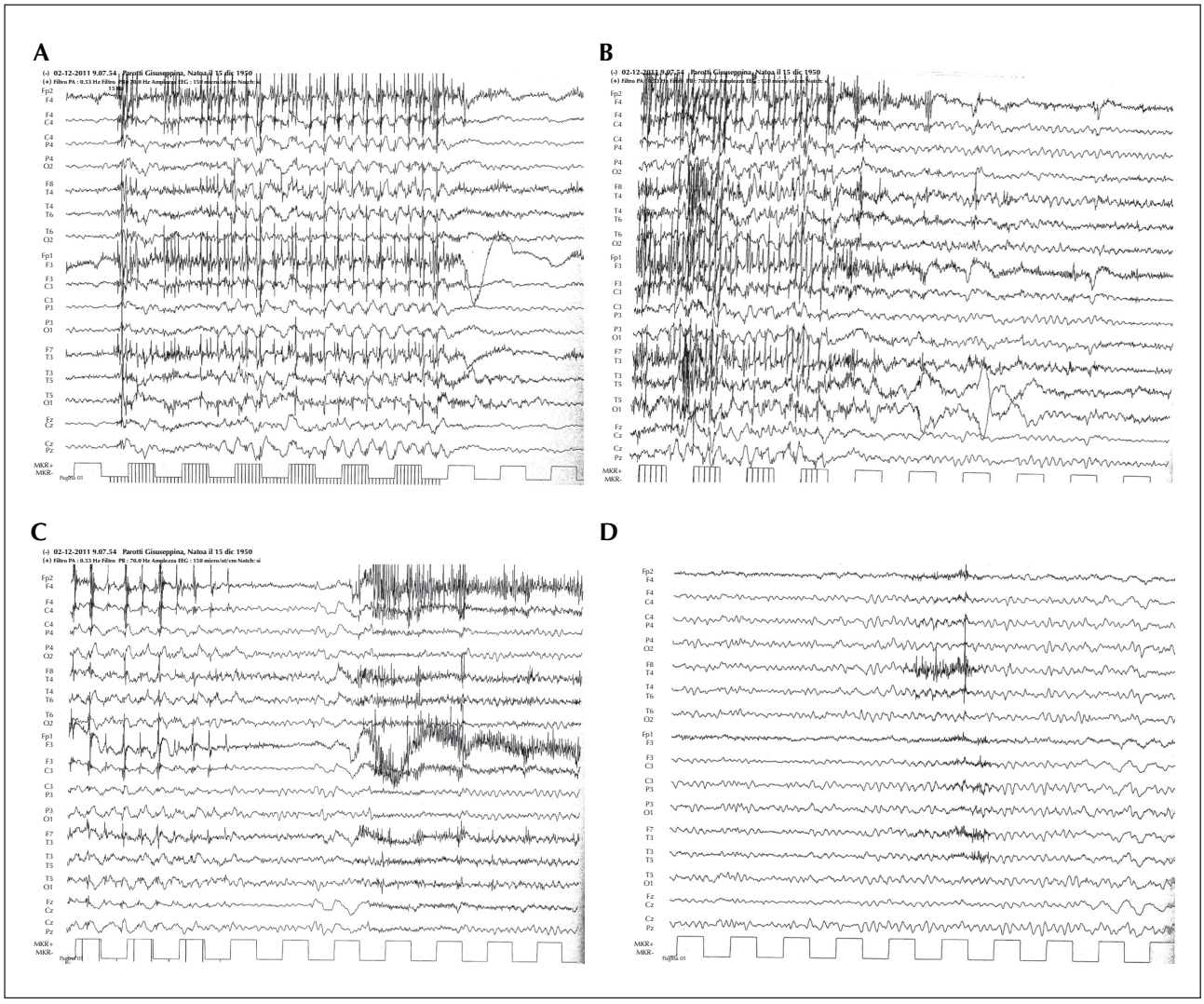

Figure 2. EEG in adult patients with MERRF. (A, B) Generalized spike and waves, as well as myoclonus, during SLI. (C, D) Paroxysmal activity in the temporal region in both hemispheres with right prevalence.

By definition, the muscle biopsy shows RRF using modified Gomori trichrome stain in typical patients (Wolf *et al*., 2009). These fibres also react intensely to the succinate dehydrogenase (SDH)-specific stain, a more sensitive indicator of excessive mitochondrial proliferation. Both RRF and some non-RRF fail to stain histochemically to COX. Muscle biopsies from MERRF patients can also show strongly SDH-reactive blood vessels (SSVs), similar to those characteristically seen in the muscles of patients with MELAS (Harding, 1990), again emphasizing the concept that the two disease entities may overlap to some extent. However, in contrast to MELAS SSVs, which stain positive for COX, MERRF SSVs are uniformly COX-negative (Lamantea *et al*., 2002). MRC activities in muscle extracts usually show defects in mtDNA-dependent complexes, particularly COX (Lombes *et al*., 1989; Silvestri *et al*., 1993).

Neuronal loss and gliosis predominate in the brains of MERRF patients, preferentially involving the cerebellum, the brainstem, and the spinal cord. In the cerebellum, neuronal loss is particularly severe in the dentate nucleus, an observation originally made by Ramsay Hunt, who described 'primary atrophy of the dentate system' in patients with '*dyssynergia cerebellaris myoclonica*' (Hunt, 1921). The inferior olivary nucleus of the medulla oblongata is the most severely affected structure in the brainstem, followed by the red nucleus and the substantia nigra in the mesencephalon. In the spinal cord, severe cell loss has been observed in the thoracic nucleus of Clarke, while milder involvement has been detected in the anterior and posterior horns of the spinal cord. Demyelination preferentially affects the superior cerebellar peduncles and the posterior columns and

lateral spinocerebellar tracts of the spinal cord, while the pyramidal system is usually spared or mildly affected.

Private mtDNA mutations and myoclonic epilepsy

Although MERRF is one of the most common mitochondrial encephalomyopathies, a substantial fraction of paediatric patients with myoclonic epilepsy and MRC defects fails to show MERRF mutations (El Sabbagh *et al*., 2010). In this group, severe myoclonus may be preceded by other seizure types, such as erratic myoclonus, focal motor seizures, myoclonic absences, or tonic seizures, but becomes predominant during the disease course. Occasionally, the disease may evolve into recurrent myoclonic *status epilepticus*. In the report by El Shabbagh *et al*. on 56 paediatric patients (El Sabbagh *et al*., 2010), only one showed photosensitivity, with spikes induced by intermittent light stimulation. Brain MRI showed hyper-intense signals on T2/FLAIR (fluid-attenuated inversion recovery) sequences of the basal ganglia (n = 8) and/or dentate nuclei (n = 3), and cerebellar atrophy (n = 5). The basal ganglia were involved irrespective of the age at onset, while cerebellar involvement was present only in patients with early-onset epilepsy, *i.e*. within the first decade of life. Myoclonic seizures were drug-resistant. Six patients died from global neurological failure leading to comatose *status*. Biochemically, eight patients had complex I deficiency, three complex V, two complex IV, one complex II, and four showed multiple defects. In 45 percent of the cases, a mtDNA mutation was identified; in *MT-ATP6* (n = 3), *MT-ND3* (n = 2), *MT-TK* (n = 1), *MT-ND5* (n = 1), and *MT-TL1* (n = 1). mtDNA depletion in muscles is only rarely associated with progressive myoclonic epilepsy (Rahman, 2012; Minassian et *al*., 2016).

Alpers-Huttenlocher syndrome

The second most common form of mitochondrial myoclonic epilepsy is Alpers-Huttenlocher syndrome (AHS) (OMIM #203700). AHS is clinically characterized by psychomotor retardation, intractable epilepsy, and liver failure. The onset is in infancy or early childhood, often with seizures and/or hypotonia. *Status epilepticus* is common and most patients die from refractory seizures and liver failure before the age of 3 (Luoma *et al*., 2004). Liver dysfunction can be present at the onset of the neurological symptomatology, or may manifest following treatment with sodium valproate for the control of seizures. Individuals with AHS typically present with focal myoclonic and complex seizures. *Epilepsia partialis continua* is also frequently seen and may lead to fatal *status epilepticus*.

The electroencephalogram at onset may point towards the diagnosis, particularly when characterized by unilateral occipital, rhythmic, high-amplitude slow activity with superimposed (poly)spikes, frequently evolving into generalized discharges (Wolf *et al*., 2009).

Other clinical features include global developmental delay and regression, progressive microcephaly, cortical visual impairment with abnormal visual-evoked potentials, and, importantly, evidence of progressive liver failure, heralded by elevated levels of liver enzymes in the blood and hepatomegaly, followed by overt liver cirrhosis. Brain MRI may be normal in the initial stage of disease, or show non-specific changes, such as progressive cerebral atrophy.

AHS was firstly diagnosed as a neuropathological entity defined by the presence of extensive necrotizing poliodystrophy; histological features include spongiosis, neuronal loss and astrocytosis affecting the cerebral cortex, particularly the calcarine cortex, which explains the cortical visual loss in this condition (Luoma *et al*., 2004; Van Goethem *et al*., 2004; Davidzon et al., 2005). Liver histology in AHS may reveal steatosis, hepatocyte loss, bile duct proliferation and fibrosis, evolving into frank cirrhosis (Harding, 1990). By and large, AHS is associated with a few recessive mutations in the *POLG* gene, encoding the catalytic, large subunit of mtDNA polymerase (polymerase gamma). More than 150 mutations have been reported in the *POLG* gene, constituting a major cause of mitochondrial disease. Mutations in this gene are also the most frequent cause of autosomal dominant progressive external ophthalmoplegia (ad-PEO). In adPEO due to *POLG* mutation, distinct features also include severe dysphagia and dysphonia and, occasionally, extra-pyramidal signs, *e.g*. parkinsonism, cerebellar dysfunction, or chorea (Luoma *et al*., 2004). Recessive mutations of *POLG* may also be responsible for autosomal recessive cases (Lamantea *et al*., 2002) or apparently sporadic PEO cases characterised by multiple mtDNA deletions (Agostino *et al*., 2003), with or without additional findings, including parkinsonism, severe peripheral neuropathy, endocrine failure, or psychotic depression (Van Goethem *et al*., 2003).

In addition, recessive *POLG* mutations are responsible for a wide spectrum of syndromes of increasing severity and precocity, including juvenile sensory ataxic neuropathy, dysarthria, ophthalmoplegia, SANDO (Horvath *et al*., 2006), childhood cerebellar ataxia and epilepsy, SCAE, and possibly infantile AHS, all characterized by exquisite sensitivity of the liver to valproate-associated damage.

As mentioned above, liver failure occurs spontaneously in AHS, due to severe, liver-specific mtDNA depletion.

The molecular basis of this clinical heterogeneity can be explained, in part, by the structural and functional complexity of the enzyme. Pol-γA, the 145 kDa catalytic subunit encoded by *POLG*, comprises an N-terminal exonuclease domain, with predominantly proofreading functions, and a polymerase domain, which performs the template-directed synthesis of the nascent mtDNA strands. The 2 most prevalent mutations in AHS, but also in SANDO and SCAE, are p.A467T and p.W748S, which are present at a frequency of approximately 1 percent in the Scandinavian population (Horvath *et al*., 2006). Rapid molecular diagnosis of AHS syndrome may therefore be achieved by screening these selected 'common' mutations in DNA extracted from blood, however, in several cases, sequence analysis of all exons and exon-intron boundaries is required to identify causative *POLG1* mutations. Liver biopsy of AHS patients shows severe mtDNA depletion while multiple mtDNA deletions are the molecular hallmark in muscle for adPEO or arPEO patients.

Recently, the combination of early encephalopathy, epilepsy, hepatopathy, and sensory axonal neuropathy was found in patients with recessive mutations in the mtDNA helicase, Twinkle (*PEO1*), which co-functions with Pol-γ in mtDNA replication (Saneto & Naviaux, 2010; Lonnqvist *et al*., 2009). As is the case for POLG-associated AHS, patients with recessive mutations in Twinkle also display mtDNA depletion in the liver.

Therapy

There is no specific therapy for MERRF or other mitochondrial encephalomyopathies associated with myoclonic epilepsy. Patients are empirically treated with '"cocktails' of vitamins and cofactors, including idebenone at high dosage (150 mg x3 daily) and L-carnitine (1 g daily) (Farge *et al*., 2007). Myoclonus can be controlled with clonazepam (0.5-1 mg three times a day) or zonisamide. As with all mitochondrial diseases, valproate has to be used with caution and always in combination with L-carnitine because of its well-documented inhibition of carnitine uptake (DiMauro *et al*., 2000). Hepatic impairment in AHS, SANDO or SCAE may be precipitated by valproate, leading to fulminant liver failure. These conditions, therefore, represent an absolute contra-indication for the use of valproate to control seizures (Tein *et al*., 1993). Lactic acidosis can be controlled by bicarbonate which, however, has only a transient buffering effect and may exacerbate the cerebral symptoms. Levetiracetam is the first choice of treatment for myoclonus in MERRF and lamotrigine may exert a neuroprotective effect (Lagrue *et al*., 2007). □

Disclosures

None of the authors have any conflict of interest to disclose.

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