Review article

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Autosomal recessive progressive myoclonus epilepsy due to impaired ceramide synthesis

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ABSTRACT – Autosomal recessive progressive myoclonus epilepsy due to impaired ceramide synthesis is an extremely rare condition, so far reported in a single family of Algerian origin presenting an unusual, severe form of progressive myoclonus epilepsy characterized by myoclonus, generalized tonic-clonic seizures and moderate to severe cognitive impairment, with probable autosomal recessive inheritance. Disease onset was between 6 and 16 years of age. Genetic study allowed to identify a homozygous non-synonymous mutation in *CERS1*, the gene encoding ceramide synthase 1, a transmembrane protein of the endoplasmic reticulum (ER), catalyzes the biosynthesis of C18-ceramides. The mutation decreased C18-ceramide levels. In addition, downregulation of CerS1 in neuroblastoma cell line showed activation of ER stress response and induction of proapoptotic pathways. This observation demonstrates that impairment of ceramide biosynthesis underlies neurodegeneration in humans.

Key words: progressive myoclonus epilepsies, epilepsy, ceramide synthase, neurodegeneration, ER stress response

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Despite great advances in molecular analytical techniques, aetiology remains undetermined in ~28 per cent of cases of progressive myoclonus epilepsies (PMEs) (Franceschetti *et al*., 2014; Minassian et *al*., 2016). We herein report clinical, neurophysiological, and genetic findings of an Algerian family with a new form of PME with autosomal recessive inheritance, in which four out of six siblings presented a peculiar clinical picture, characterized by epilepsy, action myoclonus, and moderate-to-severe cognitive impairment.

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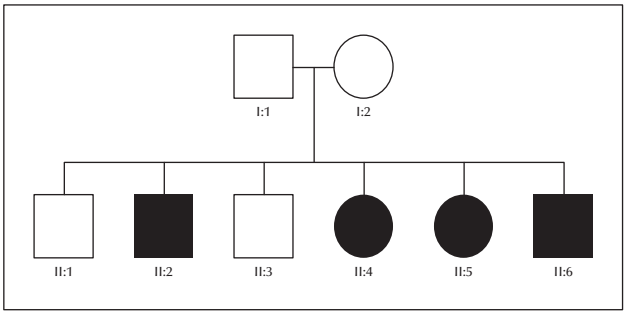

Figure 1. Family pedigree.

This family was first observed at the Hôpital Henri-Gastaut in Marseilles (France), in November 2007, by two of the authors (EF and PG), and was extensively reported by Ferlazzo *et al*. (2009). *Figure 1* shows the pedigree of the family with the four affected siblings (II:2, II:4, II:5, and II:6). The history and the clinical picture of this family appeared unusual and our first impression was that we were faced with a peculiar form of PME. In particular, we were intrigued by the presence of a severe myoclonic syndrome in most (II:2 was wheelchair-bound, and II:4 and II:5 walked with bilateral support) and the persistence of frequent generalized tonic-clonic seizures (GTCS) over the years, the dramatic reduction of action myoclonus following every GTCS, moderate-to-severe intellectual disability, and the unresponsiveness to common antimyoclonic agents (piracetam, levetiracetam and benzodiazepines).

Parents (I:1 and I:2) originated from two small but close Algerian villages and denied consanguinity. All affected siblings underwent neurological examination as well as neurophysiological evaluations, including video-EEG recordings at awakening. Sleep EEG was performed in Patients II:4 and II:6. To evaluate severity of action myoclonus, section 4 of the Unified Myoclonus Rating Scale (UMRS) was completed for all patients (normal score: 0). Serologic screening for coeliac disease was performed for Patients II.2, II:4, and II:6.

Patient II:2 underwent extensive studies including: EMG; a peripheral nerve conduction study; somatosensory, brainstem and visual evoked potentials (SSEP, BAEP, VEP); and brain MRI. He also underwent muscle biopsy to analyze the presence of abnormal mitochondria using routine methodology. This patient also underwent armpit skin biopsies in order to investigate Lafora bodies in sweat duct cells; ultrastructural studies of skin and rectal biopsy specimens were performed to search for typical neurolipofuscinosis inclusions. Biochemical assays for ceruloplasmin, hexosaminidases A and B, arylsulfatase, urine sialyloligosaccharides, leucocitary betagalactosidase, neuraminidase and betaglucocerebrosidase, as well as serum and CSF lactate and pyruvate levels, were performed. Molecular analysis for EPM1, EPM2A, EPM2B, MERRF, MELAS and *KCTD7*, were performed according to standard techniques.

Finally, homozygosity mapping was carried out to identify mutations in common using the Web-based tool, HomozygosityMapper. Based on the autosomal recessive pattern of inheritance, we carried out homozygosity mapping of the available family members using the Affymetrix Axiom Genome-Wide Human SNP Array. Genotypes of 567 k single nucleotide polymorphisms were analyzed by HomozygosityMapper software to identify runs of homozygous markers (ROHs) shared by the affected siblings.

Results

Patient II:2

This 29-year-old boy presented his first generalized tonic-clonic seizure (GTCS) at 7 years. Afterwards, GTCS presented at monthly intervals despite trials with different antiepileptic drugs. Onset of myoclonus probably occurred at the same time as the first seizure and worsened over the years; by the age of 20 years, the patient was wheelchair-bound. After each GTCS, myoclonus was reduced over 3-4 days. Lamotrigine worsened myoclonus, and levetiracetam and piracetam were ineffective. Clonazepam and phenobarbital produced a sedative effect. At consultation, the patient was being treated with VPA at 1,250 mg/day. Neurological examination revealed severe action myoclonus (UMRS score: 124). Neuropsychological tests revealed severe cognitive impairment. EEG showed background activity at 4-6 Hz with diffuse or multifocal spike- and polyspike-and-wave discharges (*figure 2*). Intermittent photic stimulation (IPS) was negative. EMG, ENG, BAEP and VEP were normal. SSEP showed giant potentials. Brain MRI showed mild atrophy of the cerebellum and brainstem. Abdominal ultrasound was normal. Fundus oculi and campimetry were normal. Muscle biopsy showed normal morphology and histochemistry without ragged red fibres. No Lafora bodies were observed in skin biopsies. No typical lipofuscin inclusions were present in skin or rectal biopsies. Biochemical assays were normal. Mutation analyses of the cystatin B gene, Lafora genes (laforin and malin), mtDNA for MERRF, MELAS or other mitochondrial diseases, were negative.

Patient II:4

This 24-year-old girl had GTCS that started at the age of 8 years and thereafter occurred monthly. Onset of myoclonus was at around 10 years and worsened

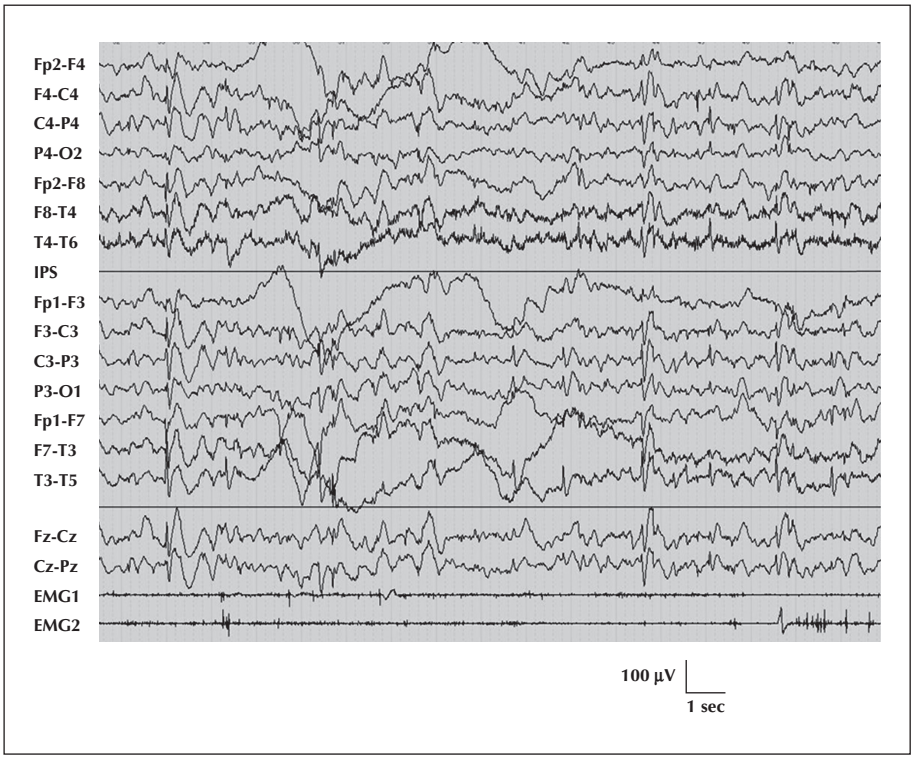

Figure 2. Patient II:2. EEG showing slow background activity in the theta-delta range and low- and mid-voltage spikes, spike- and polyspike-wave discharges, with fast spike components, both diffuse or multifocal, predominant over the centro-parieto-temporal regions. Note myoclonic jerks recorded over both deltoid muscles (EMG1: right deltoid; EMG2: left deltoid).

over the years. The girl started to attend a specialized institution for disabled persons by the age of 13 years. At consultation, she was being treated with VPA at 1,000 mg/day, which was the only drug she was ever given. Neurological examination revealed severe action myoclonus (UMRS score: 106), and she was unable to walk without support. Neuropsychological evaluation showed severe cognitive impairment. Following GTCS, a marked reduction in myoclonus severity was observed over 3 to 4 days. EEG showed slow background activity mixed with low-voltage fast activity, along with diffuse, irregular, spike- and polyspike-and-wave discharges. IPS from 10 to 20 Hz provoked a photoparoxysmal response. Sleep EEG showed spikes and polyspikes over the vertex and the centro-parietal regions during the first and second stages of non-REM sleep (*figures 3-5*).

Patient II:5

Myoclonus began at the age of 6 years. GTCS occurred at the age of 7 years and persisted weekly/monthly. At consultation, she was being treated with VPA at 1,250 mg/day. Neurological examination revealed action and stimulus-sensitive myoclonus (UMRS score: 99). A reduction in myoclonus severity was observed after each GTCS, which persisted for 3-4 days. Neuropsychological evaluation showed severe cognitive impairment. EEG showed slow background activity and multifocal fast spikes, polyspikes and polyspike-and-wave discharges (*figures 6, 7*). IPS was ineffective.

Patient II:6

This 19-year-old boy presented two GTCS at the age of 16 years (only a few months before consultation) and had never had specialized medical attention. He was described to be completely normal by his father. A very mild action myoclonus was observed according to neurological evaluation (UMRS score: 14). He attended normal school with low performance until age 13, after which he was moved to a specialized school. Neuropsychological evaluation showed moderate cognitive impairment, with visual perception impairment and reduction in spontaneous language. EEG showed slow background activity, as well as diffuse spike- and polyspike-and-wave discharges and multifocal spikes activated by non-REM sleep (*figure 8*). IPS between 6 and 20 Hz provoked a photoparoxysmal response. He was untreated at referral and was prescribed VPA up to 1,000 mg/day.

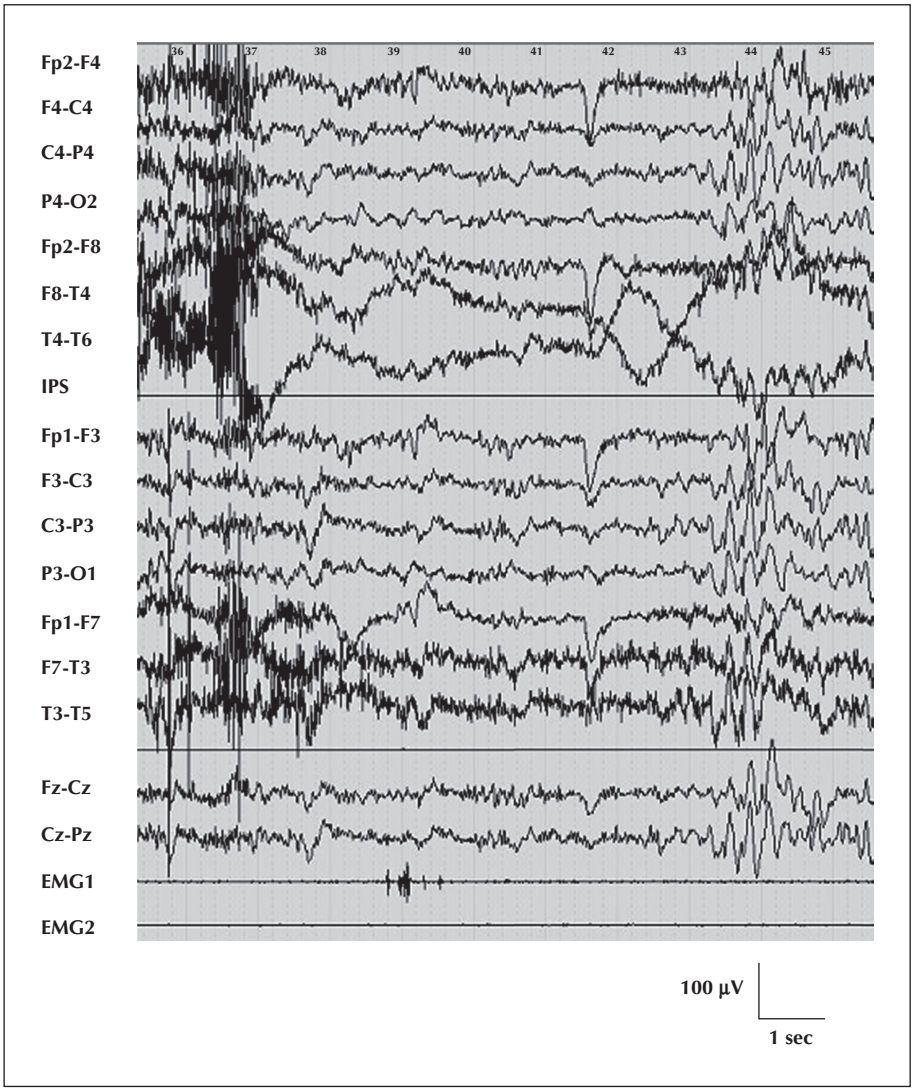

Figure 3. Patient II:4. EEG showing slow background activity at 6 Hz mixed with low-voltage fast activity, and diffuse, irregular, spike- and polyspike-and-wave discharges at 3-4 Hz. Note myoclonic jerks recorded over the right deltoid (EMG1: right deltoid; EMG2: left deltoid).

Homozygosity mapping

Homozygosity mapping revealed a unique unreported non-synonymous coding variant of the *CERS1* gene (c.549 C>G, NM_021267.3; p.H183Q, NP_067090) (Vanni *et al*., 2014). The mutation affects a critical histidine conserved in all human ceramide synthases and throughout evolution, which proved to be essential for enzymatic activity. Sanger sequencing confirmed the presence of the homozygous mutation in all affected individuals, whereas both parents and two unaffected siblings were heterozygous. By transfecting wild-type H183-tagged and mutant Q183-CerS1 V5-tagged vectors into HeLa cells, we found that the mutant and wild-type proteins were similarly expressed and that the mutant was correctly localized to the ER compartment (Vanni *et al*., 2014). However, this mutation was shown to severely impair CerS1 activity leading to down-regulation of CerS1 in a neuroblastoma cell line, triggering endoplasmic reticulum (ER) stress response and inducing proapoptotic pathways (Vanni *et al*., 2014).

Discussion

We herein describe clinical, neurophysiological and genetic features of a family with a novel neurodegenerative disorder featuring PME. The main characteristics distinguishing our family from Unverricht-Lundborg Disease (ULD), the most common form of PME, are the presence of relevant cognitive impairment and seizure persistency even in the late phase of the disease. However, EEG findings partially overlap with those of ULD

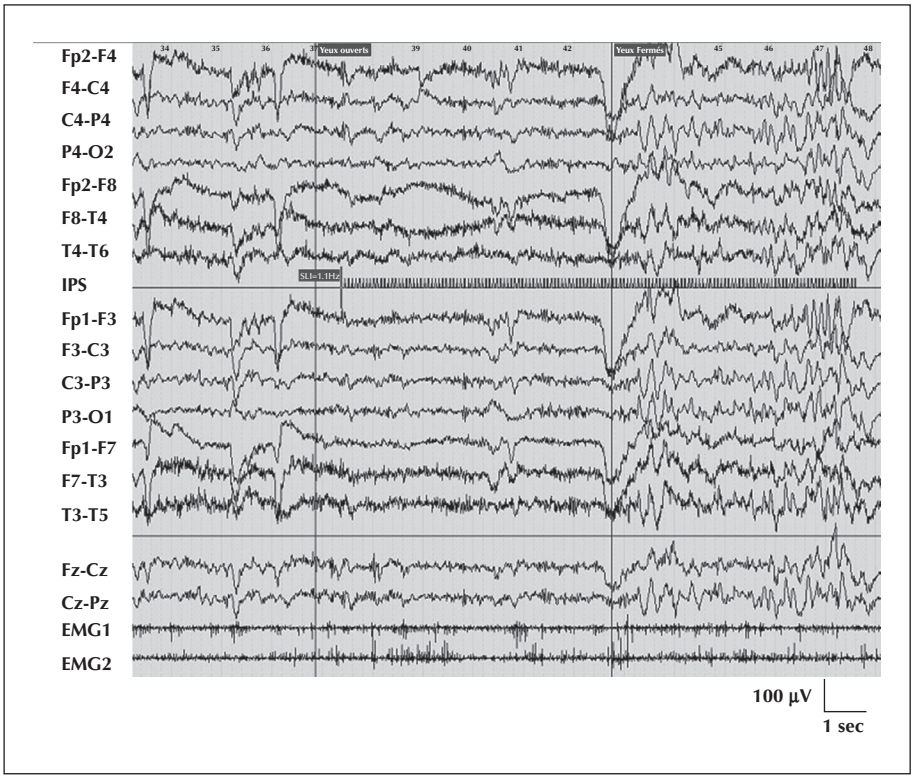

Figure 4. Patient II:4. The same EEG recording as in Fig. 3. Diffuse, irregular spike- and polyspike-and-wave discharges provoked by IPS at 14 Hz, associated with eye closure.

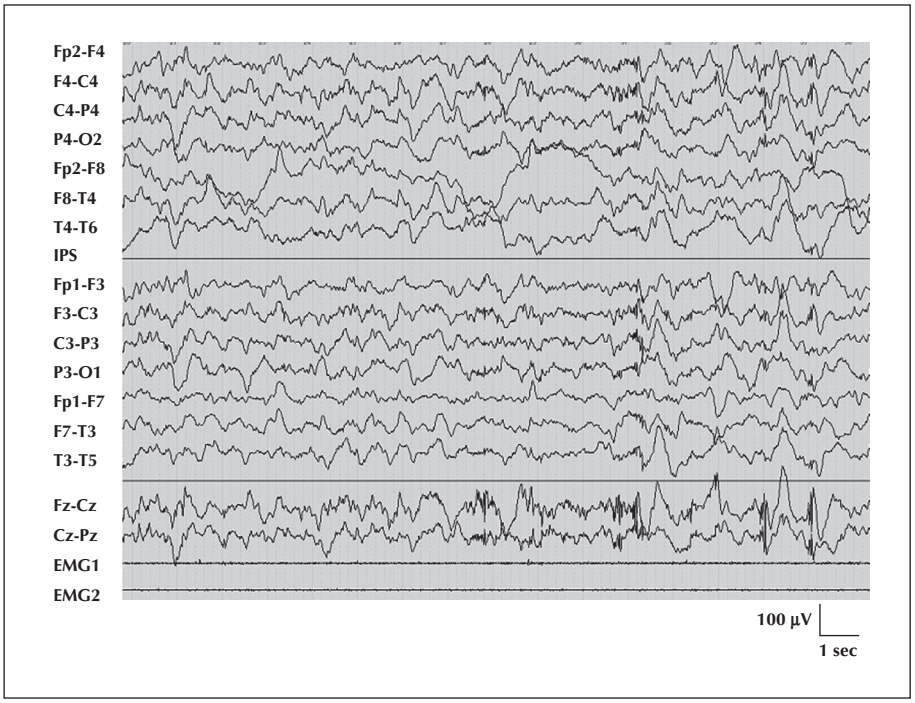

Figure 5. Patient II:4. During stages N1 and N2 of sleep, low- and mid-voltage spikes, polyspikes and polyspike-wave discharges, with fast spike components, were recorded over the vertex and both centro-parietal regions.

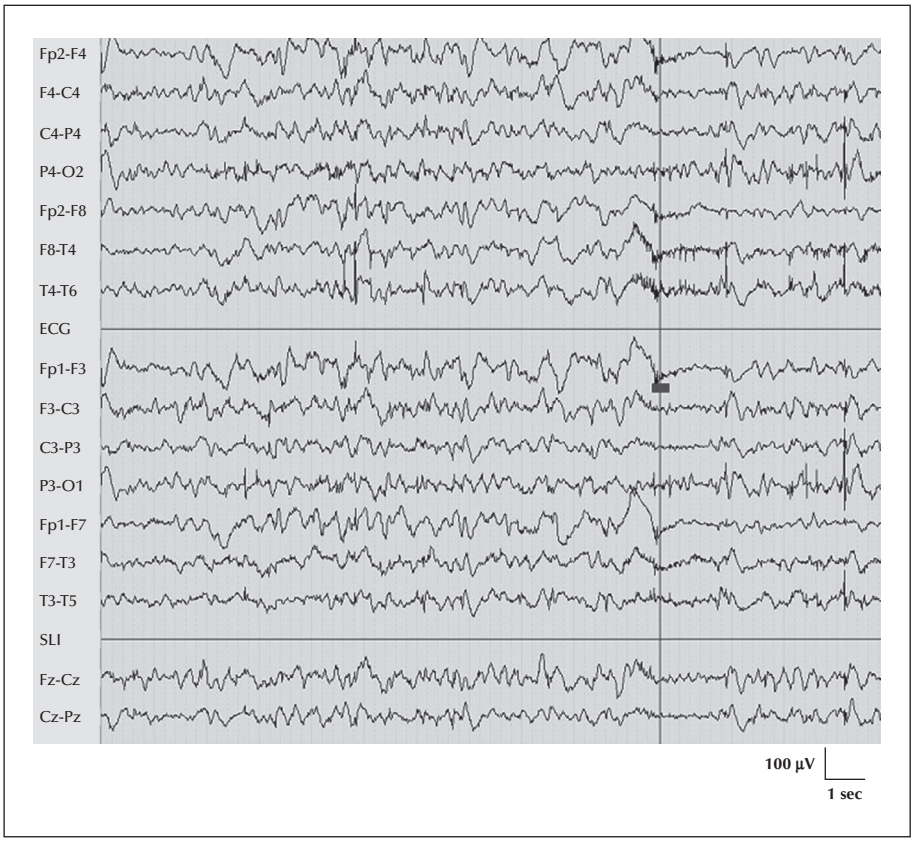

Figure 6. Patient II:5. EEG showing slow background activity in the theta-delta range mixed with low-voltage fast activity, along with multifocal fast spikes, polyspikes and polyspike-and-wave discharges.

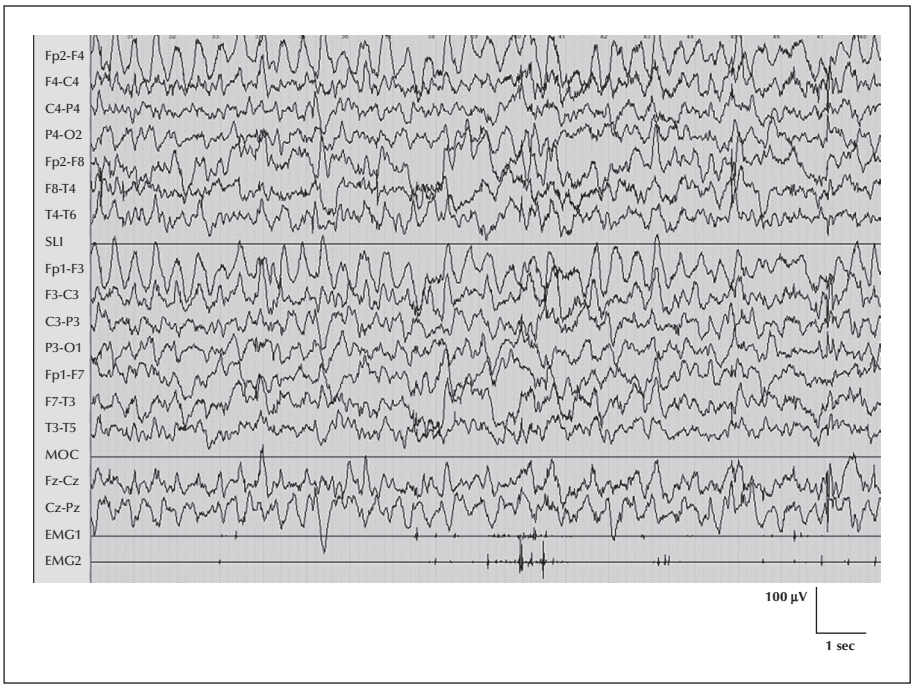

Figure 7. Patient II:5. EEG recorded after a GTCS showing continuous polymorphic delta activity over both frontal regions. Note myoclonic jerks recorded over both deltoid muscles (EMG1: right deltoid; EMG2: left deltoid).

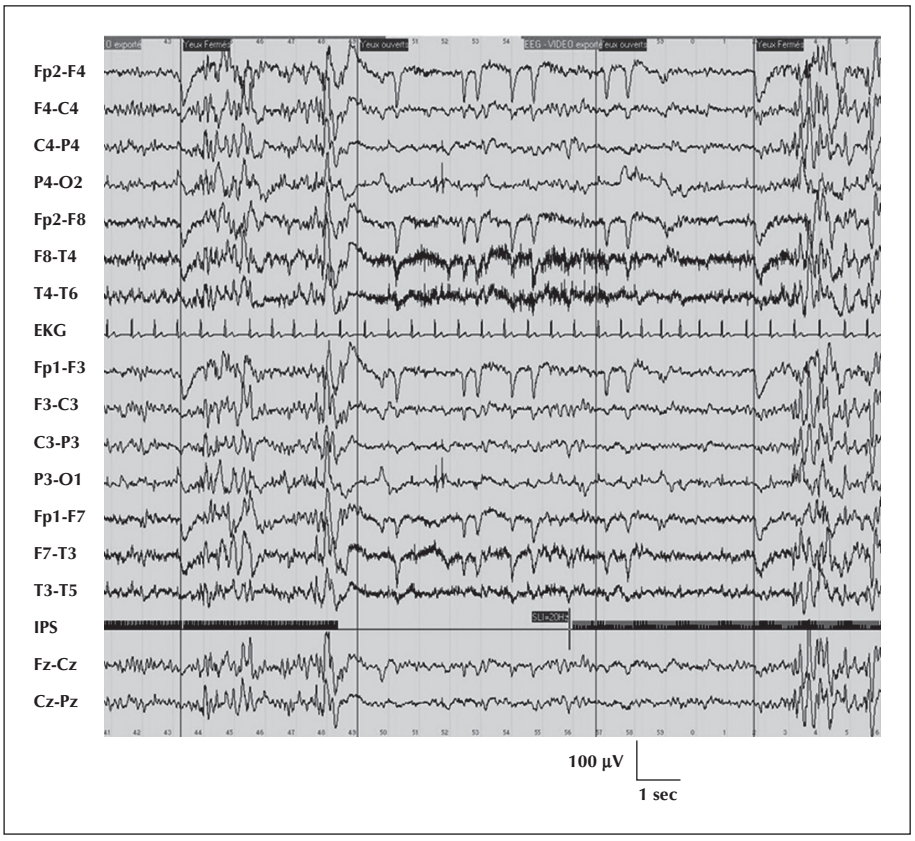

Figure 8. Patient II:6. Diffuse, irregular spike- and polyspike-and-wave discharges provoked by IPS at 16 and 20 Hz, associated with eye closure.

(Magaudda *et al*., 2006). Indeed, normal or mildly slow background activity, generalized spike- and polyspike-and-wave discharges, photoparoxysmal response at IPS between 10 and 25 Hz, bursts of low voltage, and spikes and polyspikes over the central and vertex regions during sleep are usually found in ULD. Moreover, in ULD, generalized epileptiform discharges, both spontaneous or induced by IPS, tend to decrease after 10 to 15 years of disease (Ferlazzo *et al*., 2007). For the family reported here, generalized epileptic discharges with a fast spike component, as well as a photoparoxysmal response, were observed in two out of four patients. Moreover, one affected sibling (Patient II:4) showed fast low-voltage spikes and polyspikes over the vertex and both centro-parietal regions during sleep. Unlike ULD, background activity was slow in all patients, and epileptic abnormalities persisted in all subjects for many years after disease onset.

A mutation in the *CERS1* gene, leading to the impairment of C18 ceramide biosynthesis, was shown to be the cause of this peculiar form of PME, providing evidence of the involvement of ceramide metabolism in the pathogenesis of these conditions. Moreover, we observed that impairment of CerS1 activity is associated with up-regulation of key ER stress markers, highlighting a possible link between ceramide metabolism, ER stress response, and neurodegeneration.

The significance of impaired ceramide synthesis in the development of PMEs was also shown by Mosbech *et al*. (2014) who described a novel 27-kb heterozygous deletion in the *CERS2* gene in a PME patient. This was a 30-year-old man presenting febrile and afebrile seizures at 2 years of age. Developmental delay and myoclonus became evident at the age of 13 years, and GTCS and myoclonia increased over the years despite administration of several antiepileptic drugs. At the last follow-up visit, he presented 4-8 GTCS per month and severe myoclonus. Compared to parental controls, levels of *CERS2* mRNA, protein, and activity were reduced by ~50 per cent in fibroblasts isolated from this proband, resulting in significantly reduced levels of ceramides and sphingomyelins containing the very long-chain fatty acids C24:0 and C26:0.

However, *CERS1* and *CERS2* mutations were not found in a recent whole-exome sequencing study of 84 undiagnosed PME patients with unknown aetiology (Muona *et al*., 2015) and, to date, have not been reported elsewhere (June 2015). Hence, we cannot

exclude that the mutation reported here represents a private mutation within this family.

Finally, the interest of these findings extends beyond the field of rare forms of PMEs because ceramide represents a central element in sphingolipid metabolism, since disturbed sphingolipid levels may be found in ceroid lipofuscinosis (CLN1-6 and CLN8), as well as other common neurodegenerative diseases, such as Parkinson disease, Alzheimer disease, amyotrophic lateral sclerosis, and prion diseases. □

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

The present manuscript is part of a *Epileptic Disorders*/Mariani Foundation supplement on Progressive Myoclonus Epilepsies, downloadable as a whole by visiting www.epilepticdisorders.com.

References

Ferlazzo E, Magaudda A, Striano P, Nguyen VH, Serra S, Genton P. Long-term evolution of EEG in Unverricht-Lundborg disease. *Epilepsy Res* 2007; 73: 219-27.

Ferlazzo E, Italiano D, An I, *et al*. Description of a family with a novel progressive myoclonus epilepsy and cognitive impairment. *Mov Disord* 2009; 24: 1016-22.

Franceschetti S, Michelucci R, Canafoglia L, *et al*. Progressive myoclonic epilepsies: definitive and still undetermined causes. *Neurology* 2014; 82: 405-11.

Magaudda A, Ferlazzo E, Nguyen VH, Genton P. Unverricht-Lundborg disease, a condition with self-limited progression: long-term follow-up of 20 patients. *Epilepsia* 2006; 5: 860-6.

Minassian BA, Striano P, Avanzini G. Progressive Myoclonus Epilepsies: State-of-the-Art. *Epileptic Disord* 2016; 18(Suppl. 2): S1-158.

Mosbech M-B, Olsen ASB, Neess D, *et al*. Reduced ceramide synthase 2 activity causes progressive myoclonic epilepsy. *Ann Clin Transl Neurol* 2014; 1: 88-98.

Muona M, Berkovic SF, Dibbens LM, *et al*. A recurrent *de novo* mutation in *KCNC1* causes progressive myoclonus epilepsy. *Nat Genet* 2015; 47: 39-46.

Vanni N, Fruscione F, Ferlazzo E, *et al*. Impairment of ceramide synthesis causes a novel progressive myoclonus epilepsy. *Ann Neurol* 2014; 76: 206-12.