

Atypical glycine encephalopathy in an extremely low birth weight infant: description of a new mutation and clinical and electroencephalographic analysis

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Received May 19, 2008; Accepted January 16, 2009

ABSTRACT – We present the clinical course and EEG evolution of an extreme low birth weight preterm neonate with an uncommon type of glycine encephalopathy. The patient presented with myoclonic jerks, apnea and encephalopathy three months after birth without satisfactory therapeutic response. During the first days of clinical symptoms the patient presented a paroxysmic burst-attenuation EEG pattern which progressively evolved into an established typical burst-suppression pattern within a few days. West syndrome occurred four weeks later and the patient died at seven months of extra-uterine life due to a serious respiratory infection with cardio-respiratory arrest. Genetic analysis showed a non-previously described mutation affecting a consensus splice site (IVS2-1G > C 3) in the AMT gene encoding the T protein of the glycine cleavage system.

Key words: non-ketotic hyperglycinemia, neonatal encephalopathy, neonatal myoclonic epilepsy, glycine cleavage system, impairment of glycine metabolism, glycinergic system development

Glycine encephalopathy (GE), also known as non-ketotic hyperglycemia (MIM 605899), is an autosomal recessive inborn error of glycine (Gly) metabolism which leads to severe learning difficulties and refractory epilepsy. In GE, Gly accumulates in all body fluids and tissues, especially in

the central nervous system (CNS), with particular proclivity for the cerebrospinal fluid (CSF), with a CSF/plasma Gly ratio (C:PGR) above 0.08 (normal < 0.04). The fundamental defect lies in the Gly cleavage system (GCS; EC2.1.2.10) (Kikuchi, 1973), a multi-enzyme complex located in the inner

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doi: 10.1684/epd.2009.0249

mitochondrial membrane of the liver, kidney, brain and placenta. The GCS consists of four proteins: pyridoxal-phosphate-dependent Gly decarboxylase (also called P-protein); amino-methyl-transferase (a tetrahydrofolate-dependent enzyme known as T-protein); a lipoic-acid-containing hydrogen carrier (identified as H-protein), and dihydrolipoamide dehydrogenase (or L-protein). Defects in the P, H, and T proteins have been linked to GE. Gly is a non-essential, neutral and genetically encoded amino acid. It can be either synthesized from serine or through other pathways, is actively involved in the genesis of proteins and can act as a neurotransmitter or neuromodulator. GE can be classified as either early onset, neonatal to infantile (3-12 months), or late onset; the neonatal category being the most frequently observed. In neonatal GE, after an asymptomatic period of between six hours and seven days (average of 48 hours), progressive lethargy is accompanied by hypotonia, burst-suppression EEG, myoclonic jerks, hiccups, and apnoea develop, often leading to coma and death (in up to 30% of patients). Those who survive generally suffer severe neurological damage and intractable seizures, although there are some uncommon forms of GE with better prognosis (Dinopoulos *et al.*, 2005).

We describe an unusual case of severe GE in an extremely low birth weight preterm (27 weeks) infant presenting with a long period without neurological symptoms and a new mutation in the AMT gene (that encodes the T-protein) of the GCS. EEG progression and clinical parameters were studied throughout the seven-month life-span of the patient. Brain immaturity due to the extreme preterm condition of the patient could justify, at least in part, this uncommon clinical course.

Case report

The patient was a 27-gestational-week, extremely low birth weight preterm, neonate girl (weight: 910 g [P25-50], height: 35 cm [P25-50] and head circumference: 23 cm [P10]) from non-consanguineal parents (father: 33 years old, mother: 36 years old with three previous gestations: two miscarriages and one stillbirth). The patient required both intubation (Apgar 5/9) and assisted ventilation at birth. The neonate did not present any dysmorphisms and brain ultrasound scans and neurological evaluation were normal. The patient was diagnosed with hyaline membrane disease, non-immune hemolytic jaundice and immature stage retinopathy and discharged after four weeks in the intensive care unit.

Three months after birth (corrected age of approximately 39 weeks of life; born at 27 intrauterine weeks and 12 extra-uterine weeks), the patient developed status epilepticus characterized by myoclonic seizures, apnoeas, lethargy, generalized hypotonia and absent reflexes. The

myoclonic seizures consisted of sudden, generalized contractions of axial, craneo-cervical and proximal-limb musculatures, which appeared either spontaneously or after diverse stimuli, occasionally as an isolated episode, but more frequently as prolonged clusters. The EEG changed from a burst-attenuation pattern, with interictal bursts of medium amplitude, to a burst-suppression pattern five days later (*figure 1*). During the following weeks, both the clinical picture and the burst suppression EEG pattern were refractory to the initial pharmacological therapy (phenobarbital, vigabatrin, midazolam, levetiracetam, corticoids and induced thiopental barbiturate coma). Cerebral MRI revealed delayed myelinization and polymicrogyria in an area of the right parietal lobe, but no further indication of neuronal migration abnormality (*figures 2, 3*). By the fourth month after birth, the EEG and encephalopathy features persisted, while the epileptic activity improved significantly. West syndrome was established showing typical spasms and a pattern of hirsarrhythmia in the EEG (*figure 4*). Treatments with either hydroaltesone in combination with topiramate, phenytoin or levetiracetam were unsuccessful.

At seven months of extra-uterine life the patient presented with a severe respiratory infection leading to fatal cardio-respiratory arrest.

The metabolic study showed plasma glycine at 589 $\mu\text{mol/L}$ (normal level: 104-254), CSF glycine at 167 $\mu\text{mol/L}$ (normal < 26) and a CSF/plasma glycine ratio of 0.28 (normal < 0,04, abnormal > 0,08). Treatment with sodium benzoate (500 mg/kg/day), carnitine (25 mg/kg/day) and dextrometorfan was titrated up to 35 mg/kg/day, along with a low protein diet (1.5 g/kg/day) in order to try to control seizure activity. Unfortunately, the CSF/plasma glycine ratio remained at 0.35 (276 in plasma and 95 in CSF).

A liver biopsy and a blood sample from the patient, as well as blood samples from both parents were obtained and a complete study for mutations in the four (P, H, T and L) proteins of the GCS complex was provided by the Pediatric Biochemistry Laboratory of Dr I. Maire, Hopital Debrouse (Lyon, France). Plasma branched-chain amino acid levels were normal, therefore a defect in L-protein was excluded. Glycine/CO₂ exchange enzyme analysis of the liver biopsy excluded mutations in either P or H proteins. Genetic analysis of the blood samples showed that the patient was a compound heterozygote for two alterations in the AMT gene: 1) IVS2-1G > C, an important mutation affecting a consensus splice site, and 2) a nucleotide substitution (c.-55C > T) in the 5' UTR non-coding region (that regulates translation or mRNA stability), the pathogenicity of which is unknown. The paternal exon 3 included the IVS2-1G > C mutation, while the mother possessed the respective nucleotide substitution in the 5'UTR region. To the best of our knowledge, the above-mentioned IVS2-1G > C mutation in the AMT gene has never been reported before.

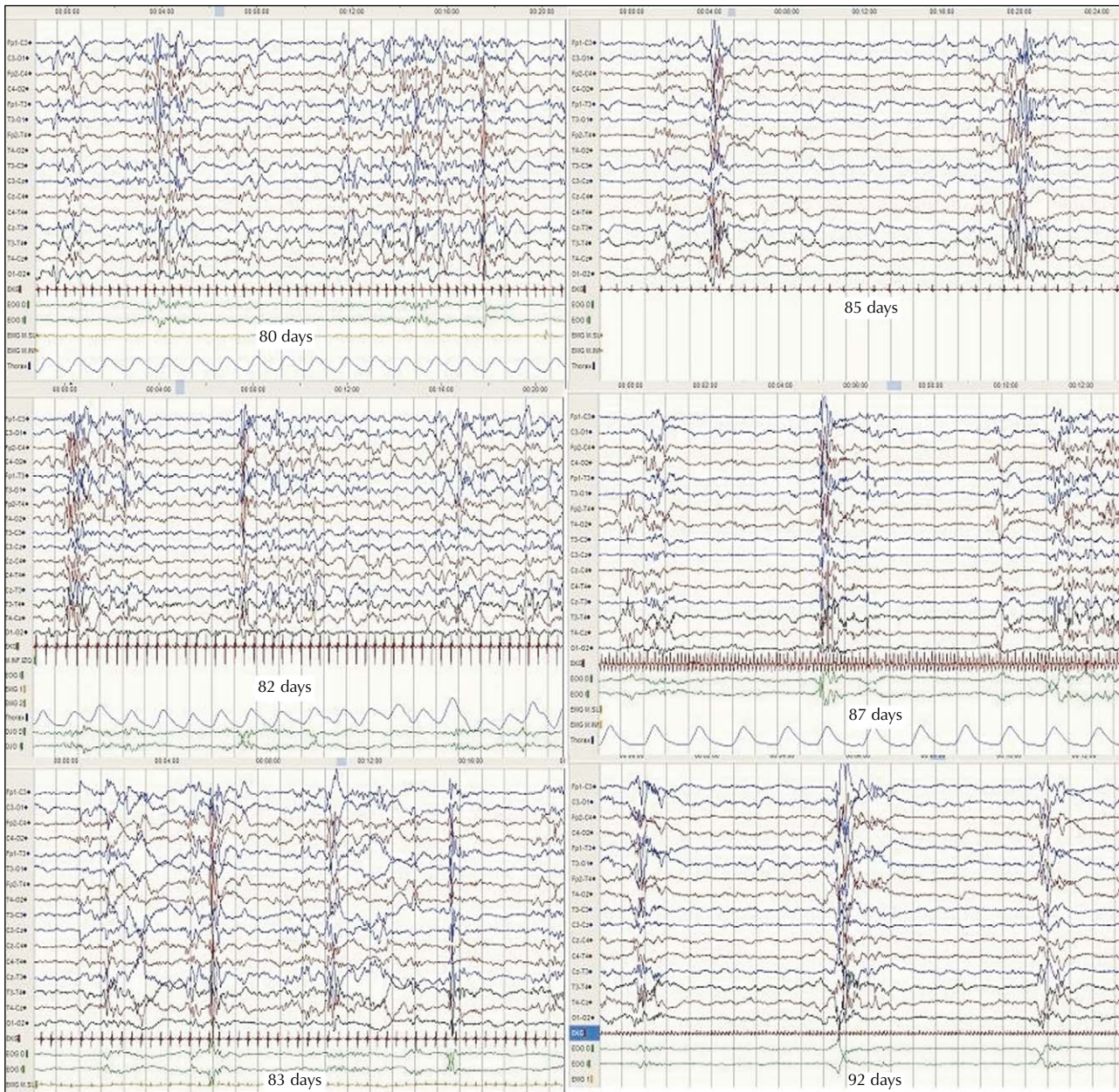


Figure 1. Initial EEG.

- Extra-uterine days 80, 82 and 83 (corrected age 39 weeks): irregular burst-attenuation pattern: spike and polyspike bursts (amplitude 120-150 μ V, duration: various seconds), only occasionally associated with clinical myoclonic jerks, appearing in a situation of generalized depression of the bioelectric cerebral activity (20-30 μ V average amplitude, with a theta / delta frequency). No sleep phase nor graphic physiological elements typical of this age could be identified. Medications administered: day 80: no medication; days 82 and 83: benzoate, carnitine, dextrometorfan.
- Extra-uterine day 85 (corrected age 39 weeks): higher amplitude burst-suppression pattern; shorter, very low amplitude interburst- intervals and well-defined rhythmicity. Medications administered: benzoate, carnitine, dextrometorfan, phenoine, corticoids, vigabatrine and phenobarbital.
- Extra-uterine day 87 (corrected age 40 weeks): well-defined burst-suppression pattern: severe bioelectric cerebral activity depression (amplitude < 10 μ V) and periodic spike and polyspike bursts (> 200 μ V), generally associated with clinical myoclonic jerks. Medications administered: benzoate, carnitine, dextrometorfan, phenoine, corticoids, vigabatrine and phenobarbital.
- Extra-uterine day 92 (corrected age 41 weeks): well-established burst-suppression pattern. Medications administered: benzoate, carnitine, dextrometorfan, phenoine, corticoids, vigabatrine and phenobarbital.

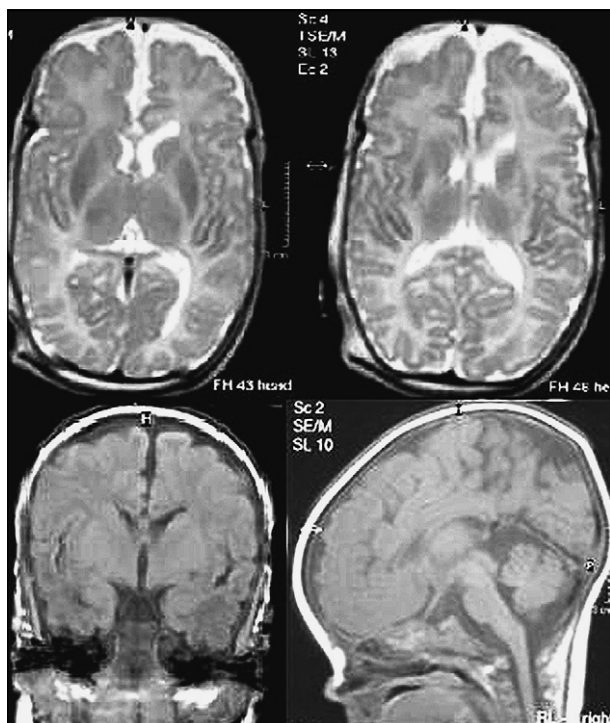


Figure 2. Selected enlarged MRIs showing the detected polymicrogyria in an area of the right parietal lobe. No further signs of neuronal migration disorder could be identified.

Discussion

The most common form of GE is symptomatic during the first days of life and shows an increased CSF-to-plasma glycine ratio. These patients generally develop progressive encephalopathy and often die. Survivors suffer profound mental retardation and intractable seizures. The atypical forms include infantile mild GE and late-onset GE with a rapid and severe course. In our case, even after adjusting the age to take into account the premature birth of the patient, there was still about a three-week delay in the onset of symptoms when compared with classical neonatal GE. Due to the existence of the apparent asymptomatic period together with the severity of the course, this case can be considered as a peculiar form of atypical GE. Extensive literature reviews have been conducted across ages to characterize the different forms of atypical GE (Dinopoulos *et al.*, 2005, Hamosh, 2001). However, they do not report any cases of extremely low birth weight preterm infants. Convulsions in the preterm neonate tend to be poorly organized due to low levels of both axonal myelination and neuronal synaptogenesis. Thus, an especially immature brain, as confirmed by our MRI study, could have been responsible, at least in part, for the long asymptomatic period before the onset of convulsions in our patient.

The role of Gly as a neurotransmitter is complex. In the brain cortex, increased Gly levels are believed to be the cause of intractable seizures, mental retardation and brain injury in GE via excitatory modulation of NMDA type glutamate receptors (Johnson and Ascher, 1987; Procter *et al.*, 1991). An enhanced Gly fast inhibitory role acting on the Gly receptor (GlyR) in the brainstem and spinal cord (Curtis *et al.*, 1968) has been thought to be responsible for lethargy, hypotonia, pyramidal signs, apnoeas, hiccups and abnormalities in breathing patterns in GE. Although the GlyR is known in the adult to be an inhibitory receptor, because the chloride equilibrium potential is usually close to, or more negative, than the cell resting potential, the intracellular chloride concentration is substantially higher in embryonic as well as in neonatal neurons (Staley, 2006), leading to GlyR activation causing a strong, supra-threshold depolarization. These large, glycine-induced depolarizations induce a calcium influx into the neuron that is necessary for the development of numerous specializations, including glycinergic synapses. The switch to the mature neuron phenotype is mediated by the expression of a potassium/chloride co-transporter, KCC2, which lowers the internal chloride concentration (Rivera *et al.*, 2003; De Cabo-de la Vega *et al.*, 2006), thereby shifting the actions of GlyRs from excitatory to inhibitory. Thus, similar to GABA, Gly can have chloride-mediated excitatory properties during development. In neonatal GE, the high levels of Gly could have an additional deleterious excitatory effect acting on already existing cortical GlyRs (Malosio *et al.*, 1991). Due to the characteristic caudal-rostral pattern of maturation of KCC2 neuronal chloride transport, GABAergic anticonvulsants seem to be more effective in controlling motor manifestations of neonatal seizures, while having no significant impact on EEG activity (Dzhalal *et al.*, 2005). In our case, however, all therapeutic options employed were unsuccessful. In more than 80% of cases, the molecular defect has been identified in the P protein (MIM 238300), yet defects in the T (MIM 238310) and H (MIM 238330) proteins have also been reported (Hamosh and Johnston 2001; Kure *et al.*, 2006). Our patient was a compound heterozygote showing two different mutations in the exon 3 of the AMT gene: one of which was not previously described (IVS2-1G > C, affecting a consensus splice site) and the other of unknown pathological consequence (a nucleotide substitution (c.-55C > T) in the 5' UTR non-coding region). The clinical consequences of AMT mutations have not yet been studied in depth, perhaps because mutations in this gene are relatively uncommon (Hamosh and Johnston, 2001; Kure *et al.*, 2006). The combination of AMT mutations found in our case seems to have led to a very severe neurological syndrome, more similar to earlier onset neonatal cases. This finding is consistent with the only report found in the literature where the effect of AMT mutations was clinically analysed (Nanao *et al.*, 1994). It seems possible that this particular

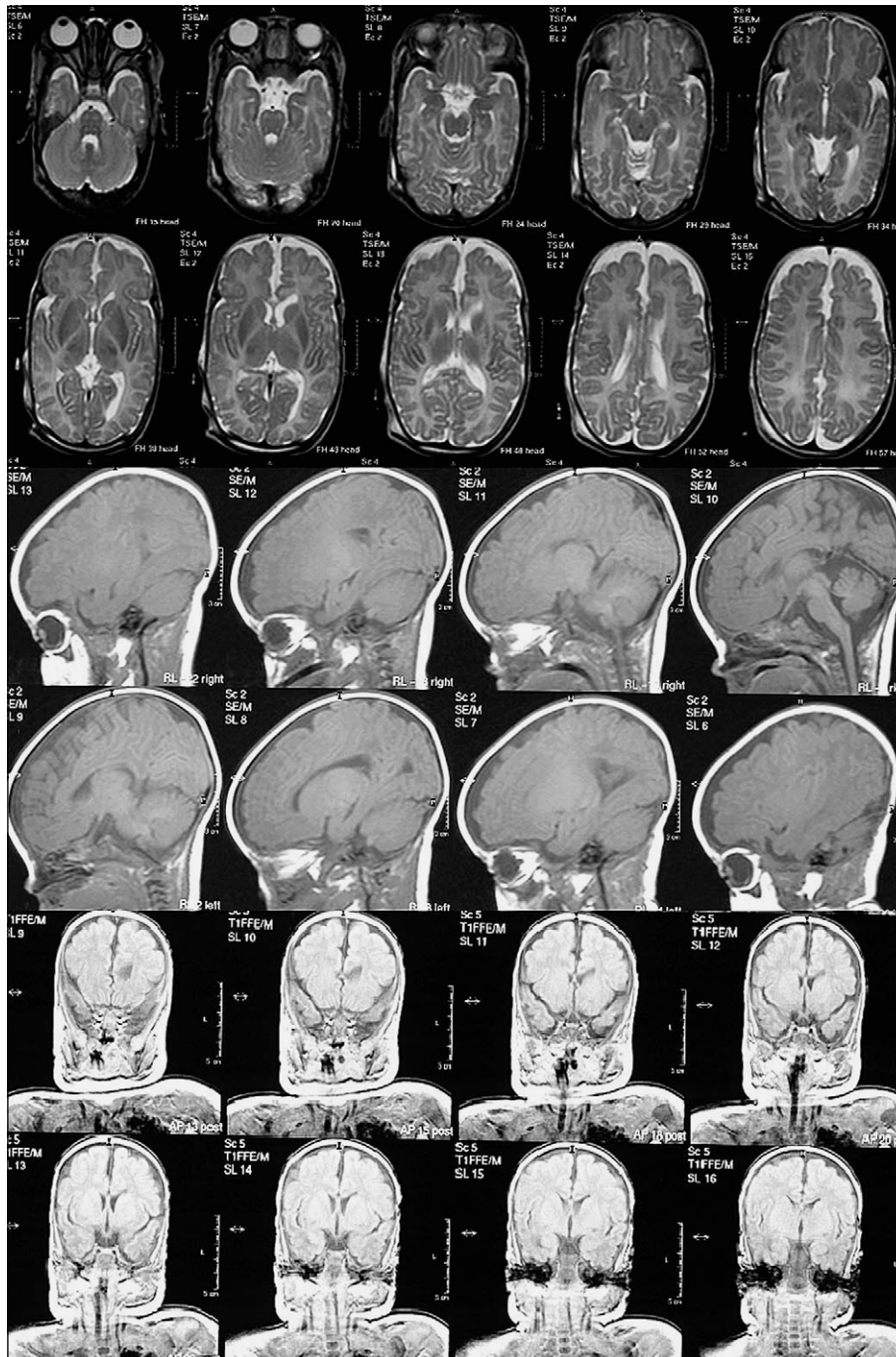


Figure 3. Complete series of MRIs obtained from the patient's brain. Images were consistent with a delay in the myelination process.

combination allowed the GCS to retain enough residual enzymatic activity for the nervous system to display a transient compensation during the early neonatal period, failing abruptly a few months later, possibly due to accumulative metabolic stress over the GCS.

In summary, this is a particularly atypical case of severe neonatal GE in an extremely low birth weight preterm infant after a neurologically asymptomatic early neonatal period, presenting a previously unknown mutation in the AMT protein. □

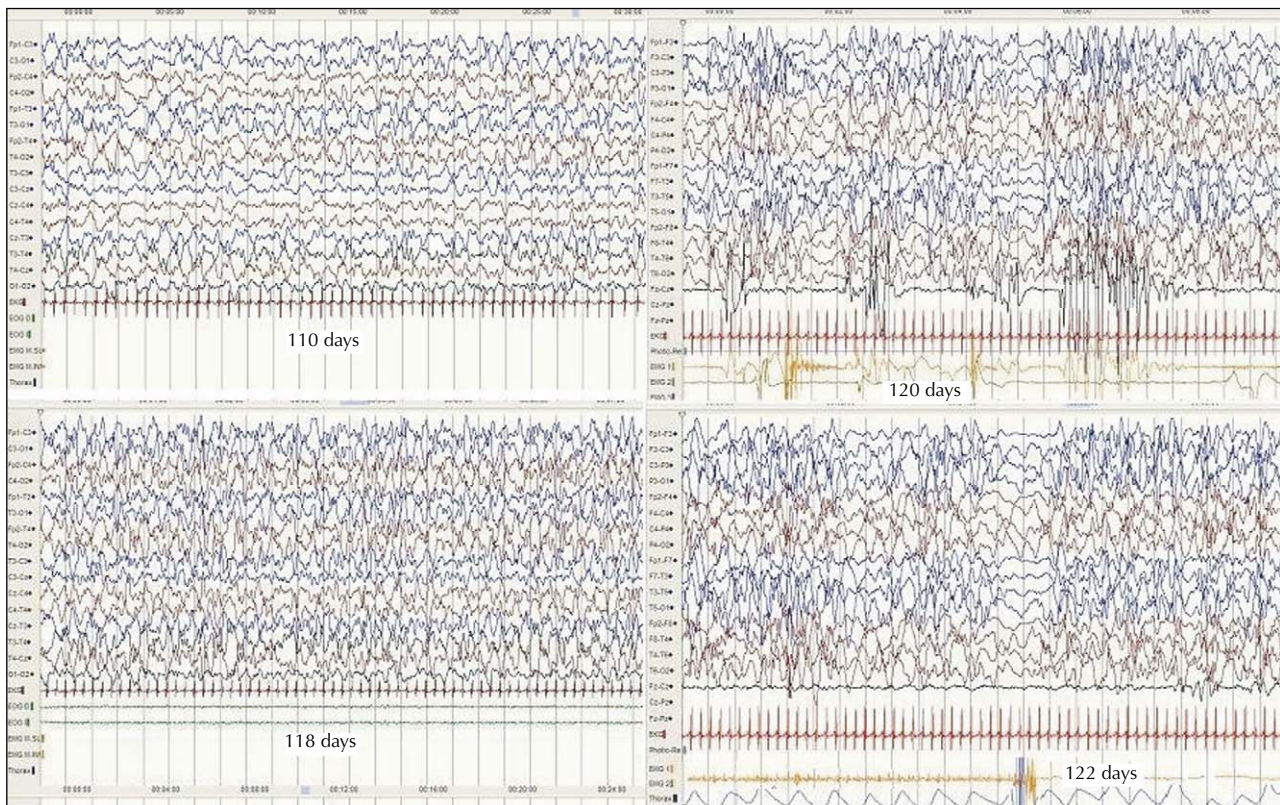


Figure 4. Final EEG. Approximately 4th extra-uterine month: irregular, disorganized bioelectric cerebral activity at theta/delta frequency, 80-100 μV amplitude. No recognizable awake/sleep phases and no responses to stimuli could be detected. In subsequent days, amplitude increased and disorganization became very significant: persistent erratic epileptiform elements occurred and critical episodes characterized by sudden, brief, generalized de-synchronicity of brain activity appeared associated with flexor spasms.

Acknowledgments.

Carlos de Cabo-de la Vega was a recipient of a FIS-FEDER grant (ref. 01/3018). Carlos de Cabo-de la Vega and José Manuel Pardal-Fernández were recipients of a JCCM grant (ref. GC 05011). We thank Alexandra L. Salewski, ND, MSc, for English revision of the manuscript.

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