Clinical commentary

RFT1-congenital disorder of glycosylation (CDG) syndrome: a cause of early-onset severe epilepsy

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ABSTRACT – RFT1-congenital disorder of glycosylation (CDG) syndrome, a recessive N-glycosylation disorder caused by mutation in the *RFT1* gene, is a very rare subtype of CDG syndrome associated with deafness, developmental delay, and non-specific epilepsy. The aim of this report is to describe the electroclinical presentation of epilepsy associated with this condition. [*Published with video sequences online*]

Key words: RFT1, congenital disorder of glycosylation (CDG) syndrome, N-glycosylation, epilepsy, EEG, early onset epileptic encephalopathy (EOEE)

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RFT1-congenital disorder of glycosylation (CDG) syndrome, a recessive N-glycosylation disorder caused by mutation in the *RFT1* gene, is a very rare subtype of CDG syndrome (with eight cases reported so far), associated with deafness, developmental delay, and non-specific epilepsy (Clayton and Grunewald, 2009; Jaeken *et al.*, 2009; Vleugels *et al.*, 2009; Ondruskova 2012). The aim of this report is to describe the early electroclinical presentation of epilepsy associated with this condition based on two new cases of RFT1-CDG syndrome.

Cases studies

Patient 1

Patient 1 was a baby girl born after a 37-week pregnancy marked by polyhydramnios that prompted foetal MRI (which was normal at 32 weeks of gestation) and amniocentesis for both fluid removal and analysis (revealing normal karyotype). She was the second child of healthy, unrelated parents of Moroccan origin. Her birth weight was 3.185 kg (P50-90), length 49 cm (P50-90), head circumference 35.3 cm (P90),
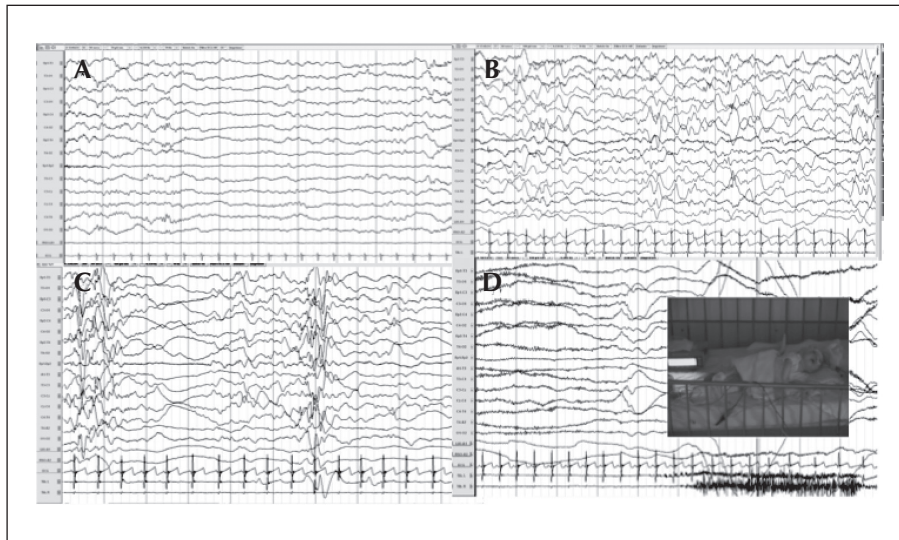
Figure 1. Video-EEG of Patient 1 at birth: (A) a continuous and labile EEG pattern without spikes at two months; (B) EEG during wakefulness showing a diffuse excess of monomorphic delta activity, alternating with multifocal sharp waves, spike-waves, and polyspike waves; (C) suppression-burst pattern in slow-sleep; and (D) paroxysmal fast activity followed by sudden tonic contractions of the arms and trunk with extension of both arms and legs, and crying, lasting 1 to 30 seconds, corresponding to tonic seizures.

and Apgar score 8/9/9. At birth, the patient was irritable and showed weak suction with feeding difficulties, requiring a nasogastric tube. She presented a hypomimic face, convergent strabismus, axial and peripheral hypertonia with brisk myotatic reflexes, and adducted thumbs. She also presented a subcutaneous limb oedema that spontaneously resolved. In the first days of life, brain MRI, abdominal and cardiac sonographic examinations, and ocular fundus were unremarkable, but brainstem evoked response audiometry (BERA) showed evidence of severe bilateral hearing loss (no peak V at 80 dB) with normal tympanometry. Metabolic screening revealed a capillary zone electrophoresis type 1 pattern of serum sialotransferrin. Because the CDG-Ix pattern associated with deafness is very suggestive of a RFT1-CDG syndrome (Jaeken, 2010), direct sequencing of the *RFT1* cDNA was performed, showing homozygosity for the missense mutation c.454A>G (p.K152E) (Jaeken *et al.*, 2009 ; Vleugels *et al.*, 2009) and heterozygosity for this mutation in both parents. EEG performed at birth was considered to be normal, showing a labile and continuous pattern in awake and agitated sleep states without spikes (*figure 1A*). At 2 months of age, the EEG background had worsened with a suppression-burst pattern during slow sleep (*figure 1B, C*) and daily numerous tonic seizures (*figure 1, D and video sequence 1*). Seizures were resistant to valproate but were transiently controlled by vigabatrin. Evolution was marked by virtually no neurological development since birth, severe feeding difficulties, and failure to thrive despite nasogastric feeding

(weight gain of only 1 kg in one year). The patient died at the age of 1 year from apparently sudden cardiorespiratory arrest.

Patient 2

Patient 2 was the second son of healthy, unrelated parents of Italian origin, with no relevant family history. He was born after a 39-week uneventful pregnancy. Birth weight was 3.5 kg (P50-90), length 51.5 cm (P50-90), head circumference 34.5 cm (P50), and Apgar score 5/7/7. At birth, he presented cyanosis and transitory respiratory distress requiring ventilation support by nasal CPAP for six hours. Neurological examination at birth was abnormal, characterized by irritability, wailing, erratic eye movements, convergent strabismus, axial and peripheral hypertonia, brisk tendon reflexes, and retraction of the knees and elbows. On Day 2, he presented recurrent tonic-clonic seizures associated with apnoea, bradycardia, and desaturation. He was intubated for ventilation and seizures were refractory to various antiepileptic drugs (phenobarbital, pyridoxine, phenytoin, levetiracetam, midazolam as continuous infusion, and vigabatrin). EEG recorded on Day 2 was considered to be normal, showing a labile and continuous pattern in awake and agitated sleep states without spikes nor seizures recorded at that time (data not shown). Brain MRI at 4 days of age demonstrated white matter non-specific lesions in the frontal periventricular white matter (*figure 2*). Metabolic investigations were unremarkable, including capillary electrophoresis of serum sialotransferrin that showed a normal
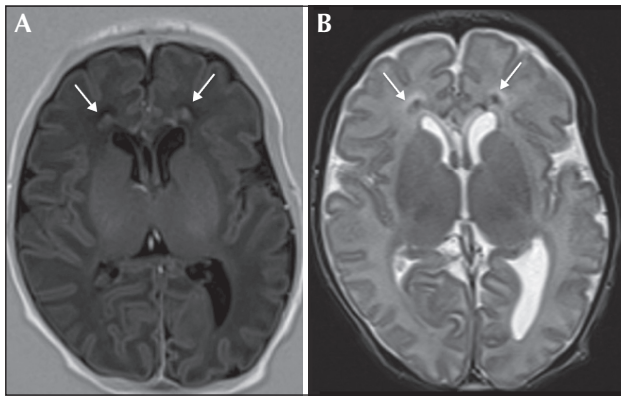
Figure 2. Brain MRI performed at Day 4 of life (Patient 1) showing non-specific white matter lesions in the frontal periventricular region with hypersignal in T1 and hyposignal in T2 weighted images (blue arrow).

pattern. At 3 months of age, he was transferred to ULB-Erasme Hospital. EEG background had worsened with a suppression-burst pattern during slow sleep and the child presented daily partial seizures (*figure 3A, B and C and video sequence 2*). BERA showed evidence of severe bilateral hearing loss (no peak V at 80 dB) with normal tympanometry. Levetiracetam was re-introduced progressively up to 60 mg/kg/d with transient efficacy during two weeks. Then, seizures recurred and a ketogenic diet associated with clonazepam was started. EEG at that time showed several focal seizures followed by asymmetric spasms in clusters during the awake state (*figure 3D and video sequence 3*). Two months later, clonazepam was stopped and topiramate was added up to 20 mg/kg/d with a dramatic decrease in focal seizures (from 20 seizures a day to one seizure a day). Then, vigabatrin add-on, up to 150 mg/kg/d, led to transient resolution of the clusters of spasms. Because of the presence of early-onset severe epilepsy associated with deafness, capillary zone electrophoresis of serum sialotransferrin was repeated and displayed, for the second time, a normal profile. Gene panel testing using DNA amplified by multiplex PCR (Ampliseq®) and next-generation sequencing on Ion PGM® showed that this patient was compound heterozygous for two mutations in the *RFT1* gene: c.1325G>A (p.R442Q) and c.110G>T (p.R37L). The first mutation (c.1325G>A (p.R442Q)) was already described in two adult siblings with RFT1-CDG (Ondruskova *et al.*, 2012). The second is a novel missense mutation, predicted to be pathogenic by prediction software (Align GVGD, PolyPhen2, SIFT, MutationTaster). This boy had virtually no neurological development since birth, with severe tetraparesis and swallowing difficulties requiring nasogastric tube feeding. Seizures unfortunately recurred despite ketogenic diet, levetiracetam, topiramate and vigabatrin, and EEG stabilized over time, showing a suppression burst pattern in slow sleep. The child died at 2 years of age because of chronic respiratory insufficiency.
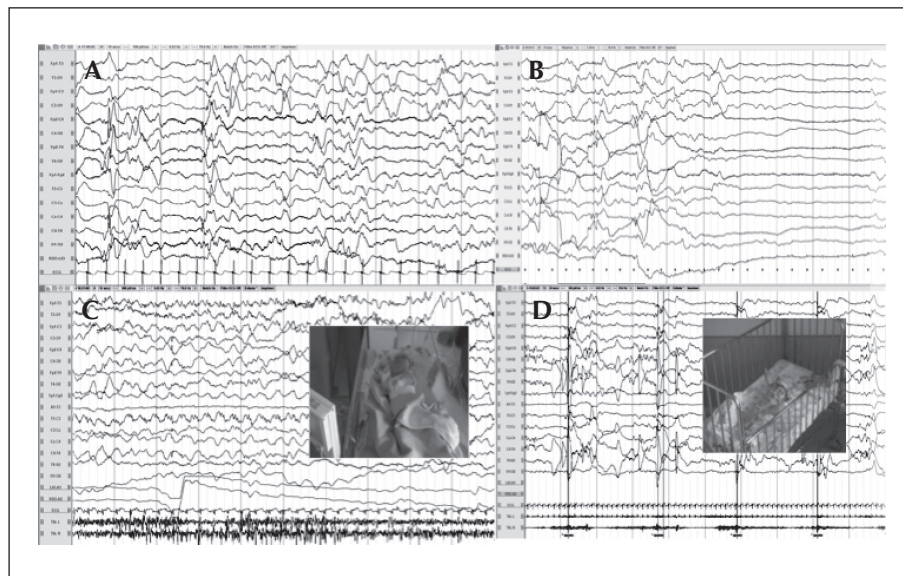

Figure 3. EEG of Patient 2 at three months: (A) a continuous EEG pattern in awake state with monomorphic hypovolted theta and delta activity mixed with high-voltage delta waves, spikes and waves, and polyspikes and waves; (B) suppression-burst in slow sleep; (C) multiple partial seizures with tonic posturing and infrequent secondary generalization; followed by (D) clusters of asymmetric spasms (with extension of the right arm and left leg).

Discussion

These two cases illustrate that RFT1-CDG syndrome is a previously unreported cause of early-onset severe epilepsy.

Genetic glycosylation disorders are multisystemic diseases causing a remarkably wide range of phenotypes involving the brain for most of them (Jaeken, 2010). Mutation in the *RFT1* gene leads to the absence of the Rft1 protein, which is located in the endoplasmic reticulum (ER) membrane. Rft1 protein facilitates the translocation of Man5GlcNAc2-PP-dolichol to the ER lumen in a bidirectional and ATP-independent manner (Helenius *et al.*, 2002). Its absence leads to the accumulation of Man5GlcNAc2-PP-dolichol in the cytosol and impaired glycosylation. Glycosylation, which employs at least 2% of the translated genome to generate thousands of molecular structures, represents a key regulatory modification in the brain, contributing to cell signalling, cell-cell interaction, and cell migration (Grünewald *et al.*, 1999; Rexach *et al.*, 2008). Epilepsy in CDG syndrome may be caused by neuronal migration errors but may also result from a disrupted balance of excitatory (mainly glutaminergic) and inhibitory neuronal activity (Jaeken, 2010). Indeed, tightly regulated activation/deactivation of voltage-gated ion channels in the cell membrane of excitable cells is required for neuron function and most channel proteins contain sialiated N-glycans (Baycin-Hizal *et al.*, 2014).

The severity of epilepsy in CDG syndromes ranges from severe refractory epilepsy to easily treated seizures in some individuals (Jaeken, 2010). Epilepsy was present in the eight RFT1-CDG reported so far, from early severe refractory epilepsy (Clayton and Grunewald, 2009; Jaeken *et al.*, 2009; Vleugels *et al.*, 2009) to easily controlled epilepsy with few seizures starting in childhood (Jaeken *et al.*, 2009; Ondruskova *et al.*, 2012). However, the electroclinical presentation of the severe early-onset cases was not characterised in the previously published cases.

The ILAE recognizes two early-onset epileptic encephalopathies (EOEEs): early infantile epileptic encephalopathy (EIEE) (also called Ohtahara syndrome) and early myoclonic encephalopathy (EME) (Berg *et al.*, 2010 ; Beal *et al.*, 2012). The two RFT1-CDG patients reported here present features suggestive of both syndromes, underpinning that they overlap, as previously pointed out by other authors (Djukic *et al.*, 2006; Ohtahara and Yamatogi, 2006; Beal *et al.*, 2012). Characteristics supporting EME are the suppression-burst pattern that was not evident at birth, but when present, was essentially recognisable in slow sleep, and the relative improvement in seizure control over time (Djukic *et al.*, 2006; Ohtahara and Yamatogi, 2006). Nevertheless, none of our patients had myoclonia, and tonic spasms were the first seizure type in Patient 1,

which are more suggestive of EIEE (Beal *et al.*, 2012). Patient 2 presented a variety of different types of seizures (tonic seizures in the beginning and partial seizures followed by clusters of spasms at 3 months of age), which is more suggestive of EME (Beal *et al.*, 2012). The term "epileptic encephalopathy" refers to conditions where the epileptic abnormalities themselves are believed to contribute to the progressive disturbance of cerebral function, such that early effective intervention may improve developmental outcome (Berg *et al.*, 2010). Whether the two patients reported here, and more generally all patients with EOEE of metabolic origin, fit with this definition of epileptic encephalopathy is highly debatable because the neurological prognosis of these patients seems predominantly caused by the pre-existing brain dysfunction (Cross and Guerrini, 2013). Therefore, the term "early-onset severe epilepsies with suppression burst" seems a more appropriate term.

Interestingly, Patient 2 had repeatedly (at birth, six months and two years) a normal serum transferrin IEF pattern while genetic analysis demonstrated mutation in the *RFT1* gene. It is well known that a normal

sialotransferrin pattern does not exclude CDG syndrome (Jaeken, 2010). For early-onset severe epilepsy with suppression-burst and deafness, this should prompt a search for *RFT1* gene mutation, even if serum sialotransferrin is normal.

In conclusion, these two cases suggest that *RFT1* gene mutation should be considered in the differential diagnosis of early-onset severe epilepsy, even in the presence of normal serum transferrin isoelectrofocusing. Other clues for diagnosis include congenital severe hypotonia with limb retractions and deafness. □

Disclosures.

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

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