Clinical commentary

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Practical clues for diagnosing WWOX encephalopathy

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ABSTRACT – The WW domain-containing oxidoreductase gene is implicated in autosomal recessive disorders of the central nervous system, expressed either as spinocerebellar ataxia or as a severe form with early-infantile epileptic encephalopathy. Here, we describe the electroclinical evolution of these disorders, adding new diagnostic clues based on a case study. The patient, a boy with early-onset epilepsy, presented with profound global developmental delay, persistent hypsarrhythmia, and epileptic spasms, associated with progressive cerebral atrophy without microcephaly. Metabolic disease was excluded. Whole-exome sequencing showed mutations in the WW domain-containing oxidoreductase gene. Our findings extend the phenotypic traits of this aggressive epileptic encephalopathy, with persistent epileptic spasms and hypsarhythmia as a part of the electroclinical phenotype, demonstrating that microcephaly is not mandatory for diagnosis, even when associated with progressive cerebral atrophy. These mutations might be more frequent than expected among early-onset epileptic encephalopathies. We present practical clues for the diagnosis of WWOX encephalopathy in order to avoid unnecessary investigations and ensure appropriate genetic counselling for the families.

Key words: WWOX, epileptic encephalopathy, progressive cerebral atrophy, persistent hypsarrhythmia, rare epilepsies

The WW domain-containing oxidoreductase (WWOX) gene was initially described to be altered in cancer cell lines and tumours (Bignell *et al*., 2010; Zack *et al*., 2013), and was cloned in 2000 (Bednarek *et al*., 2000). It is located on chromosome 16q23.1-q23.2, crossing the second most common fragile site of the human genome (*FRA16D*) (Ried *et al*., 2000).

The role of *WWOX* mutation in brain development was first described in rats (Suzuki *et al*., 2009). Following this, the first human carrying a homozygous germline loss-of-function *WWOX* mutation was identified in a large consanguineous family, and the patient was diagnosed with a new form of childhood-onset autosomal recessive cerebellar ataxia and epilepsy, previously referred to as "autosomal recessive spinocerebellar ataxia-12 (SCAR12)" under the OMIM (Gribaa *et al*., 2007; Mallaret *et al*., 2014).

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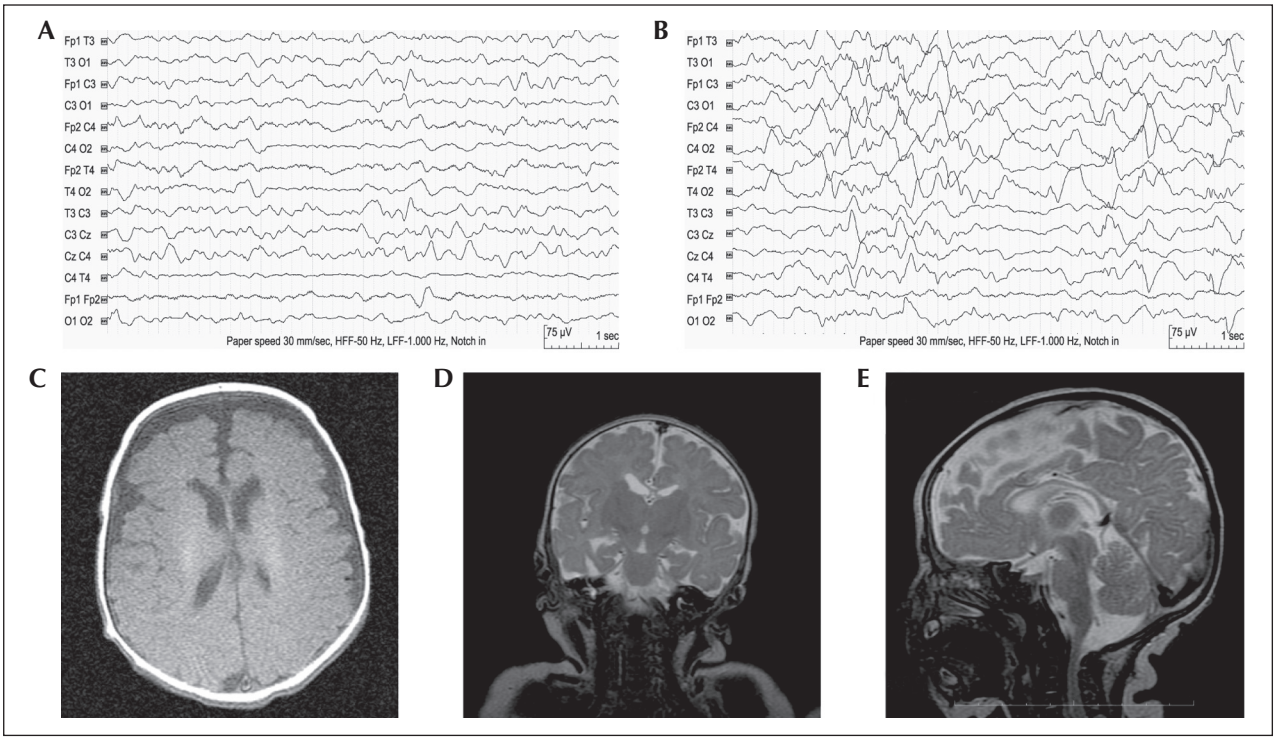

Figure 1. Investigations at age 5 weeks. (A) EEG during sleep at the age of 4 weeks showing focal discharges on left centro-median derivations, associated with asymmetry of activity, slower on the left hemisphere. (B) Asymmetric fragmented hypsarrhythmia, greater on left than right derivations. Axial T1 (C), coronal T2 (D), and sagittal T2 (E) MR images at the age of 5 weeks, showing completely formed, but thin, corpus callosum and normal myelination for age, without other gross abnormalities.

In 2014, *WWOX* mutations were described to be associated with an early-infantile epileptic encephalopathy (EIEE), which was named "EIEE-28" and later also "WWOX-related epileptic encephalopathy (WOREE)" (Abdel-Salam *et al*., 2014; Mignot *et al*., 2015).

Our aim was to describe practical clues to identify the WOREE phenotype.

Case study

Our patient was the first child of a non-consanguineous family. The pregnancy was normal, but the mother described reduced foetal movements. He was born through C-section due to maternal reasons, with a birth weight of 3,840 g (P85) and head circumference (HC) of 34.5 cm (P50). The boy had apparently hypokinetic behaviour from birth and epilepsy started at the age of 4 weeks. Seizure semiology suggested a focal onset, with head and eye deviation to the right, followed by secondary generalization, and shortly followed by flexion spasm. These were short (up to 30 seconds) stereotyped seizures, which were not clustered. The seizure frequency increased progressively from four seizures in the first day to 10 seizures in the third day, when he was admitted to our hospital.

Clinical evaluation revealed an infant with no constitutional particularities and normal HC. The following abnormal features were noticed during the neurological examination: a lack of gaze fixation, present but reduced limb movements, and slight axial hypotonia.

His electroencephalogram (EEG) showed normal background for his age and infrequent focal discharges (*figure 1A*). He was considered to have epilepsy with focal seizures and was treated with phenobarbital, with control of focal seizures, but persisting spasms, not in clusters, associated with hypsarrhythmia (*figure 1B*).

His cerebral MRI at 5 weeks of age revealed a thin corpus callosum (*figure 1C, D, E*).

Vigabatrine (VGB) was added to phenobarbital and the seizures and EEG were completely controlled for a month, however, epileptic spasms and hypsarrhythmia subsequently reappeared. Synthetic ACTH was added, with complete control of seizures, however, abnormal EEG with fragmented hypsarrhythmia during sleep remained and no motor or cognitive development was identified.

At the age of 6 months, he did not have any seizures, but developmental milestones were not reached. Gradual tapering of phenobarbital was considered. Seizures reappeared as alternant focal motor, left or right, oculogyric, generalized tonic seizures with

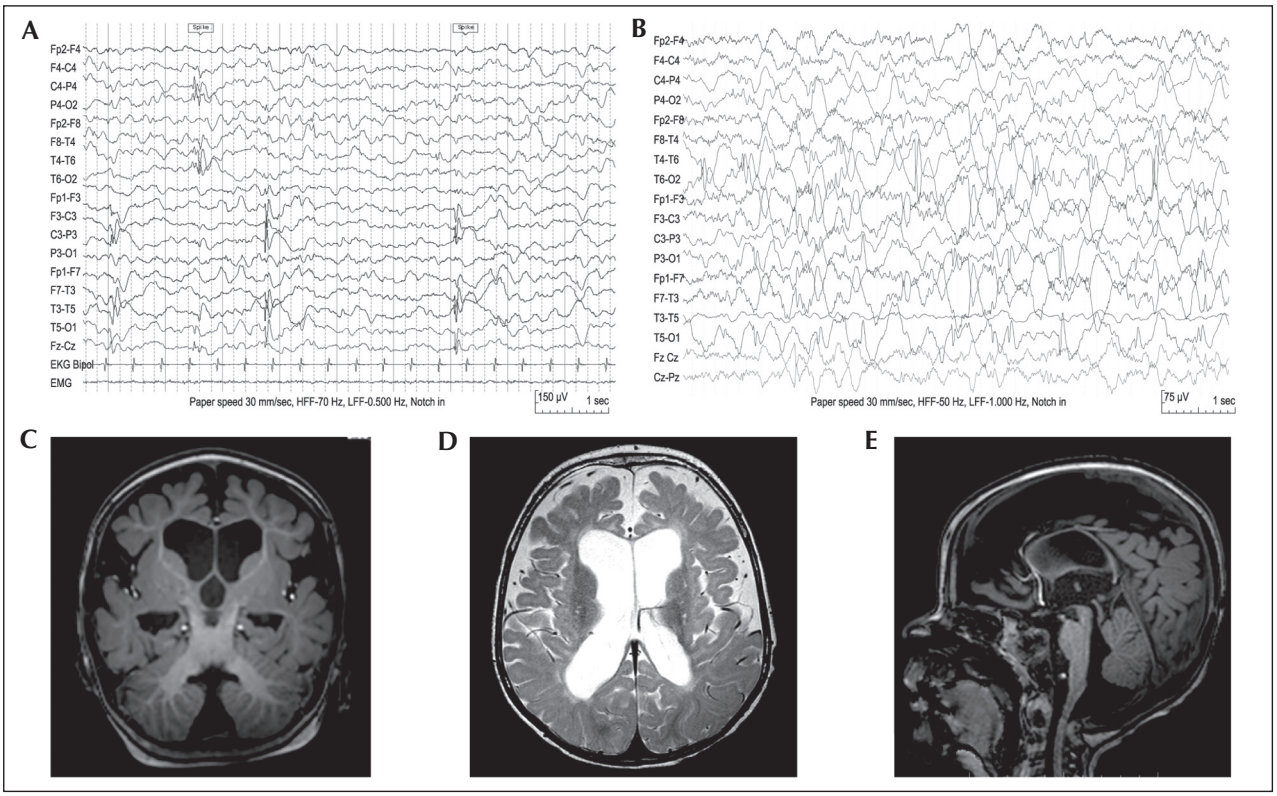

Figure 2. Investigations at age 2.6 years. (A) EEG during wakefulness at the age of 2 years showing multifocal, bilateral, asynchronous epileptiform abnormalities on slower background (not as propagation). (B) Sleep hypsarrhythmia. Coronal T1 (C), axial T2 (D), and sagittal T1 (E) MR images at 2.6 years, showing significant supratentorial atrophy, delayed myelination, an extremely thin corpus callosum, and flattening of the brainstem.

epileptic spasms. These seizures were resistant to all anti-seizure drugs.

At the age of 2.6 years, the patient still had normal HC (50.5 cm; P85) and normal somatic growth. Neurological examination revealed a lack of gaze fixation, no reaction to sound, and possible swallowing only for liquids or semi-liquid nutrients. He had reduced active movements of the limbs and extremely significant axo-rhizomelic hypotonia with distal hypertonia and bilateral pyramidal signs. He smiled occasionally without any social contact. Ophthalmological examination showed severe cerebral visual impairment. EEG during wakefulness showed multifocal, bilateral epileptiform abnormalities, which were asynchronous between hemispheres (but not propagated from one hemisphere to the other), with slower background (*figure 2A*) and fragmented hypsarrhythmia during sleep (*figure 2B*).

The second cerebral MRI performed at 2.6 years showed significant cortico-subcortical atrophy, an extremely thin corpus callosum, and flattening of the brainstem (*figure 2C, D, E*).

Considering the aspect of cerebral MRI, which showed a neurodegenerative disease affecting mainly the grey matter, metabolic disorders were taken into consideration in the differential diagnosis. Specific tests for disorders of small molecules (involving amino and organic acids, fatty acids, neurotransmitters, the urea cycle, vitamins, cofactors, and mitochondria) and large molecules (including lysosomal storage disorders, peroxisomal disorders, and glycosylation disorders) were performed. These results were negative, and a genetic aetiology of the epileptic encephalopathy was considered (possibly related to *ARX*, *SL25A22*, *FOXG1*, *STXBP1*, or *KCNQ2*, etc). However, because he did not have any specific phenotype associated with any of these mutations, whole-exome sequencing was performed.

A previously unreported heterozygous variant in intron 2 of the *WWOX* gene, c.173-1G>T, was detected. This is located in the highly conserved acceptor splice site of this intron. Software analysis (Alamut v.2.7.1) predicted a likely aberrant effect on splicing. A previously unreported heterozygous, likely pathogenic, variant in exon 8 of the *WWOX* gene, c.918del (p.Glu306Aspfs*21), was also identified. This is a deletion of 1 bp that creates a frameshift starting at codon Glu306. The new reading frame ends in a stop codon, 20 bases downstream.

The variant in intron 2 was detected in the mother, whereas the variant in exon 8 was detected in the father, both in a heterozygous state. Thus, it was confirmed that both variants were compound heterozygous in the index patient (that is, in a *trans* configuration).
Our patient had aspiration pneumonia and died at the age of almost 3 years old.

Discussion

To date, almost 20 patients (including our patient) have been reported to have CNS disorders associated with epilepsy due to autosomal recessive mutations of *WWOX* (Suzuki *et al*., 2009; Wang *et al*., 2012; Zhang *et al*., 2012; Aldaz *et al*., 2014; Tabarki *et al*., 2015).
The clinical phenotype of WOREE can be less severe than that of SCAR12, with developmental delay, ataxia, and epilepsy (six patients; Mallaret *et al*. [2014]) or as an intermediate phenotype with global developmental delay and epilepsy and onset at around 5 months old (two siblings, Patients 3 and 4; Mignot *et al*. [2015]).
Most reported patients with WOREE to date, including the present case, had a severe phenotype with early-onset epileptic encephalopathy and premature death. As clinicians, we are interested in clues for early recognition of the WOREE phenotype. All previously reported patients had early-onset seizures (at 2 weeks to 3 months old), profound global developmental delay, hypomotor behaviour, and several brain abnormalities. Most of them had optic atrophy and acquired microcephaly (Abdel-Salam *et al*., 2014; Ben-Salem *et al*., 2015; Mignot *et al*., 2015; Valduga *et al*., 2015; Tabarki *et al*., 2015).
Our patient was from a non-consanguineous family. He had hypomotor behaviour since birth, followed by profound global developmental delay with lack of eye contact, no motor abilities, severe axo-rhizomelic hypotonia with distal limb hypertonia, pyramidal signs, and premature death at almost 3 years old. Our patient and Patient 5 of Mignot *et al*. are the only ones with normal HC from all the patients reported with a severe phenotype.
Regarding his epilepsy, onset was within the "typical" period for onset of WOREE (four weeks), with focal and generalized seizures (epileptic spasms), responding temporarily to anti-seizure drugs. The particular feature of early-onset spasms (with persistence over time, not always in clusters, and not related to sleep) has not previously been described as a clinical clue for diagnosis.
The EEG of WOREE patients shows a disorganized background either with focal or generalized epileptiform discharges, or even hypsarrhythmia. In our patient, EEG evolution was concordant with a progressive degenerative disease, with initial focal abnormalities on normal background and subsequent fragmented hypsarrhythmia which was persistent in sleep, even at older ages, as a mark of severe epileptic encephalopathy. In addition to age and clinical phenotype as important factors to diagnose early epileptic encephalopathy, we also consider the lack of suppression-burst pattern on initial EEG (not described in any of the diagnosed patients) as an important feature.
In the literature, cerebral imaging of WOREE patients has revealed the presence of brain abnormalities as a thin corpus callosum (even when imaging was performed early), followed by a supratentorial generalized volume loss, and a flat appearance of the mesencephalon later on (Abdel-Salam *et al*., 2014; Ben-Salem *et al*., 2015; Mignot *et al*., 2015). Neuroimaging results of our patient were concordant with other cases, but not associated with microcephaly.
Prognosis is extremely poor for WOREE patients, with persistent epilepsy, profound global developmental delay, and early death before 3 years of age, being dependent on the type of mutation.
Our patient had a severe phenotype associated with a compound heterozygote mutation with a deletion in exon 8 of the *WWOX* gene. It seems that nonsense mutations or deletions of *WWOX* are correlated with severe phenotype, and since missense mutations may cause partial loss-of-function, they may therefore result in relatively milder phenotypes (Ben-Salem *et al*., 2015).
In conclusion, in addition to the rather consistent documented electroclinical phenotype for WOREE patients, we present other electroclinical clues for diagnosis which include persistent epileptic spasms and sleep-fragmented hypsarrhythmia associated with progressive cerebral atrophy without microcephaly. It is important for clinicians to consider this mutation early on in order to avoid unnecessary investigations and ensure appropriate genetic counselling for the family. At present, screening for *WWOX* mutation is not possible using the gene panels for epileptic encephalopathies, however, this is likely to change in the future. □

Supplementary data.
Summary didactic slides are available on the www.epilepticdisorders.com website.

Acknowledgements and disclosures.
We are indebted to the family of our patient for agreeing to publish their son's story in order to improve early diagnosis or even help identify a specific treatment.
The authors have no conflict of interest to disclose.

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TEST YOURSELF

(1) What are the typical electroclinical features of WWOX-related epileptic encephalopathy?

(2) Is there clinical heterogeneity with regards to *WWOX* mutation?

(3) What are the imaging clues for WWOX-related epileptic encephalopathy?

Note: Reading the manuscript provides an answer to all questions. Correct answers may be accessed on the website, www.epilepticdisorders.com, under the section "The EpiCentre".