Original article

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West syndrome, developmental and epileptic encephalopathy, and severe CNS disorder associated with *WWOX* mutations

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ABSTRACT – *Aims*. Mutations in the *WWOX* gene have been reported in a number of patients with various neurological disorders including spino-cerebellar ataxia, intellectual disability, epilepsy, and epileptic encephalopathy. We aimed to study the clinical, electrographic, and imaging features of two new cases with *WWOX* mutations and compare them to previously reported cases with *WWOX* mutations. *Methods*. We assessed two unrelated children from two consanguineous families who had severe neurological disorder including early-onset spastic quadriplegia, profound developmental delay, epilepsy, and West syndrome. *Results*. Based on whole-exome sequencing, we identified homozygous null mutations in *WWOX* in both children, and further addressed the genotype-phenotype correlation. In addition, we provide a detailed review of the previously reported cases of *WWOX*-related neurological disorders and compare them to the children in this report. *Conclusions*. The findings in this report expand the clinical phenotype associated with *WWOX* mutations and confirm a well characterised severe central nervous system disorder in association with biallelic null mutations in *WWOX*. This syndrome consists of profound psychomotor delay, early-onset spastic quadriplegia, and refractory epilepsy including epileptic encephalopathy, acquired microcephaly, and growth restriction. This can be associated with progressive brain atrophy, suggestive of neurodegeneration. Identification of this phenotype by clinicians may help with early diagnosis and appropriate genetic counselling.

Key words: *WWOX*, West syndrome, epileptic encephalopathy, intellectual disability, microcephaly, spasticity

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Central nervous system (CNS) diseases in children including those with severe epilepsy and motor disorders impose big challenges for diagnosis and potential treatments. Many children with undiagnosed CNS disorders are labelled with "cerebral palsy", particularly those with spasticity and seizures as the main features of their disorder. With this label, the search for an underlying diagnosis is often not pursued further (Gupta and Appleton, 2001; Leach *et al.*, 2014). The advent of next-generation sequencing has led to a molecular revolution with rapid growth in the number of identified monogenic determinants underlying many neurological disorders, including severe epilepsy and epileptic encephalopathy (Charng *et al.*, 2016; Fogel *et al.*, 2016; McTague *et al.*, 2016). Epileptic encephalopathies form a large heterogeneous group of severe childhood-onset epilepsies, associated with frequent epileptiform activity on EEG and developmental delay (Berg *et al.*, 2010). West Syndrome (WS) is one of the common epileptic encephalopathies, and is characterised by epileptic spasms along with "hypsarrhythmia", as a specific EEG pattern. Until recently, up to 30% of cases of WS had no identified cause (Osborne *et al.*, 2010). However, many genes have been identified recently in association with WS, therefore an aetiology is now known in a larger proportion of cases. One of the genes described recently in association with CNS disorders and epileptic encephalopathy is *WWOX* which encodes for a WW domain-containing oxidoreductase; a cytoplasmic protein involved in many cellular processes including growth, differentiation, and tumour suppression (Bednarek *et al.*, 2000; Chang *et al.*, 2014). *WWOX* is located on a common fragile site, FRA16D, on the long (q) arm of chromosome 16 at position 23 (16q23) and contains nine exons. Somatic *WWOX* mutations have been reported in different human cancers and its role as a tumour suppressor gene is well recognized (Bednarek *et al.*, 2001). More recently, germline mutations were described in children with neurological disorders, which indicates that *WWOX* plays an important role in CNS function and development (Abdel-Salam *et al.*, 2014; Mallaret *et al.*, 2014; Ben-Salem *et al.*, 2015; Mignot *et al.*, 2015; Tabarki *et al.*, 2015a; Elsaadany *et al.*, 2016). It is thought that *WWOX* is highly expressed in various parts of the CNS including the cerebrum, cerebellum, brain stem, and spinal cord. A portion of *WWOX* is thought to be located in the mitochondria, to carry out its oxidoreductase function (Chang *et al.*, 2014).

Here, we report two children with a severe CNS disorder and epileptic encephalopathy in the form of West syndrome, in association with homozygous null mutations in *WWOX*.

Subjects and methods

Two unrelated children with early-onset spastic quadriplegia, psychomotor retardation, and WS were evaluated at the paediatric neurology service at Tawam Hospital, Al-Ain, United Arab Emirates. Written informed consent was obtained from both families. This study was approved by Al-Ain Medical Human Research Ethics Committee according to the national regulations (protocol number 13/95-CRD 297/13), and funded by the United Arab Emirates University (grant number 31M135).

DNA samples from peripheral blood were extracted for the affected children and their parents at the hospital laboratory, as recommended by the manufacturer. Chromosomal microarray analysis (CMA) and whole-exome sequencing (WES) was performed as a service at Baylor Genetics Laboratory (www.bmgl.com), Texas, USA, according to the generally accepted international standards.

Results

Clinical data

The first child was a two-year-old boy who was born vaginally at term. Birth weight was 3.5 kg (SD: -0.23), length 48 cm (SD: -1.07), and head circumference (HC) 35 cm (SD: -0.63). There were concerns about clenched fists and limb stiffness soon after birth. Head ultrasound showed periventricular cysts, which led to suspicion of periventricular leukomalacia. At five weeks of age, parents reported paucity of spontaneous movements and the presence of sudden brief body jerks. EEG performed at six weeks of age was reported as normal. The child had poor feeding and nasogastric feeds were commenced. At the age of six months, his growth parameters were as follows: weight 5.1 kg (SD: -3.95), length 61.6 cm (SD: -2.86), and HC 39.7 cm (SD: -3.38), consistent with failure to thrive and acquired microcephaly (*figure 1A*). He had severe global developmental delay, no visual fixation or vocalisation, axial hypotonia, limb spasticity and rigidity (with variable degree), and minimal spontaneous movements (hypokinesia). Optic atrophy (bilateral) was confirmed by an ophthalmologist. At the age of seven months, he continued to have severe psychomotor delay and his parents reported arm stiffening episodes with facial redness, which they noted a few months earlier. EEG showed hypsarrhythmia and epileptic spasms and tonic seizures were captured during the EEG (*figure 2A*). Oral prednisolone was given as per UKISS protocol (Lux *et al.*, 2005) with a partial response.

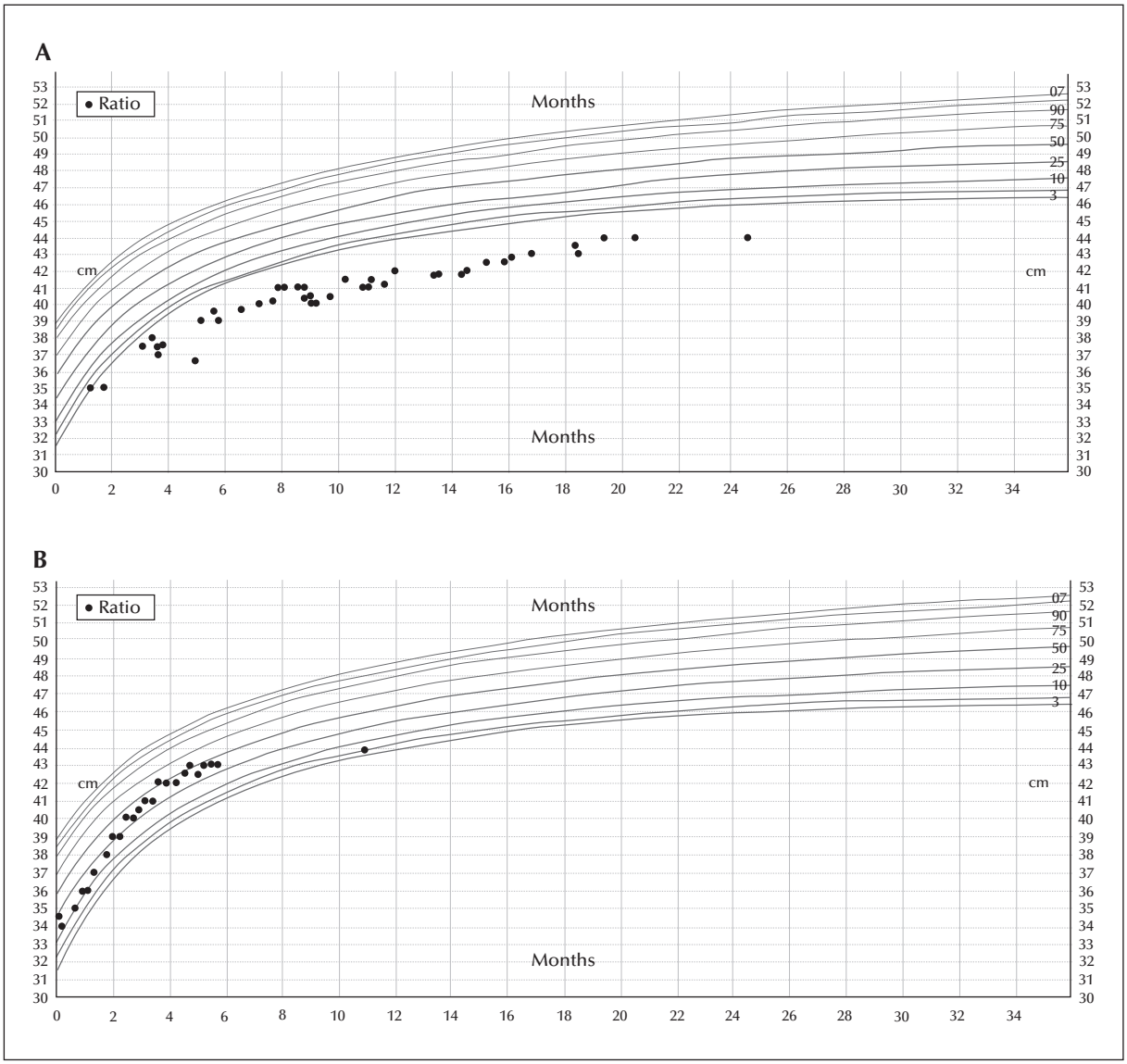

Figure 1. Head circumference growth charts for the first child (A) and second child (B), showing acquired microcephaly (2000 CDC [Centers for Disease Control and Prevention] growth charts).

Vigabatrin was added and the spasms resolved, however, he soon developed other seizure types, including focal myoclonic jerks and clonic seizures, which improved after adding levetiracetam. At two years of age, he remained profoundly delayed with infrequent mixed-type seizures and spastic quadriplegia. EEG showed slow background and infrequent multifocal epileptic activity.

Family history was notable for parents being first cousins with four healthy children. Two cousins had neurological diseases with spasticity and profound developmental delay. They eventually required ventilation and one of them died at the age of four years.

The following investigations were normal: lactate, ammonia, acylcarnitine, uric acid, homocysteine, plasma amino acids, urine organic acids, and transferrin isoforms. Cranial magnetic resonance imaging (MRI) at two months of age showed thin corpus callosum, wide Sylvian fissure, and periventricular connatal cysts in the left frontal region (Tan *et al*., 2010) (*figure 3A*).

The second child was a 12-month-old boy who was born via emergency Caesarean section due to foetal distress and meconium stained fluid at 41 weeks of gestation. He required initial resuscitation and subsequent intubation and ventilation. His birth weight

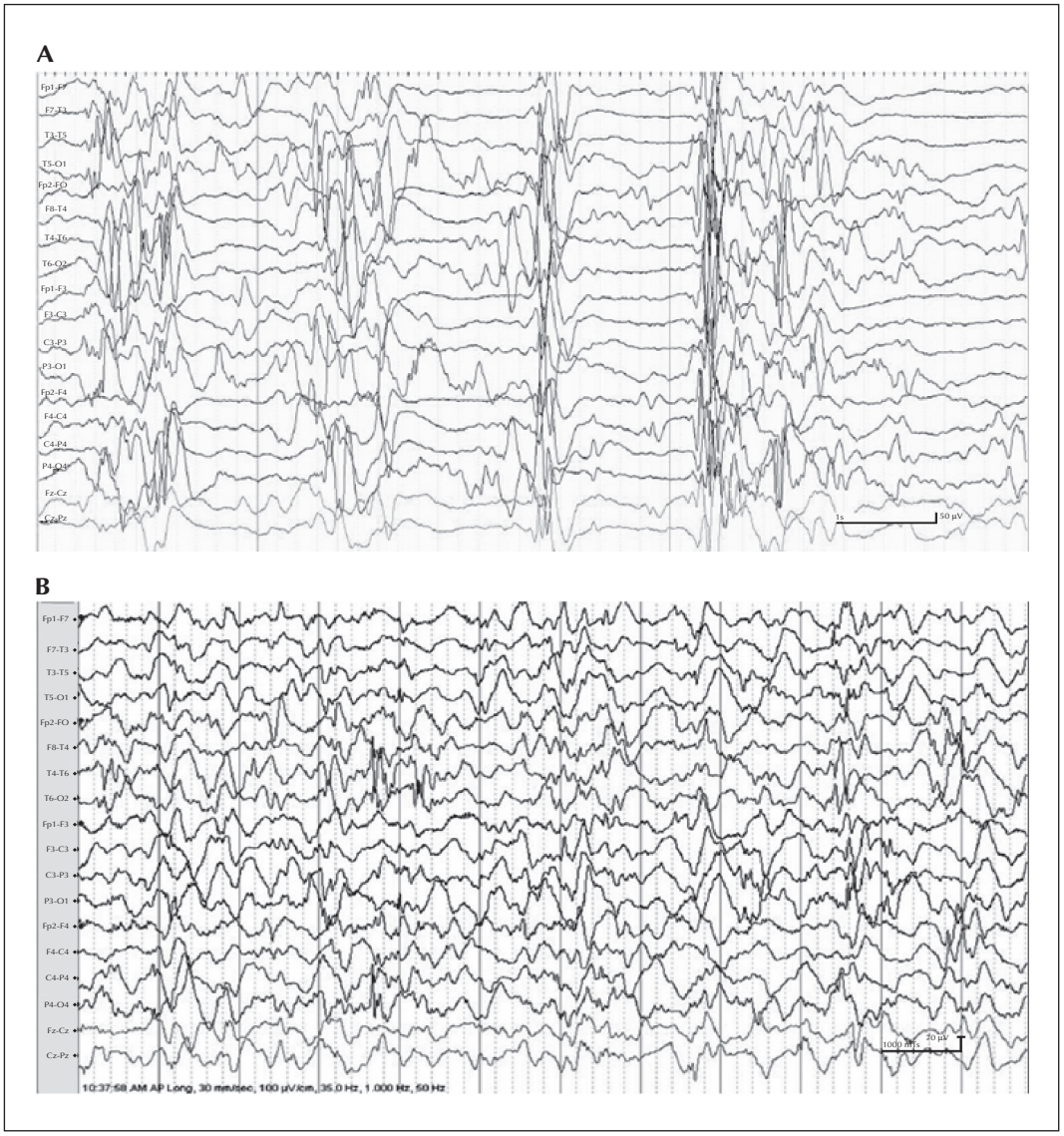

Figure 2. (A) EEG of the first child at age eight months shows high-voltage disorganized background, intermixed with multifocal high-amplitude spike and polyspikes, followed by voltage background consistent with hypsarrhythmia and burst suppression. (B) EEG of the second child at age four months showing high-voltage disorganized slow background, intermixed with multifocal high-amplitude spike and polyspikes, consistent with modified hypsarrhythmia.

was 3.745 kg (SD: 0.21), length 50 cm (SD: -0.31), and HC 34.5 cm (SD: -0.87). He was found to have limb hypertonia early in life. Seizures were noticed at five weeks of age and were focal involving lip smacking and arm jerking. EEG showed low-amplitude background of mixed delta and theta activity and frequent sharp and slow waves in the right fronto-temporal region. Phenobarbital was given and seizures improved. At four months, the child failed to attain any developmental milestones. He developed flexor spasms and EEG showed modified hypsarrhythmia in sleep (*figure 2B*) and epileptic spasms were captured. Vigabatrin was added with a partial response. He had mild spasticity in all limbs and was commenced on oral baclofen. He required tracheostomy at four months due to repeated failure of extubation and the need for assisted ventilation.

The child was evaluated again at the age of 11 months. He had profound psychomotor delay and remained ventilator-dependent. He had subtle right hemi spasms occurring in clusters despite being on four antiepileptic medications including vigabatrin, sodium valproate, clobazam, and phenobarbital. Clinical assessment revealed no visual fixation or

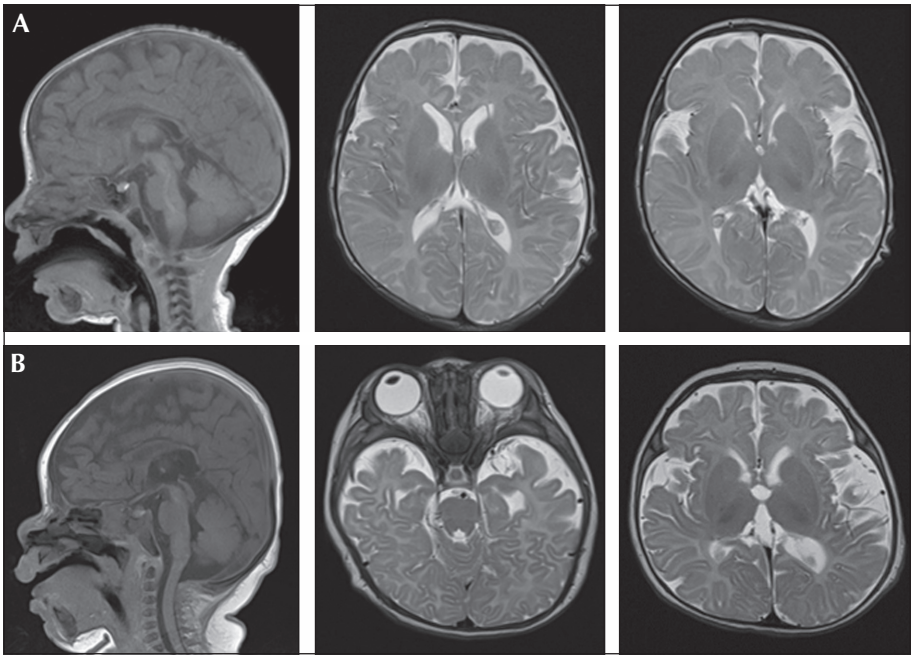

Figure 3. Cranial MRI of the two patients (left: sagittal T1; middle and right: axial T2). (A) The first child at age two months showing hypoplastic corpus callosum, particularly in the anterior portion, wide Sylvian fissure, and small connatal cysts adjacent to the frontal horn of left lateral ventricle. (B) The second child at age two months showing thin corpus callosum, mild ventricular dilatation, and wide CSF spaces, particularly in frontal and temporal regions (indicating reduced cerebral volume) and bilateral posterior polymicrogyria.

communication, axial hypotonia, and limb hypertonia and hyperreflexia. Growth parameters were as follows: weight 7.4 kg (SD: -2.79), length 68 cm (SD: -2.44) and HC 44 cm (SD: -1.62), consistent with failure to thrive and acquired microcephaly (*figure 1A*).

The child was the first born to first-cousin parents, with unremarkable family history.

The following investigations were normal: echocardiogram, lactate, ammonia, acylcarnitine profile, uric acid, homocysteine, plasma amino acids, urine organic acid, and urine sulfocystine. Cranial MRI at two months of age revealed bilateral parietal polymicrogyria, dysplastic thin corpus callosum, wide CSF spaces and reduced white matter volume, particularly in the temporal lobe (*figure 3B*).

Genetic testing

WES in the first child revealed a novel homozygous deletion affecting exons 3 to 4 of the *WWOX* gene (NM_016373.3), at position chr16;78143675-78149052. This finding was confirmed by CMA, which revealed homozygous copy number loss within chromosome band 16q23.1 of approximately 11 Kb in size, including exon 3 and 4 of *WWOX*, consistent with the WES findings. Parental CMA revealed a heterozygous copy number loss involving *WWOX* in both the mother and the father. No other relevant variants or copy number variations were detected by WES or CMA.

For the second child, CMA did not identify any copy number changes associated with known microdeletion or microduplication syndromes. However, WES revealed a previously reported homozygous pathogenic splice site mutation, c.606-1G>A, in *WWOX* (NM_016373.3) at position chr16;78453766, intron 6 (Tabarki *et al*., 2015a). This was confirmed by Sanger sequencing. Both parents were heterozygous for this mutation. No other relevant variants were detected by WES.

Discussion

The genetics of epileptic and neurodevelopmental encephalopathies is a rapidly growing field and the recent advances in next-generation sequencing have helped in identifying many monogenic variants in association with epileptic encephalopathy, with autosomal recessive, autosomal dominant or X-linked inheritance (McTague *et al*., 2016).

Recent reports have revealed the importance of *WWOX* in children with neurological disorders including epilepsy. Here, we report two new cases with *WWOX*-related CNS disorder and present a

summary of all reported patients from previous studies (*table 1*).

The two children presented in this report had early-onset epilepsy (myoclonic seizures in the first child and focal motor seizures in the second child) and epileptic encephalopathy in the form of WS along with severe neurological disorder in the form of progressive microcephaly, early-onset spasticity, and severe psychomotor retardation. The developmental outcome was unfavourable with profound impairment despite improvement of epileptic activity (*e.g.* in the first child). This supports the new concept of "developmental and epileptic encephalopathy" in genetic encephalopathies (Scheffer *et al.*, 2017), as compared to the traditional concept regarding epileptic encephalopathy in which it was thought that "the epileptic activity itself contributes to cognitive and behavioural impairments beyond that expected from the underlying pathology alone (*e.g.* cortical malformation)" (Berg *et al.*, 2010). We believe that, in these children, the underlying cause itself (mutations in *WWOX*) caused both the severe cognitive impairment and the epileptic encephalopathy. In support of this hypothesis is the fact that the cognitive and psychomotor impairment preceded the onset of epileptic encephalopathy and did not improve with achievement of better control of epileptic activity in some of these cases.

Biallelic missense mutations in *WWOX* were reported in association with a form of childhood-onset cerebellar ataxia with epilepsy and intellectual disability and, in addition, lower limb spasticity was present in some of these patients (Gribaa *et al.*, 2007; Mallaret *et al.*, 2014). This phenotype is milder than that of the children described in this report. This is likely due to the nature of the mutations that were missense rather than null, which result in partial rather than complete loss of function of the protein.

Reviewing the previous reports of children with various biallelic null mutations (nonsense, splice site and deletions) in *WWOX* shows that many of these children had a clinically distinct form of severe CNS disorder consisting of profound global developmental delay, spastic quadriplegia of early onset, refractory epilepsy, progressive microcephaly, and growth restriction (*table 1*) (Abdel-Salam *et al.*, 2014; Ben-Salem *et al.*, 2015; Mignot *et al.*, 2015; Tabarki *et al.*, 2015a; Valduga *et al.*, 2015; Elsaadany *et al.*, 2016). Other features in some cases include ophthalmic involvement such as optic atrophy (present in Case 1 in this report), retinal disease (Abdel-Salam *et al.*, 2014; Ben-Salem *et al.*, 2015; Mignot *et al.*, 2015; Tabarki *et al.*, 2015a; Elsaadany *et al.*, 2016), and early death (Mignot *et al.*, 2015; Tabarki *et al.*, 2015a; Valduga *et al.*, 2015). Many children in these reports had early-onset epileptic encephalopathy, most commonly West syndrome (*table 1*).

Of note is that some children had compound heterozygous mutations in the form of deletion and missense, for example, the two siblings in the study of Mignot *et al.* (Case 3 and 4) (*table 1*). These children had profound developmental delay as well as severe epilepsy and epileptic encephalopathy, however, they did not have spastic quadriplegia microcephaly or abnormality on cranial MRI. This could be considered an intermediate phenotype which supports genotype-phenotype correlation in cases with *WWOX* mutations. Interestingly, all reported patients with *WWOX* mutations so far, whether biallelic null, biallelic missense or compound null and missense mutations, had severe developmental delay/intellectual disability (ID) and epilepsy (23/23 for both features) (*table 1*), thus potentially adding *WWOX* to the growing and heterogeneous list of genes associated with ID, as well as epilepsy.

Most children with *WWOX* biallelic null mutations in previous reports, as well the cases presented here, had loss of volume in supratentorial structures including thinning of the corpus callosum, progressive brain atrophy involving grey and white matter, particularly in frontal and temporal regions, as well as widening of sylvian fissures evident on brain imaging (Abdel-Salam *et al.*, 2014; Mignot *et al.*, 2015; Tabarki *et al.*, 2015a; Elsaadany *et al.*, 2016). *WWOX* is known to be involved in control of cell survival or death through different mechanisms and interactions with various molecules involved in the regulation of cell signalling, gene transcription, and lipid metabolism (Chang *et al.*, 2014). It is postulated that *WWOX* plays a critical role in neuronal development, differentiation, and protection, and that loss of *WWOX* function leads to neuronal injury and neurodegeneration by mechanisms that are still unclear, but possibly involve mitochondrial dysfunction and apoptosis (Chang *et al.*, 2014; Tabarki *et al.*, 2015b), hence leading to brain atrophy and loss of volume.

The rare finding of polymicrogyria in Case 2 and a previously reported case (Ben-Salem *et al.*, 2015) indicates that *WWOX* may play a role in neuronal migration, and more cases and research are needed to confirm the role of *WWOX* as a gene implicated in malformation of cortical development (Parrini *et al.*, 2016).

The findings in this report support a clinically recognisable form of CNS disease in association with *WWOX* biallelic null mutations with the following features: severe early-onset disease with profound psychomotor delay, spastic quadriplegia, refractory seizures including epileptic encephalopathy, particularly WS, progressive microcephaly, and growth restriction. Other common features include poor vision and optic atrophy, variable degrees of rigidity and hypokinesia,

Table 1. Clinical, electrographic, imaging and genetic features in patients with mutations in *WWOX*.

Study	**Gribaa *et al.*, 2007, Mallaret *et al.*, 2014**						**Abdel-Salam *et al.*, 2014**	**Ben Salem *et al.* 2014**	**Mignot *et al.*, 2015**				
Families/number of cases	2/6						1/1	1/1	4/5				
Case	1	2	3	4	5	6	1	1	1	2	3	4	5
Age[&]/sex	19y/F	18y/F	16y/M	10y/F	NA/M	NA/F	12m/F	5m/M	4y/F	6m/F	24m/M	24/F	10m/F
Severe developmental delay/ID	+	+	+	+	+	+	+	+	+	+	+	+	+
Spastic quadriplegia (SQ)	-	-	-	-	-	-	+	+	-	+	-	-	+
Early onset SQ <3 m	na	na	na	na	na	na	NA	NA	na	NA	na	na	NA
Microcephaly	NA	NA	NA	NA	NA	NA	+	+	Possible	+	-	-	-
Growth restriction	NA	NA	NA	NA	NA	NA	+	+	NA	NA	NA	NA	NA
Early death <3y	-	-	-	-	-	-	+	na[@]	-	+	-	-	+
Ataxia	+	+	+	+	+	+	-	-	-	-	-	-	-
Ophthalmic involvement	+Nystagmus	+Nystagmus	+Nystagmus	+Nystagmus	-	-	+Optic atrophy Abnormal ERG	+Optic atrophy	+Poor eye contact	+Abnormal ERG	-	-	+Abnormal ERG
Other clinical features	Delayed walking and talking Dysarthria Hyporeflexia				Spastic lower limbs			SNHL-unilateral	Hypokinesia	Rigidity Dystonia Hypokinesia Apnea			Rigidity Hypokinesia
Epilepsy	+	+	+	+	+	+	+	+	+	+	+	+	+
Early onset <3m	-	-	-	-	-	-	+	+	+	+	-	-	+
Seizure type	GTC	GTC	GTC	GTC	GTC	GTC	Hemiconvulsions Myoclonic	Focal tonic IS	Focal tonic	Focal and generalised	Focal SG Myoclonic	Focal SG	IS Tonic
EEG	N	Generalised SSW	Occipital paroxysms	N	NA	NA	Generalised bursts of SSW	Hyps	Slow disorganised BG Occipital SSW	Slow disorganised BG Multifocal SSW	Slow BG Frontal SSW	Slow BG Multifocal SSW	Modified hyps
EE	-	-	-	-	NA	NA	+	+	+	+	+	+	+
EE type	na	na	na	na	na	na	WS	WS	NA	NA	NA	NA	NA
Number of AEDs	1	2	1	2	NA	NA	2	2	NA	NA	NA	NA	NA
AED response	Partial	Poor	Good	Good	na	na	Partial	Good	Partial	Partial	Partial	Partial	Partial

Table 1. Clinical, electrographic, imaging and genetic features in patients with mutations in *WWOX* (*Continued*).

Study	**Gribaa *et al.*, 2007, Mallaret *et al.*, 2014**						**Abdel-Salam *et al.*, 2014**	**Ben Salem *et al.* 2014**	**Mignot *et al.*, 2015**				
Cranial MRI#	-	-	+	+	-	-	+	+	+	+	+	+	+
MRI abnormality	na	na	+	+	na	na	+	+	+	+	-	-	+
Age at MRI	na	na	12 y	4 y	na	na	NA	NA	4 y	6 m	24 m	6 and 24 m	10 m
Cerebral atrophy/ reduced volume	na	na	-	-	na	na	+	+	+	-	-	-	+
Location of atrophy	na	na	na	na	na	na	Temporal	NA	NA	na	na	na	Generalised
Thin corpus callosum	na	na	-	-	na	na	+	-	+	+	-	-	+
Sylvian fissure widening	na	na	-	-	na	na	-	NA	-	-	-	-	+
Other imaging findings			Mild cerebellar vermis atrophy Posterior white matter hyper-intensities	Mild cerebellar vermis atrophy			Simple gyral pattern Hippocamp. dysplasia	Polymicro-gyria (right Front-opariatal)	Mild ventricular enlarge-ment				
Summary of MRI abnormalities	Not done	Not done	Mild cerebellar vermis atrophy Posterior white matter hyperin-tensities	Mild cerebellar vermis atrophy	Not done	Not done	Supraten-torial atrophy Simplified gyral pattern Thin CC	Cerebral atrophy Polymicro-gyria (right fron-topariatal)	Reduced WM volume Thin CC	Mild myelina-tion delay Thin CC	Normal	Normal	Progressive cerebral atrophy Thin CC Wide sylvian fissures
WWOX variant allele 1			c.139C>A (p.P47T)			c.1114G>C (p.G372R)	c.160G>T (p.R54*)	Deletion exon 5	Deletion exon 1-5	Deletion exon 6	c.45_48delGGAC (p.D16Sfs*63)		Deletion exon 1-9
WWOX variant allele 2			c.139C>A (p.P47T)			c.1114G>C (p.G372R)	c.160G>T (p.R54*)	Deletion exon 5	Deletion exon 6-8	c.1005G>A (p.W335*)	c.140C>G (p.P47R)		c.889A>T (p.K297*)
WWOX mutation zygosity			Hom missense			Hom missense	Hom nonsense	Hom deletion	CH: deletions	CH: dele-tion/nonsense	CH: frameshift/missense		CH: dele-tion/nonsense

Table 1. Clinical, electrographic, imaging and genetic features in patients with mutations in *WWOX* (*Continued*).

Study	**Tabarki *et al.*, 2015a**					**Valduga *et al.*, 2015***	**Elsaadany *et al.*, 2016**		**This study**		**Total[$]**
Families/number of cases	2/5					1/1	1/2		2/2		13/23
Case	1	2	3	4	5	1	1	2	1	2	23
Age[&]/sex	11m/NA	NA	NA	NA	NA	22m/F	7y/F	20m/F	24m/M	13m/M	
Severe developmental delay/ID	+	+	+	+	+	+	+	+	+	+	23/23
Spastic quadriplegia (SQ)	+	+	+	+	+	+	+	+	+	+	14/23
Early onset SQ <3 m	+	+	+	+	+	+	NA	+	+	+	9/9
Microcephaly	+	+	+	+	+	+	-	-	+	+	12/17
Growth restriction	+	+	+	+	+	+	+	+	+	+	12/12
Early death <3y	+	+	+	+	+	+	-	na[@]	na[@]	na[@]	9/19
Ataxia	-	-	-	-	-	-	-	-	-	-	6/23
Ophthalmic involvement	+Abnormal ERG	+Abnormal ERG	+Abnormal ERG	+Abnormal ERG	+Abnormal ERG	+Poor eye contact	+Optic atrophy	+Poor eye contact	+Optic atrophy	+Poor eye contact	19/23
Other clinical features						Cardiomyopathy	Respiratory failure		Rigidity Hypokinesia	Respiratory failure Hypokinesia	
Epilepsy	+	+	+	+	+	+	+	+	+	+	23/23
Early onset <3m	+	+	+	+	+	+	+	+	+	+	15/23
Seizure type	Multifocal IS	Multifocal IS	Multifocal IS	Focal SG	Focal SG	Myoclonic IS	Myoclonic	Focal tonic IS	Tonic IS Focal myoclonic	Focal motor IS Hemispasms	
EEG	NA	NA	NA	NA	NA	Normal /Hyps	Abnormal BG Frequent SSW	Consistent with EE	Hyps /Slow BG multifocal SSW	Modified hyps	
EE	+	+	+	+	+	+	-	+	+	+	16/21
EE type	WS LGS	WS LGS	WS	NA	NA	WS	na	NA	WS	WS	
Number of AEDs	NA	NA	NA	NA	NA	4	4	3	3	4	
AED response	Poor	Poor	Poor	Poor	Poor	Partial	Poor	Partial	Partial	Poor	
Cranial MRI #	+	+	-	-	-	+	+	+	+	+	16/23

Table 1. Clinical, electrographic, imaging and genetic features in patients with mutations in *WWOX* (*Continued*).

Study	Tabarki *et al.*, 2015a					Valduga *et al.*, 2015*	Elsaadany *et al.*, 2016		This study		Total[$]
MRI abnormality	+	+	na	na	na	+	+	+	+	+	14/16
Age at MRI	0 m and 11 m	NA	na	na	na	NA	2 m and 5 m	NA	2 m	2 m	
Cerebral atrophy/ reduced volume	+	+	na	na	na	+	+	+	-	+	10/16
Location of atrophy	Posterior	posterior	na	na	na	Sylvian fissure	Fronto temporal	Fronto temporal	na	Fronto temporal	
Thin corpus callosum	+	+	na	na	na	+	+	+	+	+	11/16
Sylvian fissure widening	-	+	na	na	na	+	+	NA	+	+	6/13
Other imaging findings	Connatal cysts	Symmetric thalamic lesions							Connatal cysts	Polymicrogyria (bilateral posterior)	
Summary of MRI abnormalities	Progressive atrophy of periventricular WM, CC and upper brainstem	Periventricular WM loss posteriorly Thin CC Flat upper brain stem	Not done	Not done	Not done	Thin CC Wide sylvian fissures	Hypomyelination Progressive frontotemporal atrophy Thin CC	Hypomyelination Progressive frontotemporal atrophy Thin CC	Thin CC Wide sylvian fissures	Frontotemporal atrophy Thin CC Wide sylvian fissures Polymicrogyria	
WWOX variant allele 1	c.606-1G>A					Deletion exon 1-6	c.131G >A (p.W44*)		Deletion exons 3- 4	c.606-1G>A	
WWOX variant allele 2	c.606-1G>A					Deletion exon 1-6	c.131G >A (p.W44*)		Deletion exons 3- 4	c.606-1G>A	
WWOX mutation zygosity	Hom splice-site					Hom deletion	Hom nonsense		Hom deletion	Hom splice site	

Abbreviations: +: present; -: absent; m: month; y: year, F: female; M: male; NA: not available; na: not applicable; ID: intellectual disability; SQ: spastic quadriplegia; ERG: electroretinogram; SNHL: sensory neural hearing loss; GTC: generalised tonic clonic; IS: infantile spasms; SG: secondary generalised; EEG: electroencephalogram; N: normal; SSW: spike and slow wave; hyps: hypsarythmia; BG: background; EE: epileptic encephalopathy; WS: West syndrome; LGS: Lennox- Gastaut syndrome; AEDs: antiepileptic drugs; MRI: magnetic resonant imaging; CC: corpus callosum; WM: white matter; Hom: homozygous; Het: heterozygous; CH: compound heterozygous. *The prenatally terminated case in this study was not included in this table as clinical features are mostly not applicable. [$]The total is calculated based on available data; cases with NA or na for any feature were subtracted from the total. [&]When the age was not specified in these reports, clues were used to determine age, *e.g.* age at MRI study. [@]na- not applicable as children were not three years old at the time this report was written. [#]When the MR images were available in the manuscript of these studies, extra interpretation was performed by JS to include features related to this table that were not described in the manuscript or figure legends in these reports.

feeding difficulties, and early death. Reduced volume of cerebral white and grey matter can be seen on cranial imaging with predilection to frontal and temporal areas including widening of sylvian fissures. Although there is overlap of this phenotype with many other neurodegenerative disorders, including those associated with other genes, identifying the features of *WWOX*-related CNS disease in children with early-onset epilepsy may help early diagnosis of affected children using targeted genetic testing, which may save time and cost.

Our findings support the role of advanced genetic testing as an important tool in the diagnosis of neurological disorders in children, particularly those with severe epilepsy and epileptic encephalopathy. Some children with spastic quadriplegia and epilepsy are labelled with cerebral palsy, particularly those with periventricular cysts or white matter loss, similar to the first child in this report. It is important to identify a potential genetic autosomal recessive disorder in these patients. Accurate and early genetic diagnosis is important and has many implications for the affected families, including specific recurrence risk, counselling for future pregnancies, and the potential for pre-implantation genetic diagnosis.

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

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None of the authors have any conflict of interests to declare.

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TEST YOURSELF

(1) What are the most consistent features in all reported cases with different types of *WWOX* mutations?

(2) List the clinical features associated with biallelic null type (nonsense, splice site, deletion) mutations of *WWOX*.

(3) West syndrome is the most common type of epilepsy associated with biallelic null mutations of *WWOX*- true or false?

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".