■ Original article

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Epilepsy in patients with *WWOX*-related epileptic encephalopathy (WOREE) syndrome

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ABSTRACT

Objective. Epileptic encephalopathy (EE) is difficult to diagnose and manage. It can be caused by a variety of disorders, and its aetiology may guide management and prognosis. The human gene for WW domain-containing oxidoreductase (*WWOX*) has been associated with epileptic encephalopathy, which presents in infancy with seizures, psychomotor delay, microcephaly, and optic atrophy.

Methods. We report nine patients with *WWOX*-related EE from six families. We provide detailed descriptions of clinical presentations, imaging findings, neurophysiological manifestations, and related mutations. Whole-exome sequencing (WES) was used to identify the mutations in the *WWOX* gene.

Results. We established correlations between genotype and phenotype in our cases and previously reported cases.

Significance. Our data support previously reported findings regarding *WWOX*-related EE, indicating the importance of the human *WWOX* gene in brain development and the association between *WWOX* mutations and EE. Our study also highlights the power of WES, particularly in clinically challenging cases.

Key words: *WWOX*, W44X, seizure, encephalopathy, developmental delay, whole-exome sequencing (WES)

The WW domain-containing oxidoreductase (*WWOX*) gene is located on chromosome 16q23.1-q23.2 and was first implicated in cancer [1]. It is recognized as an important tumour-suppressing gene [2]. Three different *WWOX*-related phenotypes caused by germline pathogenic variants have been described: disorder of sex differentiation (DSD), spinocerebellar ataxia (SCA), and epileptic encephalopathy (EE) [3-5]. Autosomal recessive infantile EE, or *WWOX*-related epileptic encephalopathy (WOREE) syndrome, is caused by biallelic pathogenic variants in the *WWOX* gene [6]. WOREE is characterized by early-onset drug-resistant seizures that lead to severe EE [7]. Focal seizures, focal to bilateral tonic-clonic seizures, and epileptic spasms were reported in a recent review of 59 patients with WOREE syndrome [8]. In this article, we report on nine additional patients with homozygous pathogenic mutations in the *WWOX* gene who presented with WOREE syndrome. We focus on the characteristics of epilepsy in these nine patients from our cohort and in 61 patients described in the previous literature.

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Methods

This was a retrospective case series that included paediatric patients with WOREE syndrome who were seen in the paediatric neurology department of the King Fahad Specialist Hospital Dammam (KFSHD), KSA. The study was approved by the KFSHD institutional review board. An electroencephalogram (EEG) was performed for all the patients, and the EEGs were read by a paediatric epileptologist (AM, RA). All patients' magnetic resonance imaging (MRI) scans were reviewed by a paediatric neuro-radiologist. Molecular genetic testing was conducted at the CGC Genetics Laboratory (Portugal, Spain). Next-generation sequencing panels were used, including whole-exome sequencing (WES), a WES-based next-generation sequencing (NGS) panel of 597 genes that included copy number variant (CNV) analysis, and targeted single-gene molecular analysis. The paediatric neurologists and clinical geneticist discussed all pathogenic and likely pathogenic variants in the context of the clinical phenotype. Gene-specific testing was performed on the parents and siblings of the probands for segregation analysis. The patients' genomic DNA sequence analyses were performed using next-generation sequencing on the Illumina platform; target regions were captured using oligonucleotide probes (Human All Exon V6, Agilent Technologies). After the region was aligned to reference the *Homo sapiens* genome (UCSC hg19), single nucleotide variants (SNV), insertions, deletions, and copy number variations (CNVs) were called and annotated. Variants were classified according to the classifications of the American College of Medical Genetics and Genomics (ACMG) [9].

We searched electronic databases, including PUBMED and Google Scholar, to identify all cases of WOREE syndrome reported in the literature. All searches were performed on August 16th, 2021. The literature search was supplemented by a manual search of the reference lists of included articles (performed by SB) to identify potentially relevant studies. The key words "*WWOX*" and "epilepsy" and "WOREE syndrome" were searched. We extracted the following data from all included articles: patient's gender, first symptom, age at seizure onset, seizure type(s), history of status epilepticus, anti-seizure medications (ASMs), and EEG findings.

Results

We identified nine patients in our cohort who had a homozygous pathogenic mutation in the *WWOX* gene and presented with WOREE syndrome. Sixty-one patients with *WWOX* gene mutation and WOREE syndrome, reported in 19 studies, were identified from the literature search. The clinical characteristics and genetic mutations of our patients are presented in *tables 1, 2*. The characteristics of epilepsy in our patients and the 61 patients reported in previous studies are presented in *table 3* [4-7, 10-23]. In our cohort, two (22.2%) patients were female and seven (77.8%) were male. In the previously reported cases, 40 (65.5%) patients were female and 21 (34.5%) were male. All included patients had epilepsy. Seizure was the presenting symptom in all patients. In our cohort, the age at seizure onset ranged from 21 days to 180 days (mean = 97.6); in previously reported cases, it ranged from one to 720 days (mean = 91.2). Fifty-five (78.6%) patients presented within the first three months of life. Brain MRI showed diffuse volume loss in most included patients. Three patients had thinning of the corpus callosum, and one patient had an absent corpus callosum. One patient had T2/FLAIR hyperintensity in the left subcortical parahippocampal gyrus, and another patient had subcortical band heterotopia. The MRI of Patient 4, who also has leukaemia, showed smooth dural thickening, suggesting leukaemic infiltration, and increased DWI signal intensity in the bilateral central tegmental tract. The MRI findings are shown in *figure 1*.

Patient 1.1

Patient 1.1 is a 10-year-old boy who was born at term via normal vaginal delivery to first-degree consanguineous Saudi Arabian parents. There is a history of polyhydramnios, but he presented with normal foetal movements and a birth weight of 3.5 kg. The mother has hypothyroidism, which was treated during pregnancy, and the baby had transient neonatal hypothyroidism. The patient's sister (Patient 1.2) has a similar condition, and there is a family history of epilepsy without developmental delay in the maternal uncles and one maternal cousin. Patient 1.1's first seizure occurred at the age of six months, and he has two types of seizures: behavioural arrest with or without blinking for seconds and generalized tonic-clonic (GTC) seizures. He was treated with 50 mg/kg of levetiracetam per day. He was referred to our centre at the age of five years and nine months. His EEG showed normal background activity with spike discharges in the right mid and posterior temporal regions. His seizures were well controlled on levetiracetam, occurring once, every six to ten months. His longest seizure-free period was 21 months, and breakthrough seizures responded well to an increased dose of levetiracetam. He has profound developmental delay and autistic features. He can stand and walk with support but has poor weight bearing on the lower extremities and is currently in a wheelchair. He has severe language delay and cannot utter any words;

▼ **Table 1.** Clinical characteristics of patients in our cohort.

Clinical Characteristics	Family 1		Family 2		Family 3		Family 4	Family 5	Family 6
Case	Patient 1.1	Patient 1.2	Patient 2.1	Patient 2.2	Patient 3.1	Patient 3.2	Patient 4	Patient 5	Patient 6
Age (y/m)	10 y	15 y 5 m	3 y 9 m	5 y	4 y 5 m (deceased)	5 y 6 m	2 y 5 m	4 y 6 m	5 y 2 m
Ethnicity	Saudi	Saudi	Saudi	Saudi	Saudi	Saudi	Saudi	Saudi	Saudi
Gender	Male	Female	Male	Female	Male	Male	Male	Male	Male
Consanguinity	Yes	Yes	Yes	Yes	No	No	Yes	Yes	yes
Psychomotor delay	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	yes
DTR	2+	3+	3+	2+	3+	1+	1+	Absent	2+
Tone	Hypertonia	Hypertonia	Marked hypertonia and scissoring	Marked hypertonia	Marked hypertonia, arthrogryposis, kyphoscoliosis	Marked hypertonia, arthrogryposis, kyphoscoliosis	Hypotonia	Hypertonia, joint deformities	Hypertonia
Microcephaly	No	No	Yes	Yes	Yes	Yes	No	Yes	Yes
Ambulation	No	Yes, unsteady	No	No	No	No	No	No	No
Optic atrophy	No	No	Yes	Yes	Not fixating or following	Not fixating or following	No	Yes	yes
Epilepsy	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	yes
Brain MRI	Mild generalized atrophy	T2/FLAIR hyperintensity in left subcortical parahippocampal gyrus	CC thinning	Generalized volume loss, asymmetrical ventricular dilatation, thinning of CC	Thinning of CC, periventricular leukomalacia	N/A	Generalized volume loss, bilateral central tegmental tract T2/FLAIR/DWI hyperintensity	Absent CC	Brain atrophy and subcortical band heterotopia

Y: year; m: month; CC: corpus callosum; N/A: not available.

▼ **Table 2.** Genetic results of patients in our cohort.

Patient ID	Gene	Nucleotide (protein)	Zygosity	Genetic test	Type of mutation
Patient 1.1	*WWOX*	c.139C>A (p.Pro47Thr)	Homozygous	WES	Missense
Patient 1.2	*WWOX*	c.139C>A (p.Pro47Thr)	Homozygous	Familial mutation testing	Missense
Patient 2.1	*WWOX*	c.160C>T (p.Arg54*)	Homozygous	WES	Nonsense
Patient 2.2	*WWOX*	c.160C>T (p.Arg54*)	Homozygous	WES	Nonsense
Patient 3.1	*WWOX*	c.606-1G>A	Homozygous	WES	Splice site
Patient 3.2	*WWOX*	c.606-1G>A	Homozygous	Familial mutation testing	Splice site
Patient 4	*WWOX*	c.139C>A (p.Pro47Thr)	Homozygous	WES-based NGS panel of 597 genes including CNV analysis	Missense
Patient5	*WWOX*	c.160C>T (p.Arg54*)	Homozygous	WES-based NGS panel of 597 genes including CNV analysis	Nonsense
Patient 6	*WWOX*	c.689A>C (p.Gln230Pro)	Homozygous	WES	Missense

WES: whole-exome sequencing; NGS: next-generation sequencing; CNV: copy number variant.

only sounds, laughs, and screams. He knows his parents and can grasp and release objects but is totally dependent on his family. His head circumference (HC) is 50 cm, which is between the fifth and tenth percentile, and his weight is 32 kg. He has no neurocutaneous stigmata or dysmorphic features. He is hypertonic and has poor eye contact, hand flapping, rocking body movements, deep tendon reflexes (DTRs) of 2+, a downward planter reflex, and no clonus. He also has Planovalgus deformity. His hearing and ophthalmological assessment were normal. Routine blood tests and basic metabolic screens for blood and urine, serum ammonia and lactate, and cerebrospinal fluid (CSF) neurotransmitters were unremarkable. His chromosomal analysis and comparative genomic hybridization (CGH) array were normal except for 11% excessive homozygosity. Brain MRI revealed mild generalized brain atrophy and no grey–white matter abnormality. Magnetic resonance spectroscopy (MRS) showed a glutamate–glutamine peak. A positron emission tomography (PET) scan showed relative hypometabolism in the right temporal lobe. WES detected a variant, c.139C>A (p.Pro47Thr), with apparent homozygosity in the *WWOX* gene. On his last follow-up visit in the clinic, at the age of nine years and nine months, his seizures were controlled on levetiracetam.

Patient 1.2

Patient 1.2 is a girl. She is 15 years and five months old, and she is the sister of Patient 1.1. She was born at term via normal vaginal delivery to first-degree consanguineous Saudi Arabian parents. Her birth weight was 3 kg. At birth, her crying was delayed, and she required oxygenation. She and her mother were discharged on the second day with no complications. Her first seizure occurred at the age of six months in the form of epileptic spasms. She also has other types of seizures, including generalized tonic, generalized tonic-clonic, and myoclonic seizures. Her epileptic spasms resolved at the age of five years. She had status epilepticus once at the age of four years. She was first seen in our centre when she was 10 years old; at that time, she was on phenobarbitone, topiramate, and lamotrigine. Long-term video EEG monitoring showed frequent generalized and multifocal epileptic discharges and captured generalized tonic-clonic and tonic seizures. She is currently taking carbamazepine, levetiracetam, and clobazam. Developmentally, she is globally delayed and has poor attention and a poor ability to follow complex commands. She is able to ambulate independently with slow movements with flexed posture but needs guidance. She has intellectual disability and attends a special school. Speech is poorly understood

▼ **Table 3.** Epilepsy characteristics of patients in our cohort and reported in the literature.

Reference (year of publication)	Patient number	Sex	First symptom	Seizure onset	Seizure types	Status Epilepticus	ASMs	EEG findings
Our cohort	1.1	M	Seizure	6 months	Focal unaware GTC	No	LEV	SW in right temporal lobe
	1.2	F	Seizure	6 months	Epileptic spasm GTC Myoclonic Generalized tonic	Yes	CBZ, LEV, CLOB	Generalized and MF SW discharges
	2.1	M	Seizure	2 months	Epileptic spasms Myoclonic	No	VGB	BG: slow Clinical and electrographic myoclonic seizures
	2.2	F	Seizure	2 months	Epileptic spasms	No	VGB	BG: slow Left temporal and parietal spikes
	3.1	M	Seizure	2 months	Myoclonic Focal unaware to bilateral tonic-clonic Subclinical seizures	Yes	PHB, CLOB, LEV KGD	BG: slow MF spikes Non-convulsive SE
	3.2	M	Seizure	NA	Subclinical seizures Tonic	Yes	PHB, LEV, VPA	BG: slow Generalized SW discharges
	4	M	Seizure	6 months	Myoclonic Focal unaware to bilateral tonic-clonic	No	LEV, CLOB	BG: mild slowing Multifocal spikes
	5	M	Seizure	40 days	Epileptic spasms Focal tonic-clonic Myoclonic	Yes	VPA, VGB, TOP, CZP	Hypsarrhythmia Bursts of polyspike discharges - myoclonic seizure
	6	M	Seizure	21 days	Right focal tonic Epileptic spasms Subclinical seizures	No	VGB KGD	BG: suppression-burst pattern SW discharges in left temporal-parietal-occipital & midline central-parietal regions
Su *et al.* (2020) [8]	1	F	Seizure	55 days		No	PHB, TOP	Hypsarrhythmia

▼ **Table 3.** Epilepsy characteristics of patients in our cohort and reported in the literature (*continued*).

Reference (year of publication)	Patient number	Sex	First symptom	Seizure onset	Seizure types	Status Epilepticus	ASMs	EEG findings
					Focal clonic Epileptic spasms			
	1	F	Seizure	12 months	GTC	No	VPA	Normal
	2	F	Seizure	9 months	GTC	No	PHB, VPA	BG: mixed beta and theta activity Burst of generalized SW, MF spikes
Gribaa *et al.* (2007) [5]	3	M	Seizure	9 months	GTC	No	PHB	Occipital paroxysms suggestive of benign occipital epilepsy with fixation-off sensitivity
	4	F	Seizure	9 months	GTC	No	PHB, VPA	Normal
Mallaret *et al.* (2014) [10]	1	M	Seizure	<2 years	GTC	NA	NA	NA
	2	F	Seizure	<2 years	GTC	NA	NA	NA
Abdel-Salam *et al.* (2014) [4]	1	F	Seizure	2 months	Hemi-clonic Myoclonic	No	VPA, LTG	Generalized epileptic discharges Bursts of slow SW discharges
Ben-Salem *et al.* (2015) [11]	1	M	Seizure	2 weeks	Asymmetric tonic seizures Epileptic spasms	No	VPA Steroids	Hypsarrhythmia
	1	F	Seizure	2 months	Focal tonic	No	NA	BG: slow disorganized Slow waves/ occipital paroxysms
	2	F	Seizure	7 weeks	GTC Brachio-facial	No	NA	BG: slow disorganized Bioccipital sharp and slow waves Electrographic seizures in biocciptal-temporal regions
Mignot *et al.* (2015) [6]	3	M	Seizure	5 months	Focal to bilateral tonic-clonic Tonic Myoclonic	No	NA	BG: slow SW anteriorly
	4	F	Seizure	5 months	Focal/multi-focal to bilateral tonic-clonic	No	NA	BG: slow MF spikes
	5	F	Seizure	2 months	Epileptic spasms Tonic	No	NA	Bioccipital SW Modified hypsarrhythmia

▼ **Table 3.** Epilepsy characteristics of patients in our cohort and reported in the literature (*continued*).

Reference (year of publication)	Patient number	Sex	First symptom	Seizure onset	Seizure types	Status Epilepticus	ASMs	EEG findings
Valduga *et al.* (2015) [12]	1	F	Seizure	2 months	Epileptic spasms Myoclonic	No	VPA, VGB, CLOB Hydrocortisone	Hypsarrhythmia Slow SW discharges
	2	M	Seizure	Prenatal 3rd trimester	Rhythmic foetal kicks	No	None	Not done
Tabarki *et al.* (2015) [13]	1	F	Seizure	2 months	Multifocal Epileptic spasms	No	NA Refractory	LGS pattern
	2	F	Seizure	3 months	Multifocal Epileptic spasms	No	NA Refractory	LGS pattern
	3	F	Seizure	2 months	Multifocal Epileptic spasms	No	NA Refractory	NA
	4	M	Seizure	2 months	Focal to bilateral tonic-clonic	No	NA Refractory	NA
	5	F	Seizure	3 months	Focal to bilateral tonic-clonic	No	NA Refractory	NA
Elsaadany *et al.* (2016) [14]	1	F	Seizure	7 weeks	Myoclonic	No	PHB, CZP, PHT LEV	BG: abnormal SW discharges
	2	F	Seizure	7 weeks	Focal Generalized tonic Epileptic spasms Myoclonic	No	PHB, CLOB, TOP	BG: slowing MF spikes Discontinuity of BG in sleep
Tarta-Arsene *et al.* (2017) [15]	1	M	Seizure	4 weeks	Focal to bilateral Epileptic spasms Generalized tonic	No	PHB, VGB ACTH	BG: slow MF spikes Hypsarrhythmia
Johannsen *et al.* (2018) [16]	1	F	Seizure	2 months	Epileptic spasms Tonic Myoclonic	No	NA Refractory	Hypsarrhythmia
	2	F	Seizure	2 months	Epileptic spasms Tonic Myoclonic	No	NA Refractory KD	Hypsarrhythmia

▼ **Table 3.** Epilepsy characteristics of patients in our cohort and reported in the literature (*continued*).

Reference (year of publication)	Patient number	Sex	First symptom	Seizure onset	Seizure types	Status Epilepticus	ASMs	EEG findings
Serin *et al.* (2018) [17]	1	F	Seizure	2 months	Epileptic spasms Focal non-motor BA	No	VGB, VPA, LEV	Modified hypsarrhythmia
Piard *et al.* (2018) [7]	1	F	Seizure	20 days	Focal clonic Absence Focal tonic-clonic GTC	No	NA Refractory	BG: slow Bilateral parieto temporal epileptiform activity
	2	F	Seizure	20 days	Focal Generalized Epileptic spasms	No	NA Refractory	BG: disorganized MF spikes, posterior predominant
	3	F	Seizure	2 days	Hemiclonic Epileptic spasms	NA	NA Refractory	Abnormal activity and bitemporal paroxysmal
	4	F	Seizure	First day	Focal GTC	No	NA Refractory	BG: disorganized and slow Occipital spikes
	5	M	Seizure	1 month	Focal to bilateral tonic-clonic Myoclonic	No	NA Refractory	BG: slow Spikes in temporal region
	6	F	Seizure	3 months	Clonic Epileptic spasms Tonic	Yes	NA Refractory	BG: slow disorganized Bursts of generalized sharp waves, greater on right side SW in bilateral fronto-temporal regions SE originating from left fronto-temporal area
	7	F	Seizure	2 months	Hemicorporeal generalized tonic GTC Absence Focal	Yes	NA Refractory	BG: very slow Occipital slow waves Seizures from occipital area
	8	M	Seizure	1 month	Focal Generalized tonic Epileptic spasms	No	NA Refractory	BG: slow Generalized spikes Hypsarrhythmia
	9	M	Seizure	1 day	Epileptic spasms	No	NA Refractory	Hypsarrhythmia
	10	M	Seizure	7 months	Generalized	No	NA Refractory	SW discharges predominantly bilateral posterior regions
	11	F	Seizure	5 days	Epileptic spasms	No	NA Refractory	Modified hypsarrhythmia

▼ **Table 3.** Epilepsy characteristics of patients in our cohort and reported in the literature (*continued*).

Reference (year of publication)	Patient number	Sex	First symptom	Seizure onset	Seizure types	Status Epilepticus	ASMs	EEG findings
	12	F	Seizure	11 weeks	GTC	Yes	NA Refractory	NA LGS pattern
	13	F	Seizure	7 weeks	Epileptic spasms	NA	NA Refractory	Hypsarrhythmia
	14	M	Seizure	N/A	Epileptic spasms	NA	NA Refractory	NA
	15	M	Seizure	3 months	Focal clonic Generalized Dialeptic Myoclonic	No	NA Refractory	BG: slow Focal temporal spikes MF spikes
	16	M	Seizure	2.5 months	Focal clonic Generalized Dialeptic Myoclonic	No	NA Refractory	BG: slow Focal temporal spikes MF spikes
	17	F	Seizure	1 day	Focal clonic Epileptic spasms Myoclonic	No	NA Refractory	Hypsarrhythmia
	18	F	Seizure	2.5 months	Tonic Epileptic spasms	No	NA Refractory	BG: disorganized MF SW discharges
	19	F	Seizure	3 weeks	Focal Tonic	No	NA Refractory	MF spikes Generalized fast spikes LGS pattern
	20	F	Seizure	2 months	Focal GTC Absence	Yes	NA Refractory	BG: slow MF spikes Rhythmic delta
Shaukat *et al.* (2018) [18]	1	M	Seizure	5 weeks	Epileptic spasms Tonic seizures Focal myoclonic Clonic	No	Prednisolone VGB, LEV	BG: disorganized slow MF Spike- polyspikes with burst suppression hypsarrhythmia
	2	M	Seizure	5 weeks	Focal clonic Epileptic spasms Focal hemi spasms	No	PHB, VGB, VPA, CLOB	BG: slow Sharp-slow waves in the right fronto-temporal region Hypsarrhythmia
Ehaideb *et al.* (2018) [19]	1	M	Seizure	2 months	Focal to bilateral tonic-clonic Epileptic Spasms	No	NA Refractory	NA
	2	M	Seizure	2 months	Epileptic spasms	No	NA	Burst suppression
	3	F	Seizure	2 months	Epileptic spasms	No	NA	Hypsarrhythmia

▼ **Table 3.** Epilepsy characteristics of patients in our cohort and reported in the literature (*continued*).

Reference (year of publication)	Patient number	Sex	First symptom	Seizure onset	Seizure types	Status Epilepticus	ASMs	EEG findings
Davids *et al.* (2019) [20]	1	F	Seizure	2 weeks	Myoclonic GTC	No	PHB, TOP Pyridoxine	BG: slow Generalized bursts of SW
Weisz Hubshman *et al.* (2019) [21]	1	F	seizures	3 months	NA Recurrent seizures	No	NA	NA
	2	F	Seizure	3 weeks	Tonic clonic	No	NA	Bilateral temporal spikes and sharp waves
	3	M	Seizure	2 weeks	Focal tonic	No	NA Refractory	Right fronto-central epileptic discharges
	4	F	Seizure	3 weeks	Epileptic spasms	No	NA	MF epileptic discharges Modified hypsarrhythmia
	5	F	Seizure	1 month	Focal Tonic	No	NA Refractory	MF spikes and sharp waves
	6	M	Seizure	6 weeks	Epileptic spasms	No	NA Refractory	Right centro-temporal epileptic discharges
Yang *et al.* (2019) [22]	1	F	Seizure	19 days	Tonic Focal to bilateral clonic Epileptic spasms	No	PHB LEV	Fast activity and epileptic discharges in left central-parietal regions
He *et al.* (2019) [23]	1	M	Seizure	1 month	Focal tonic Epileptic spasms Tonic upward gazing	No	NA Refractory	Irregular spike-polyspike-slow-wave discharges Fast activity in bioccipital regions

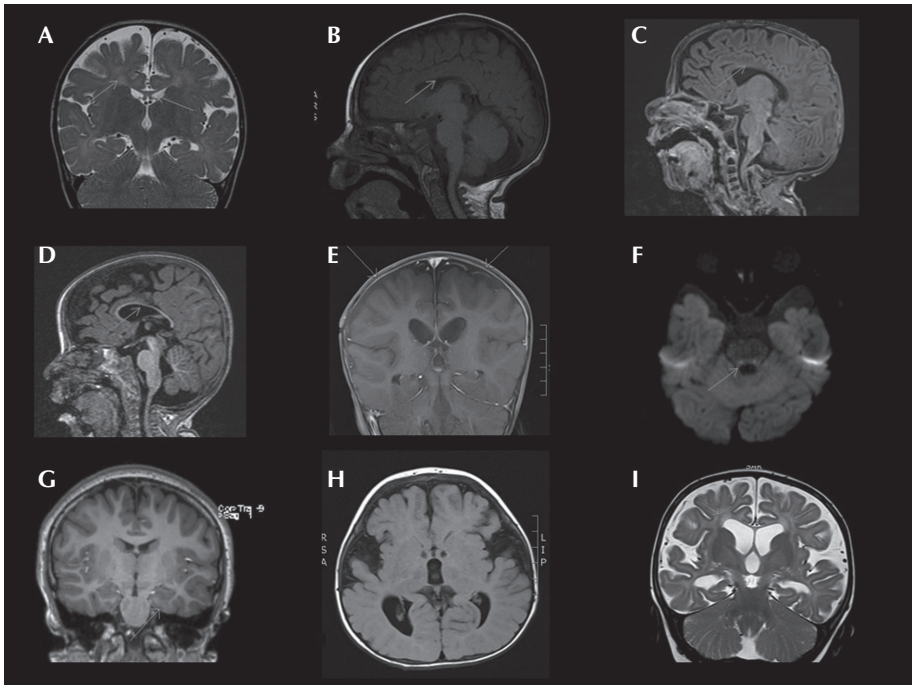
M: male; F: female; NA: not available; GTC: generalized tonic-clonic; LGS: Lennox-Gastaut syndrome; SE: status epilepticus; LEV: levetiracetam; CBZ: carbamazepine; CLOB: clobazam; VGB: vigabatrin; BG: background; MF: multifocal; PHB: phenobarbital; VPA: valproic acid; KGD: ketogenic diet; NCSE, non-convulsive status epilepticus; TOP: topiramate; CZP: clonazepam; PHT: phenytoin; ACTH: adrenocorticotropic hormone; SW: spike and wave.

(2–3 words/sentence), and her tremors and ataxia are getting worse.

She has coarse facial features and a short neck. She can fixate and follow visual stimuli. Her HC is 54 cm (<50th percentile). She has two *café-au-lait* spots, severe spasticity in all limbs, DTRs of 3+, action tremors, and an ataxic gait. Metabolic screens for blood and urine, ammonia, and lactate (serum and CSF) and basic laboratory tests were all unremarkable. The variant, c.139C>A (p.Pro47Thr), was detected with apparent homozygosity in the *WWOX* gene. Brain MRI showed indications of focal abnormality in the left subcortical parahippocampal gyrus. She currently experiences generalized tonic seizures that last less than a minute, three to four times per week. She was last seen in our clinic when she was 15 years old due to concerns about an increase in falls and unsteadiness. She is currently receiving physical and occupational therapy as part of a rehabilitation programme.

Patient 2.1

Patient 2.1 is a boy; he is currently three years and nine months old. He was born at term via normal vaginal delivery to consanguineous Saudi Arabian parents. His birth weight was 2.4 kg. He was admitted to the neonatal intensive care unit (NICU) for a few days due to suspected sepsis. His family history includes a similar condition. He has a history of recurrent



■ **Figure 1.** Brain MRI findings of patients in our cohort.

vomiting and failure to thrive. As a baby, he never smiled or cooed. At the age of two months, he developed his first seizure in the form of epileptic spasms; an EEG showed hypsarrhythmia. He was started on vigabatrin, which effectively controlled his seizures. A follow-up EEG showed generalized slowing and myoclonic seizures. He has large ears, a depressed nasal bridge, an abnormally small palpebral fissure, and no neurocutaneous stigmata. He had generalized muscle wasting and microcephaly; his HC is 43 cm. He does not fixate or follow visual stimuli. He has marked hypertonia, scissoring of the lower limbs, DTRs of 3+, and no clonus. An ophthalmological assessment showed bilateral optic nerve atrophy. His basic laboratory tests, metabolic blood and urine screening, and very long chain fatty acids (VLCFAs) were all unremarkable. His TORCH screen was non-reactive. Brain MRI showed moderate thinning of the corpus callosum; his MRS was normal. WES showed a homozygous c.160C>T p. (Arg54) mutation in the *WWOX* gene. He was last seen in our clinic one year ago; at that time, his seizures were controlled on vigabatrin.

Patient 2.2

Patient 2.2 is a five-year-old girl, the sister of Patient 2.1. She was born at term via normal vaginal delivery to consanguineous Saudi Arabian parents. Her birth weight was 2.3 kg. She presented with severe failure to thrive and vomiting. Her seizures started at the age of two months in the form of epileptic spasms and were treated outside the hospital with levetiracetam. She was first seen in our hospital at the age of 18 months, with her younger brother. An EEG showed hypsarrhythmia, and she was started on vigabatrin. She responded well to vigabatrin, and her seizures were under control. Her EEG showed significant background asymmetry with slow, high-amplitude activity over the left posterior head region, as well as generalized slowing and intermittent rhythmic delta slowing, intermixed with spikes over the left temporal and parietal head regions. She has profound developmental delay. She is non-verbal and non-ambulatory and does not fixate or follow visual stimuli, nor does she respond to sounds. She has hypertelorism, large ears, a high arched palate, and no neurocutaneous stigmata. She is microcephalic, with an HC of 40 cm; she weighs 5.3 kg (<third percentile). She has marked hypertonia, DTRs of 2+, contracture at the joints, and severe generalized muscle wasting. Her basic laboratory tests, TORCH screening, blood and urine metabolic screening, serum ammonia, lactate, and VLFFAs were all unremarkable. Brain MRI showed generalized asymmetrical brain tissue volume loss with asymmetrical

ventricular dilatation and thinning of the corpus callosum. WES showed a homozygous c.160C>T p.(Arg54) mutation in the *WWOX* gene. She was last seen in our clinic one year ago; at that time, her seizures were controlled on vigabatrin.

Patient 3.1

Patient 3.1 is a boy, four years and five months old. He was born at term via a normal vaginal delivery to non-consanguineous Saudi Arabian parents. He has congenital arthrogryposis and undescended testes. His brother has a similar condition. Seizure onset occurred at the age of two months. He has two types of seizures, myoclonic and focal tonic unaware seizures, with and without bilateral tonic-clonic activity. His seizures were refractory to multiple ASMs, including phenobarbital, clonazepam, and topiramate. He was referred to us at the age of six months. His EEG showed generalized slowing and intermittent multifocal epileptic discharges, which were larger in the bilateral frontal and temporal regions. A long-term video EEG taken when he was 14 months old showed frequent multifocal epileptic discharges. It also captured his habitual myoclonic seizures. A ketogenic diet was initiated, and he responded well to this treatment. At the age of four years, he was admitted to the paediatric intensive care unit (PICU) with hypoactivity and sepsis. An EEG showed focal non-convulsive status epilepticus in the form of continuous rhythmic spikes and wave discharges arising from the right fronto-central regions; intravenous diazepam aborted it. He has profound global developmental delay. He is non-verbal and non-ambulatory and does not fixate or follow visual stimuli. He has microcephaly, with an HC of 44 cm. He is inattentive and has no dysmorphic features or neurocutaneous stigmata. He has no spontaneous movement, marked hypertonia, DTRs of 3+, and severe kyphoscoliosis with arthrogryposis. Metabolic screening for blood and urine, ammonia, and lactate were all unremarkable. His chromosomal analysis was normal, and his TORCH screen was non-reactive. Brain MRI showed thinning of the corpus callosum and periventricular leukomalacia. The pathogenic variant, c.606-1G>A (p.?), was detected with homozygosity in the *WWOX* gene. He passed away at the age of four years and five months while in the PICU for sepsis.

Patient 3.2

Patient 3.2 is a boy; he is five years and six months old and the brother of Patient 3.1. He was born at term via normal vaginal delivery to non-consanguineous Saudi Arabian parents. He has epilepsy, profound developmental delay, arthrogryposis, chronic lung disease, and gastro-oesophageal reflux disease.

He is microcephalic and has an HC of 41 cm. He has no neurocutaneous stigmata. He has a high arched palate and low-set ears. He has profound delay; he is non-verbal, only making sounds, and non-ambulatory; he does not fixate or follow visual stimuli. He has spasticity in all limbs, a hyperextended neck, and DTRs of 1+. He has contracture in multiple joints and clubbed feet. His basic laboratory tests, blood and urine metabolic screening, lactate, and ammonia were unremarkable. The pathogenic variant, c.606-1G>A (p.?), was detected with apparent homozygosity in the *WWOX* gene. He was last seen in our clinic at the age of 16 months. He was referred back to the primary hospital and has received no follow-up care in our hospital since then.

Patient 4

Patient 4 is a boy, two years and five months old. He was born at term via normal vaginal delivery to consanguineous Saudi Arabian parents. His mother has a history of polyhydramnios during pregnancy and sickle cell anaemia. There is also a family history of febrile seizures, epilepsy, and developmental delay in two cousins. Seizure onset occurred at the age of six months. He has two types of seizures: myoclonic jerks and left focal unaware motor tonic to bilateral tonic-clonic seizures. He is on multiple ASMs, including levetiracetam, valproic acid, and topiramate. An EEG showed mild generalized slowing. He has global developmental delay and recently started sitting alone, saying up to 10 single words, interacting socially with his parents and sibling, and playing with his toys. His HC is 47.5 cm. He has a depressed nasal bridge, a flat forehead, a single palmer crease in his left hand, and a *café-au-lait* spot. During the examination, he was attentive, fixating and following visual stimuli. He is hypotonic with DTRs of 1+. His plantar reflex is downward with no clonus. His ophthalmological examination was normal. Metabolic screening for blood and urine, ammonia, and lactate were all unremarkable. He was later diagnosed with acute lymphoblastic leukaemia after a complete blood count revealed pancytopenia. The pathogenic c.139C>A p.(Pro47Thr) variant was detected with homozygosity in the *WWOX* gene. Brain MRI showed generalized volume loss and high T2/FLAIR/DWI signal intensity in the bilateral central tegmental tract; his MRS was normal. A renal ultrasound revealed tiny left-sided non-obstructive renal calculi with mild hydronephrosis. He was last seen when he was admitted for chemotherapy for acute lymphoblastic leukaemia in March 2021. At that time, his myoclonic

jerks and focal unaware seizures were controlled on levetiracetam and topiramate.

Patient 5

Patient 5 is a boy, four years and six months old. He was born at term via normal vaginal delivery to consanguineous Saudi Arabian parents. His birth weight was 3 kg. He has a significant family history of similar conditions; one brother died with brain atrophy at the age of one and a half years, and a sister has brain atrophy and has been on a ventilator in another hospital since she was four months old. Both siblings are undiagnosed. Seizure onset occurred at the age of 40 days, presenting as right focal tonic-clonic with the eyes deviated to the right side and blinking. He later developed epileptic spasms. He was referred to our hospital at the age of four years and four months. An EEG showed a slow background with multifocal epileptic discharges, which were larger in the right posterior head regions. Multiple generalized-onset epileptic spasms were captured. Vigabatrin was added to his ASMs; he was already on valproic acid, topiramate, and clonazepam. He has profound developmental delay and regression. He is currently non-verbal and non-ambulatory; he does not roll over or follow commands. He can only make sounds. His examination revealed microcephaly with a HC of 46 cm. He has no neurocutaneous stigmata. He has generalized muscle wasting and hypertonia. He cannot move his limbs against gravity; his DTRs could not be elicited due to joint deformities. An ophthalmological examination revealed bilateral optic atrophy. All basic laboratory tests and metabolic screening for blood and urine, ammonia, and lactate were unremarkable. Brain CT showed an absent corpus callosum. MRI was not performed. The pathogenic variant, c.160C>T p.(Arg54*), was detected with probable homozygosity in the *WWOX* gene. He is currently admitted to another hospital and on mechanical ventilation.

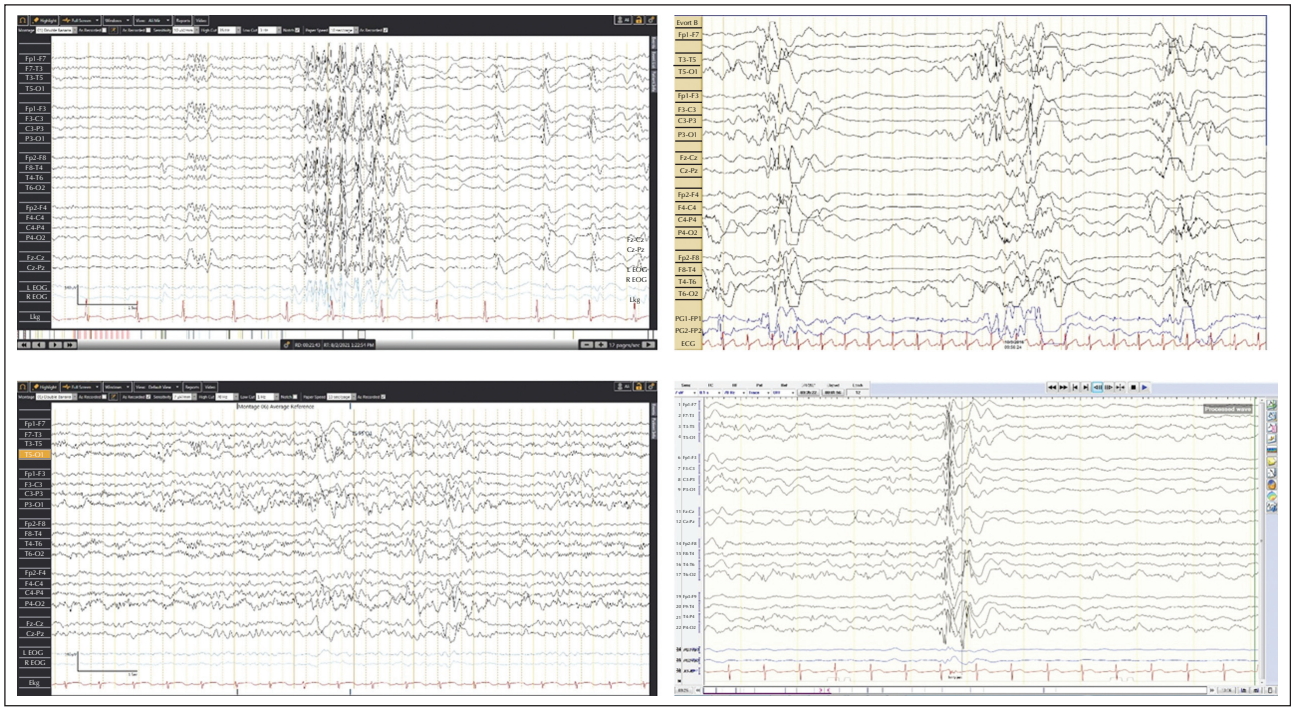
Patient 6

Patient 6 is a boy, five years and two months old. He was born at term via normal vaginal delivery to consanguineous Saudi Arabian parents. His birth weight was 3.1 kg. There is a family history of a similar condition; one sibling, who has passed away, presented with early-onset neonatal seizures and global developmental delay. Patient 6's first symptom was a seizure, presenting at the age of three weeks in the form of epileptic spasms and right focal tonic seizures. He was first seen in our clinic at the age of three months. An EEG showed a suppression-burst background pattern and frequent spikes and wave discharges arising from the left temporal-parietal-occipital regions and the midline central-parietal regions. Both epileptic spasms and right focal tonic seizures were captured. He was started on vigabatrin, which effectively controlled his seizures. He was started on a ketogenic diet at the age of 11 months, which he tolerated very well and is still following. He has breakthrough seizures during febrile illness. An ophthalmological examination revealed optic atrophy.

He is globally delayed, non-verbal, and non-ambulatory; he does not fixate or follow visual stimuli. He can roll over, turn his head to one side, and move his extremities; he can also recognize his parents, laugh, and smile. He is microcephalic with an HC of 45 cm; he is also hypertonic and has an upward planter and DTRs of 2+. His basic laboratory tests and metabolic screening for blood and urine, serum ammonia, lactate, and VLFFAs were all unremarkable. Brain MRI showed supratentorial tissue volume loss with subcortical band heterotopia. His MRS was unremarkable. WES revealed c.689A>C p. (Gln230Pro), a homozygous mutation in the *WWOX* gene. When he was last seen in our clinic a few weeks ago, he had been seizure-free for approximately nine months. His treatment plan included a repeat EEG and possible weaning off the ketogenic diet.

Epilepsy

The details of epilepsy in 70 patients with WOREE syndrome are presented in *table 3*. Seizure onset typically occurs within the first three months of life and is usually the presenting symptom in these patients. Some patients had seizures on the first day of life [7], and one patient was presumed to have had prenatal seizures during the third trimester, as the mother experienced rhythmic foetal kicks [12]. Later onset at the age of 12 months has also been reported [5]. The most common seizure type was epileptic spasms (50%, 35/70), followed by focal seizures (40%, 28/70), generalized tonic-clonic seizures (26%, 18/70) and myoclonic seizures (23%, 16/70). Other types of seizures reported were tonic, hemi-clonic, absence, multifocal, and subclinical seizures. Status epilepticus was noted in eight patients. Most of the patients were refractory to ASMs. The EEG results in most of these patients were suggestive of epileptic encephalopathy. Most EEGs showed background slowing with either generalized or multifocal epileptiform discharges. The background of 18 patients showed hypsarrhythmia; a suppression-burst pattern was observed in two patients. Two patients (reported by Gribaa *et al.*) had normal EEGs; these patients experienced seizure onset at the ages of nine months and 12 months, and both experienced generalized tonic-clonic seizures [5]. Some interesting EEGs are shown in *figure 2*.



■ **Figure 2.** EEG findings of patients in our cohort.

Genetics

Four different types of homozygous mutations in the *WWOX* gene were identified in our cohort. WES was utilized to identify the mutations in five patients, familial mutation testing was used for two patients, and a WES-based NGS panel of 597 genes, including CNV analysis, was used for two patients. Three patients have the c.139C>A (p.Pro47Thr) missense mutation, three patients have the c.160C>T p. (Arg54*) nonsense mutation, two patients have the c.606-1G>A (p.?) splice site mutation, and one patient has the c.689A>C p. (Gln230Pro) missense mutation (*table 2*). The brother of Patient 6 harbours a familial heterozygous missense pathogenic c.689A>C p.(Gln230Pro) variant and was labelled as a carrier, although his clinical presentation was similar to his brother's and he passed away at the age of 10 years. Further testing is underway to determine whether the heterozygous variant is a disease-causing compound heterozygous variant.

Discussion

We report nine patients presenting with WOREE syndrome who have homozygous pathogenic mutations in the *WWOX* gene. Sixty-one patients with *WWOX* gene mutation and WOREE syndrome were identified in previously published literature. In the present article, we present a comprehensive description of epilepsy in this entire cohort of 70 patients.

Epilepsy is a core feature of WOREE syndrome, and seizure is the presenting symptom of this disease. Most of the patients (78.6%, 55/70) presented within the first three months of life, although there are a few reports of an earlier seizure onset. One patient was reported to have prenatal seizures during the third trimester, presenting in the form of rhythmic foetal kicks [12]. In a case series of 20 patients, seizure onset occurred on the first day of life in three patients, on the second day of life in one patient, and on the fifth day of life in one patient [7]. In two case series of six patients, the age at seizure onset was delayed, occurring between the ages of nine months and two years [5, 10]. Patients who present with seizures during the first six months of life often pose a challenge to physicians, and a thorough work-up is needed to investigate the aetiology. Early recognition of WOREE syndrome could help avoid unnecessary testing and may make it easier for physicians to inform families about the natural history and prognosis of this disease.

Patients with WOREE syndrome can present with a variety of different seizure types. The most common

type is epileptic spasms, which occur in 50% of patients. The other common seizure types were focal (40%), generalized tonic-clonic (26%), and myoclonic seizures (23%). Eight patients had a history of status epilepticus, and three patients had subclinical seizures. Most of the patients who developed epileptic spasms experienced seizure onset during the first two months of life. Two patients whose only seizure type was epileptic spasms, reported in a case series by Piard *et al*., developed spasms on Days 1 and 5 of life, respectively. EEGs showed hypsarrhythmia in one of these and modified hypsarrhythmia in the other [7]. The preponderance of epileptic spasms and focal seizures in this population suggests that vigabatrin could be an effective treatment. In this cohort, nine patients with epileptic spasms were treated with vigabatrin, but the data on its effectiveness are not well established. Most of the patients were refractory to ASMs, but in five patients, seizures were controlled with one ASM. The roles of a ketogenic diet and vagus nerve stimulation in treating WOREE syndrome are not yet well described. Our data suggest that patients with WOREE syndrome typically present with early-onset refractory epilepsy and epileptic encephalopathy. Although the aetiology of epileptic spasms is heterogeneous, more and more genetic causes are being identified, and *WWOX* gene mutations should be considered while evaluating these patients.

The EEG results in most patients were suggestive of EE. Most EEGs showed background slowing with either generalized or multifocal epileptiform discharges. The backgrounds of 18 patients showed hypsarrhythmia, and a suppression-burst pattern appeared in two patients. A suppression-burst pattern on EEG, early-onset refractory seizures, developmental delay, tonic seizures/spasms, and abnormal brain MRI are also features of Ohtahara syndrome. Therefore, *WWOX* gene mutations might play a role in the aetiology of Ohtahara syndrome.

Conclusion

We summarize the characteristics of epilepsy in 70 patients with WOREE syndrome. Early seizure onset is common in this population, and seizure is the usual presenting symptom in this disorder. Epileptic spasms are the most common seizure type, followed by focal, generalized tonic-clonic, and myoclonic seizures. In patients with WOREE syndrome, epilepsy is refractory to ASMs, and no specific single medication or combination of ASMs is particularly effective. Our data suggest that further research is needed to develop a systematic approach to understand the characteristics of epilepsy in this disease as well as targeted, personalized therapies. ■

Key words

- *WWOX* (WW domain-containing oxidoreductase)-related epileptic encephalopathy is characterized by early-onset drug-resistant seizures leading to severe epileptic encephalopathy.
- Our study highlights the characteristics of nine patients from our cohort and 61 patients collected from the literature.
- Although the aetiology of epileptic spasms is heterogeneous, genetic causes are increasingly being identified and *WWOX* gene mutations should be considered in the evaluation of these patients.

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Patient consent.
Patient consent was obtained and approved by the local ethics committee.

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