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A Chinese patient with epilepsy and *WWOX* compound heterozygous mutations

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ABSTRACT – Early infantile epileptic encephalopathy type 28 is a refractory epilepsy with early onset, poor prognosis, and hereditary causes. WW domain-containing oxidoreductase (*WWOX*) gene mutation can result in epileptic encephalopathy, but the mechanism remains unclear. We present the case of a patient with epilepsy and *WWOX* compound heterozygous mutations. The seizures manifested as tonic-clonic, convulsive and were refractory to drugs. Magnetic resonance imaging showed a widened subarachnoid space and thin corpus callosum. The patient died from asphyxia at the age of one year and 23 days. Peripheral blood was taken from the patient and his parents, and whole-exome sequencing was investigated to determine possible gene mutation. Two compound heterozygous mutations were identified: c.172+1G>C (with no amino acid change) and c.984C>G (amino acid change: p.Tyr328Ter). The pathophysiology of epileptic encephalopathy related to the *WWOX* gene remains to be determined, and further studies are required to elucidate possible mechanisms.

Key words: epileptic encephalopathy, infantile epilepsy, *WWOX* gene, compound heterozygous mutations, Whole-exome sequencing

Early infantile epileptic encephalopathy type 28, caused by mutation of the WW domain-containing oxidoreductase (*WWOX*) gene, is a type of refractory epilepsy and a serious autosomal recessive inherited neurological disease (Ben-Salem *et al*., 2015), with an early onset age and poor prognosis. The *WWOX* gene was first located at 16q21-q23 in a large family with four affected children in Saudi Arabia (Gribaa *et al*., 2007). The *WWOX* gene is located in one of the most vulnerable fragile chromosomal sites in humans, referred to as "FRA16D" (Bednarek *et al*., 2001). This gene contains nine exons, encoding a 414-amino acid protein containing two N-terminal WW domains, a short-chain dehydrogenase/reductase at the C-terminal, and a nuclear localization sequence (Bednarek *et al*., 2000). The protein is widely present and highly expressed in the prostate, bones, lungs, endocrine tissues, and brain (Aqeilan *et al*., 2007).

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Case study

The male child presented with disease onset at one month of age. Convulsions involved raising the right limb during sleep, which lasted for a few seconds. A few days later, the seizure would reoccur with convulsion involving raising of the left limb. These symptoms lasted a few seconds with a frequency of 3-5 times a day. At 2.5 months of age, the form of seizure changed to clusters of spasms, with several spasms in each cluster, occurring 1-2 times per day. At nine months of age, the spasms disappeared, but both eyes appeared to deviate slightly upwards after opening. The patient had no marked response to antiepileptic drugs.

The weight of the patient was 3.6 kg at birth, 5 kg at the age of three months, and 8.5 kg at the age of 10 months. The patient was born at 37 weeks and two days; the Caesarean section was reported with Grade III amniotic fluid pollution. No history of hypoxia during the perinatal period was reported. Head circumference at the age of three and 10 months was 38 cm and 41 cm, respectively. The patient developed sepsis at the age of two months and seven days, and was hospitalized for two weeks. No epilepsy was reported in his parents, and no members of his family had a related medical history. He died from suffocation due to pneumonia at one year and 23 days of age. The patient was unable to follow objects but could raise his head at the age of two months. He was consistently unable to roll over, sit alone, or speak during his short life.

Methods

Whole-exome sequencing (WES) was performed based on Illumina technology sequencing, using Agilent SSELXT Human All Exon V6 to capture and build libraries, with an applied paired-end sequencing strategy. Raw data were >10G, with Q30 ≥80%. Bioinformatics analysis and mutation screening were based on original data from the sequencer, converted from .bcl to .fastq using bcl2fastq, and files were aligned with Human Reference Genome GRCh38/hg38 using BWA, Samtools and Picard software. Later, the generated .bam files were locally re-aligned using GATK series, and the repetitive sequences were omitted and mutations detected. We used Annovar to annotate the variation of .vcf mutation files. Pathogenic mutations were screened according to ACMG classification guidelines and the clinical phenotypes of the patients. The pathogenic mutation sites associated with clinical phenotypes, detected by second-generation sequencing, were validated by first-generation Sanger sequencing. On detecting a positive mutation, samples from both parents were investigated simultaneously.

Results

EEG (10-20 international system with 500-Hz sampling rate; Nihon Kohden, Tokyo, Japan) of the patient at the age of 10 months is presented in *figure 1A-D*. Short range, low/medium-amplitude 4/5-Hz theta activity can be seen on both sides of the occipital region, and the left and right sides are basically symmetrical. Interictal EEG shows irregular spike-slow-wave and polyspike-slow-wave discharge in the posterior region of both sides, more clearly in the bilateral occipital region. Ten seizures were detected during EEG monitoring at the age of 10 months. Both eyes appeared with a slightly upward gaze, lasting for three to five seconds. Ictal EEG onset showed a low-amplitude fast wave in the posterior region of both sides, which was on the left side seven times and on the right three times.

Gene detection results showed *WWOX* (NM_016373) compound heterozygous mutations (*figure 2A, B*). The nucleotide substitution, c.172+1G>C (at chr16:78142385), with no change in amino acid but affecting a splicing site, was derived from the mother. The nucleotide substitution, c.984C>G (at chr16: 78466577), creating the amino acid change, p.Tyr328Ter, a nonsense mutation, was derived from the father. Axial T2-weighted imaging (3 mm thickness) showed a widened subarachnoid space, and sagittal T1-weighted imaging showed a thin corpus callosum (*figure 3A, B*).

Discussion

Many published studies have established the association between the *WWOX* gene and cancer, but few cases related to epileptic encephalopathy are reported. The case reported in the present study represents the first Chinese patient with epileptic encephalopathy and *WWOX* gene mutation and the second reported patient with epilepsy and *WWOX* compound heterozygous mutations.

To date, all autosomal recessive mutations detected in the *WWOX* gene are associated with early epileptic seizures, psychomotor development retardation, and ataxia caused by intellectual disability. Patients also have microcephaly and spastic quadriplegia, which usually occur in very young babies (1.5 months), called "WWOX-related epileptic encephalopathy" (Gribaa *et al*., 2007; Abdel-Salam *et al*., 2014; Mallaret *et al*., 2014; Mignot *et al*., 2015).

WWOX is expressed in the uterus on Days 12-14 during the development of the nervous system, including the brain, and later in the cerebral cortex and cerebellum during adulthood (Chen *et al*., 2004). WWOX-related epileptic encephalopathy has a poor response to drugs, and MRI shows brain atrophy,

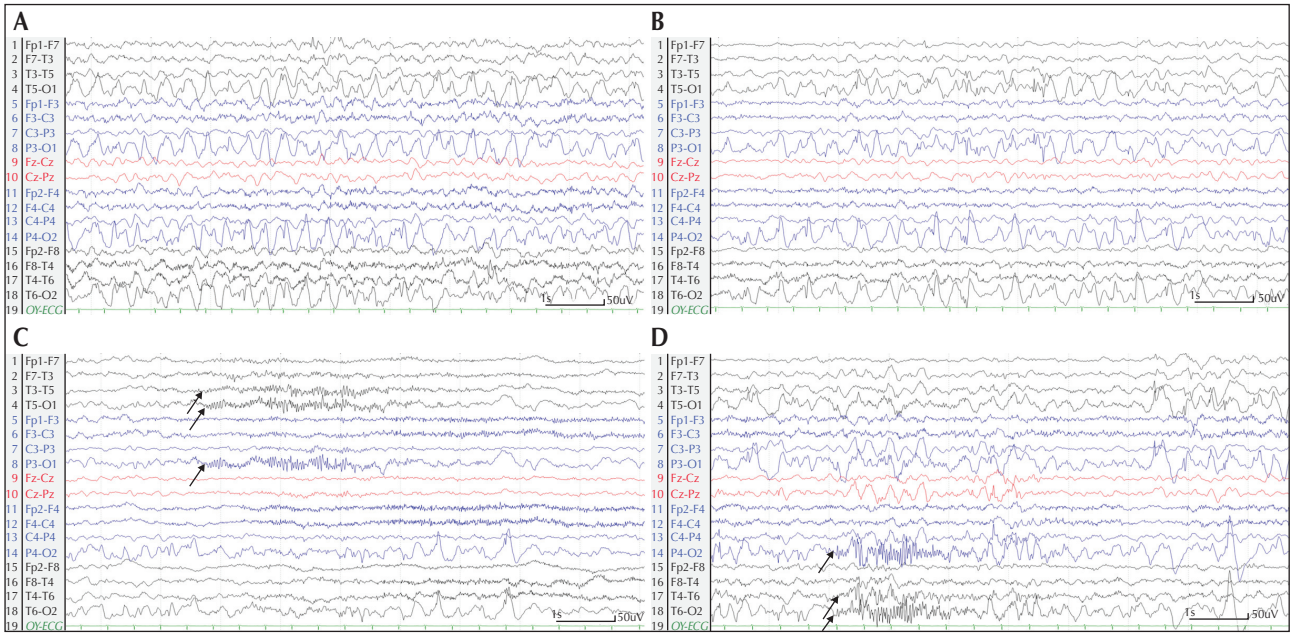

Figure 1. EEG at the age of 10 months (high frequency filter = 70 Hz, low frequency filter = 1.6 Hz, sensitivity =10 uV/mm, and display speed = 30 mm/sec). (A) Background showing 4/5-theta activity at the bilateral occipital region. (B) Interictal EEG showing spike-slow waves and polyspike-slow waves in the posterior region in both sides. Ictal EEG shows a low-amplitude fast wave in the posterior region in both sides ([C] arrows pointing to the left side; [D] arrows pointing to the right side).

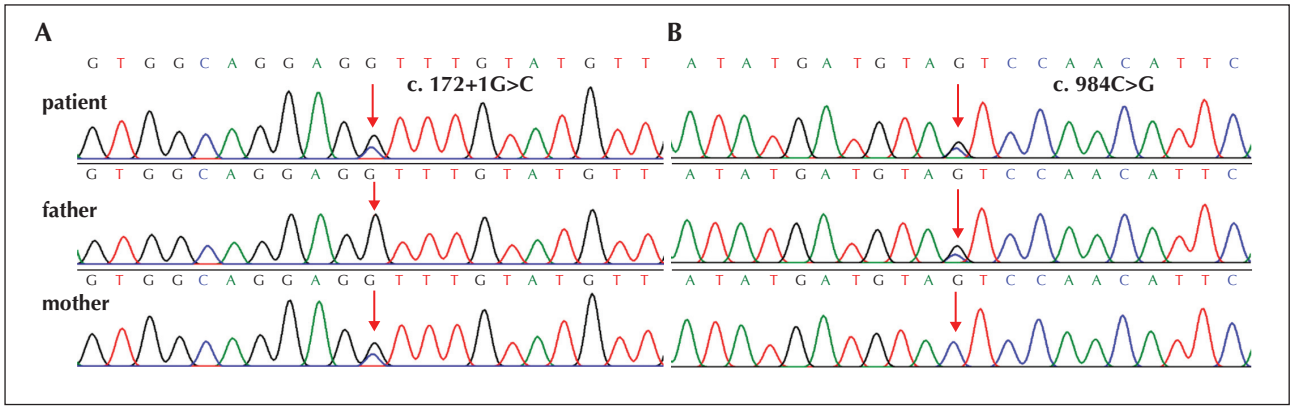

Figure 2. *WWOX* gene sequence showing the compound heterozygous mutation c.172+1G>C in the patient and his mother (A), and the c.984C>G mutation in the patient and his father (B) (red arrows indicate the mutation site).

widening of the subarachnoid space, a thin corpus callosum, delayed myelination, among other features. Ehaideb *et al*. (2018) studied 28 patients with *WWOX* mutation, including 20 female and eight male patients. The youngest onset age was two weeks, and oldest onset age was five months, with an average of two months. Only one patient had a compound heterozygous mutation with an onset age of one month. In the present study, the patient with compound heterozygous mutations also had onset at the age of one month. The mechanism of action of WWOX in the nervous system remains unclear, but may involve abnormal signalling proteins, neural pathway disorders, neuronal differentiation, and mitochondrial dysfunction or apoptosis (Tabarki *et al*., 2015, Tanna *et al*., 2018). In cases of WWOX deficiency, diseases of the central nervous system may be caused by abnormal interactions, such as those with the dishevelled segment polarity protein 2 and Tau protein which are involved in cortical development and neurodegeneration (Del Mare *et al*., 2009). Knockout mice showed growth retardation and died early, but no epileptic seizure activity was observed (Abdel-Salam *et al*., 2014).

This case report adds to the list of *WWOX* mutations related to epilepsy. Future studies of patients with *WWOX* mutations and epilepsy should be conducted

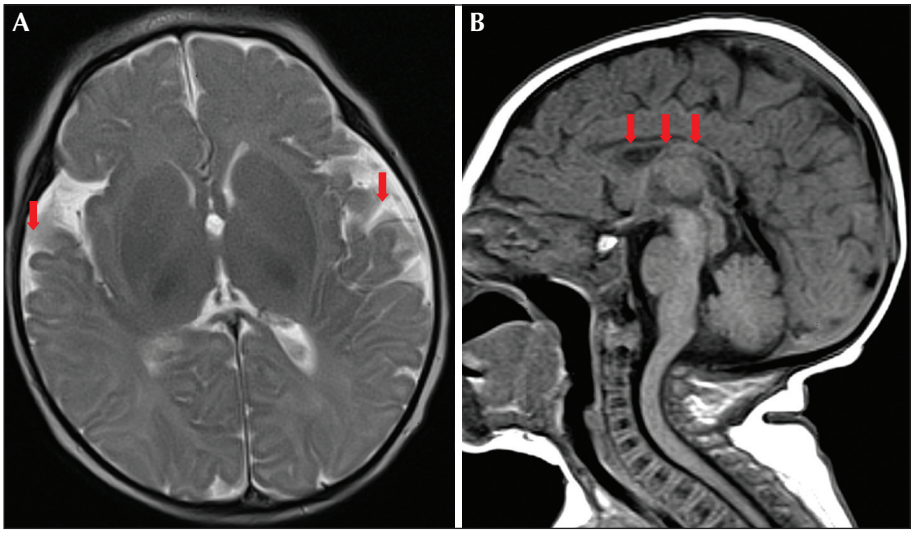

Figure 3. (A) Axial T2 brain MRI showing thinning of the corpus callosum. (B) Sagittal T1 brain MRI showing a widened subarachnoid space.

in order to further investigate genotype/phenotype correlations, and better establish the prevalence of *WWOX* mutations. □

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

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TEST YOURSELF

(1) Describe the structure and expression of the *WWOX* gene?

(2) What diseases are caused by *WWOX* gene mutation?

(3) What does MRI show in cases of *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".