Clinical commentary

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Biallelic *SZT2* variants in a child with developmental and epileptic encephalopathy

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ABSTRACT – Developmental and epileptic encephalopathy is a group of conditions characterized by the co-occurrence of epilepsy and intellectual disability, in which there is additional developmental impairment independent of epileptic activity. Biallelic variants of *SZT2*, a known seizure threshold regulator gene, have been linked to a wide spectrum of clinical features, ranging from severe intellectual disability with refractory seizures to mild intellectual disability without seizures. Here, we describe a child with developmental and epileptic encephalopathy whose genetic testing led to the identification of novel biallelic variants of *SZT2*, a paternally inherited c.2798C>T, p.(Ser933Phe) variant and a maternally inherited c.4549C>T, p.(Arg1517Trp) variant. Our patient showed common clinical and radiographic features among patients with *SZT2*-related encephalopathy. However, neonatal-onset seizures and suppression-burst EEG activity, not previously associated with *SZT2*-related encephalopathy, were observed in this case. Although the seizures were controlled with carbamazepine, the developmental consequences remained profound, suggesting that the developmental impairments might be attributed to a direct effect of the *SZT2* variants rather than the epileptic activity. We propose that *SZT2* variants should be regarded among those that are believed to cause neonatal-onset developmental and epileptic encephalopathy with a suppression-burst pattern on EEG.

Key words: developmental and epileptic encephalopathy, electroencephalogram, epilepsy, intellectual disability, suppression-burst, *SZT2*

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Epileptic encephalopathy (EE) is a group of severe epilepsies often associated with developmental plateauing or regression. The underlying concept of this condition is that the epileptic activity itself interferes with development; therefore, suppression of the epileptic activity may have the potential to improve the developmental consequences of the disorder (Scheffer *et al*., 2017). However, many severe

genetic disorders also have developmental consequences arising directly from the effect of the genetic variants, in addition to the effects of the frequent epileptic activity on development. Thus, the new concept of "developmental and epileptic encephalopathy" (DEE) was proposed to refer to such a condition in which there is additional developmental impairment, independent of the epileptic activity (Scheffer *et al*., 2017). It is therefore suggested that the term "DEE" can be used to describe instances when the developmental consequences may remain profound even after the epileptic activity subsides, relatively early on in a child's history (Scheffer *et al*., 2017).

Recently, biallelic variants in the seizure threshold 2 (*SZT2*) gene were reported in patients with EE with dysmorphic features and intellectual disability (Basel-Vanagaite, *et al*. 2013; Venkatesan, *et al*. 2016; Imaizumi, *et al*. 2018; Kariminejad, *et al*. 2018; Nakamura, *et al*. 2018; Pizzino, *et al*. 2018; Tsuchida, *et al*. 2018). Based on a chemical mutagenesis screen, *SZT2* was identified as a gene that confers a low seizure threshold in mice (Frankel, *et al*. 2009). *SZT2* is expressed in various tissues with the highest level of expression in the brain, beginning in the embryonic period, and is therefore proposed to play an important role in epileptogensis and brain function (Frankel, *et al*. 2009).

Here, we describe the case of a four-year-old girl patient with DEE who harbours biallelic *SZT2* variants. Her initial seizures occurred in the early neonatal period and showed a suppression-burst pattern on electroencephalogram (EEG); her seizures were effectively controlled with an antiepileptic drug. However, her psychomotor development was severely delayed, suggesting that developmental impairments might be attributed to the direct effects of the *SZT2* variants rather than the epileptic activity.

Case study

A four-year-old girl, the only child of healthy, non-consanguineous parents, was born at 40 weeks of gestation after an uneventful pregnancy. Her birth weight, length, and head circumference were 3,348 g (+0.8 SD), 48.2 cm (+0.6 SD), and 35.5 cm (+1.9 SD), respectively. There were no signs of perinatal distress. Four days after birth, she developed focal seizures with impaired awareness, which were characterized by staring and eye blinking, followed by tonic or clonic seizures affecting one or both limbs on the left side of her body. Her seizures typically lasted for 30-40 seconds and occurred multiple times per day. She occasionally had bilateral tonic-clonic seizures. The patient's interictal EEG results indicated a suppression-burst pattern, comprising bursts of high-amplitude spikes and polyspikes that alternated at a regular rate with periods of electric suppression (*figure 1*). Ictal recordings revealed fast focal, rhythmic activities that originated from the right central regions and eventually spread to the right hemisphere (*supplementary figure 1*). The seizures were unresponsive to phenobarbital and midazolam, whereas carbamazepine (CBZ) treatment effectively decreased the frequency of the seizures. When the dose was eventually increased (5 mg/kg body weight, daily), the seizures were controlled; after 15 days of CBZ treatment, no further seizures occurred. Initial brain magnetic resonance imaging (MRI) performed on Day 6 showed a thin corpus callosum (CC) and a persistent cavum septum pellucidum (CSP) (*figure 2A, C*). At one year of age, the patient's follow-up MRI also demonstrated diffuse brain atrophy with dilated ventricles (*figure 2B, D*) and the EEG showed multifocal epileptiform discharges.

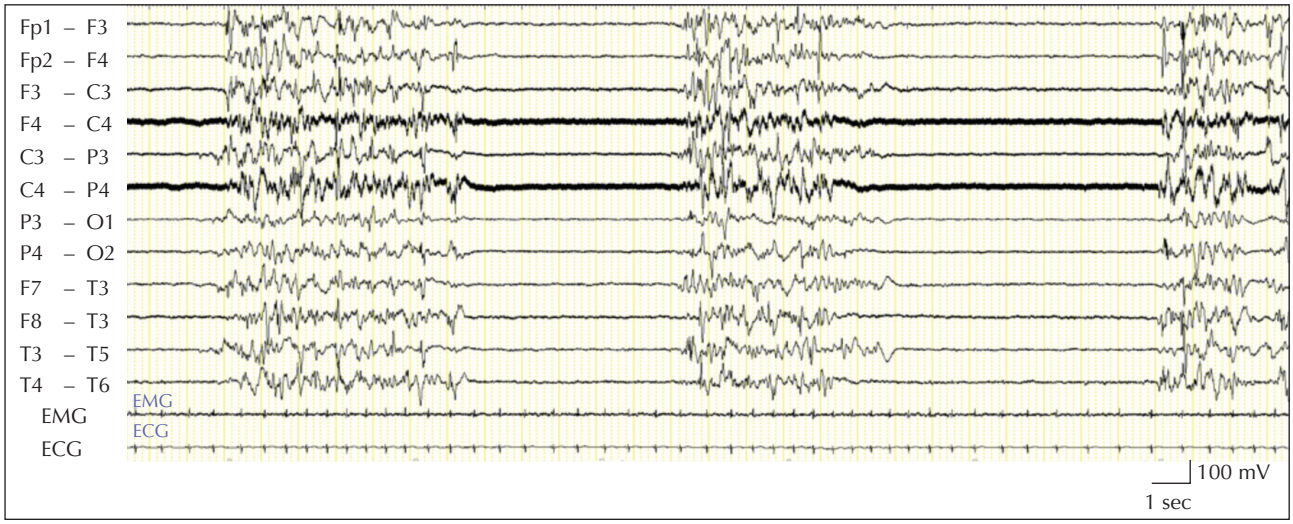

Figure 1. EEG recording, five days after birth, indicates a suppression-burst pattern.

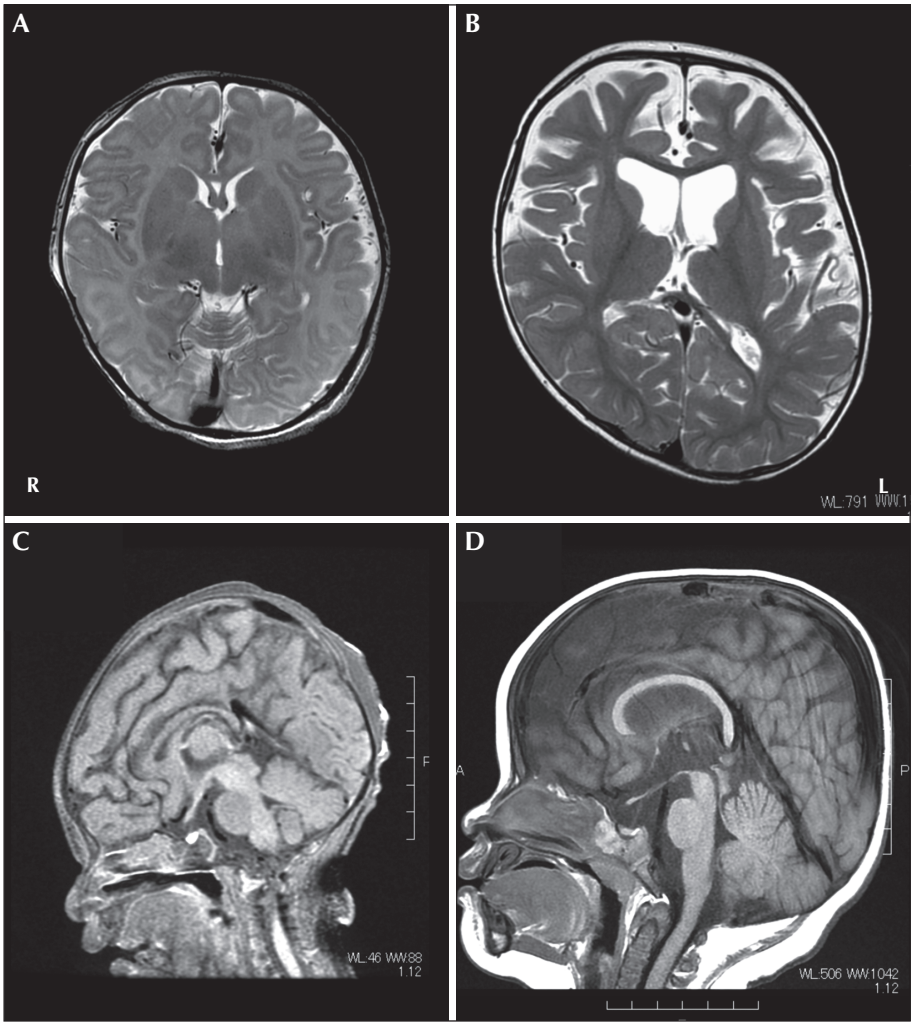

Figure 2. Brain MRI of the patient with biallelic *SZT2* variants. Initial MRI on Day 6 demonstrates a thin corpus callosum and persistent cavum septum pellucidum (A, C). The follow-up MRI at one year of age demonstrates diffuse brain atrophy with dilated ventricles (B, D). (A, B) T2-weighted axial images; (C, D) T1-weighted sagittal images.

Additionally, she had dysmorphic facial features, including a high forehead, slightly down-slanting palpebral fissures, and laterally extended eyebrows. A neurological examination revealed generalized hypotonia, but deep tendon reflexes of her limbs were intact and no pathological reflexes were detected. Extensive metabolic investigations, including serum amino acid quantification, serum acylcarnitine profile quantification, and urine organic acid quantification, revealed no abnormalities. The patient was able to sit up unassisted but was unable to walk. Her speech did not contain meaningful words, and she interacted poorly with others.

A cytogenetic study and SNP array test showed normal results. To determine a possible underlying genetic aetiology, whole-exome sequencing was conducted in the patient and her asymptomatic parents. We found that the patient harboured compound heterozygous variants of *SZT2*, the only gene in which rare biallelic variants were observed in the exome. The patient harboured a paternally inherited NM_015284.3:c.2798C>T (p.Ser933Phe) variant and a maternally inherited NM_015284.3:c.4549C>T (p.Arg1517Trp) variant; neither variant has been previously reported. The paternal variant, p.Ser933Phe, is predicted to be tolerated based on *in silico* analysis using Polyphen-2 and SIFT algorithms. However, amino acid Ser933 is conserved from human to Xenopus, suggesting that it is important for protein function. This variant is not listed in the Genome Aggregation Database (gnomAD) or database of single nucleotide polymorphisms (dbSNP); therefore, it is considered a variant of unknown significance

(VUS). The other maternal variant, p.Arg1517Trp, is located within the GATOR1-binding region (amino acids 935-1734), relevant to SZT2 function (Peng *et al*., 2017), and is predicted to be deleterious based on *in silico* analyses. This variant is registered in the dbSNP as rs79351309, with an allele frequency of 0.00007% (18/244586 alleles), and all alleles are reported to be heterozygous. Therefore, the maternal variant, p.Arg1517Trp, is likely to be pathogenic.

Discussion

To date, biallelic *SZT2* variants have been found in 13 patients presenting with EE and/or intellectual disabilities, from 11 families (Basel-Vanagaite, *et al*. 2013; Falcone, *et al*. 2013; Venkatesan, *et al*. 2016; Imaizumi, *et al*. 2018; Kariminejad, *et al*. 2018; Nakamura, *et al*. 2018; Pizzino, *et al*. 2018; Tsuchida, *et al*. 2018). Brain MRI of these patients demonstrated characteristic findings that included CC deformities in seven patients (five cases with thick CC and two cases with thin CC) and persistent CSP in five patients (*supplementary table 1*). In the present case, one possible pathogenic *SZT2* variant, an *in trans* VUS, was associated with common clinical features among patients with *SZT2*-related EE, including early-onset seizures, a profound delay in psychomotor development, and dysmorphic facial features. In addition, her brain MRI demonstrated a thin CC and persistent CSP. These clinical and radiographic features suggested that the genetic cause of her DEE was likely to be *SZT2*. The VUS of *SZT2* was possibly responsible for the patient's phenotype, however, the pathogenic extent of this variant is unclear. Functional validation assays are required to confirm whether the *SZT2* VUS contributes to the clinical phenotype, however, these assays are currently unavailable.

The clinical features of patients with *SZT2* variants ranges from mild intellectual disability without seizures to severe intellectual disability with refractory seizures. Among the 13 patients reported with *SZT2* variants, 10 patients developed epilepsy (*supplementary table 1*) (Basel-Vanagaite, *et al*. 2013; Venkatesan, *et al*. 2016; Imaizumi, *et al*. 2018; Kariminejad, *et al*. 2018; Nakamura, *et al*. 2018; Pizzino, *et al*. 2018; Tsuchida, *et al*. 2018). The initial seizures in these patients developed during infancy or early childhood at a median age of eight months (range: two months to 10 years). Focal seizures with impaired awareness or bilateral tonic-clonic seizures were the most frequently observed seizure type. EEG studies showed focal or multifocal epileptic discharges; a suppression-burst pattern has not been observed previously. Four patients were seizure-free following treatment with valproic acid, lamotrigine, clobazam, or phenobarbital, however, the seizures were refractory to multiple antiepileptic drugs in the other six patients. In the present case, the initial seizures, which were mainly focal to bilateral tonic-clonic seizures, occurred in the early neonatal period. EEGs showed a suppression-burst pattern at the onset of seizures and multifocal paroxysmal abnormalities thereafter. Treatment with CBZ controlled seizures effectively. However, her psychomotor development was severely delayed, and she was unable to crawl, engage in eye pursuit, or use meaningful words. Serial MRI demonstrated brain atrophy, which appeared to progress independently of the seizures. This suggests that the progressive brain atrophy may be attributed to a direct effect of the *SZT2* variants rather than the epileptic activity. There was no significant difference in clinical severity between the present case and previous cases without neonatal period-onset epilepsy and suppression-burst pattern (*supplemental table 1*). A loss-of-function variant of *SZT2* has also been shown to cause intellectual disability without seizures (Falcone *et al*., 2013). Thus, SZT2 is likely to play a crucial role in brain structure and function as well as epileptogenesis.

In conclusion, the present case expands the clinical spectrum of *SZT2*-related encephalopathy and suggests that *SZT2* variants should be regarded as causative of neonatal-onset DEE with suppression-burst on EEG. □

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

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

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TEST YOURSELF

(1) What is the difference between "epileptic encephalopathy" and "developmental and epileptic encephalopathy"?

(2) What are the characteristic features of patients with biallelic *SZT2* variants?

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".