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Early-life epileptic encephalopathy secondary to *SZT2* pathogenic recessive variants

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ABSTRACT – Advances in genetic testing have led to the identification of increasing numbers of novel gene mutations that underlie infantile-onset epileptic encephalopathies. Recently, a mutagenesis screen identified a novel gene, *SZT2*, with no known protein function that has been linked to epileptogenesis in mice. Thus far, two clinical reports have identified children with different recessive mutations in *SZT2* and varying clinical phenotypes. One case report described patients with epileptic encephalopathy and the other noted patients with cognitive deficiencies, but normal MRI and no epilepsy. This case report identifies novel mutations (a compound heterozygous frameshift and a nonsense variant) in the *SZT2* gene with distinct clinical and radiographic findings relative to those previously reported. Our patient presented with intractable epilepsy at 2 months of age. Seizures were refractory to numerous antiepileptic medications and the patient finally achieved seizure cessation at age 3 years with a combination of divalproex and lamotrigine. Our patient had similar facial dysmorphisms (macrocephaly, high forehead, and down-slanted palpebral fissures) to a previous case with truncating mutation. While developmental delay and cognitive deficiencies were present, our case had unique MRI findings suggesting migrational abnormalities not previously reported in other cases.

Key words: SZT2, seizures, infants, epileptic encephalopathy, neuronal migration, periventricular heterotopia

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Infantile-onset epileptic encephalopathies are characterized by recurrent, often intractable, seizures and global developmental impairments (Mastrangelo and Leuzzi, 2012). Examples of these encephalopathies include Ohtahara syndrome, early myoclonic epileptic encephalopathy, and Dravet syndrome (Mastrangelo and Leuzzi, 2012; Nordli, 2012). This diverse group of conditions can be a result of aetiologies that include structural brain malformations and metabolic

and causes. With advances in genetic testing, many causative genes have been identified, including Aristaless-related homeobox (*ARX*), cyclin-dependent kinase-like 5 (*CDKL5*), syntaxin-binding protein 1 (*STXBP1*), polynucleotide kinase 3′-phosphatase (*PNKP*), sodium channel neuronal type 1α subunit (*SCN1A*), protocadherin 19 (*PCDH19*), and pyridoxamine 5-prime-phosphate oxidase (*PNPO*) (reviewed in Mastrangelo and Leuzzi, 2012). While mutations in some genes, such as *PNPO,* are linked to a fairly narrow phenotype with characteristic clinical and EEG features (Mills *et al*., 2014), others such as *ARX* have a broader clinical spectrum (Charzewska *et al*., 2013). Other genes have been linked to a phenotype characterized by intractable epilepsy, intellectual disability, facial dysmorphisms, and migrational abnormalities. These include the C6orf70 gene (Conti *et al*., 2013) and the *RAI1* gene, the causative gene of Smith-Magenis syndrome (Capra *et al*., 2014).

Recently, a mutagenesis screen identified a novel gene, *SZT2,* with no known protein function that has been shown to affect seizure threshold in mice (Frankel *et al*., 2009). The mutant allele of *SZT2* contains a splice donor mutation downstream of exon 32, with predicted transcriptional read-through and a translational frameshift and premature stop codon (Frankel *et al*., 2009). Thus far, two clinical reports have identified children with different recessive mutations in *SZT2* and varying clinical phenotypes (Basel-Vanagaite *et al*., 2013; Falcone *et al*., 2013). In one report, epileptic encephalopathy in two unrelated individuals with similar MRI findings, that included short and thick corpus callosum and persistent cavum septum pellucidum, was described (Basel-Vanagaite *et al*., 2013). In another, three children with macrocephaly and cognitive deficiencies, but no epilepsy and normal MRI and EEG, were described (Falcone *et al*., 2013).

This case report identifies novel recessive variants in the *SZT2* gene (a compound heterozygous frameshift and a nonsense variant) with distinct clinical and radiographic findings relative to those previously reported. We compare our case with those previously reported and identify common and distinctive elements that may provide more insight into the clinical features of *SZT2* mutation causing early-onset epileptic encephalopathy. We also describe antiepileptic medications used in our patient to control seizures.

Case study

The patient is currently 3 years old. He first presented to our hospital at age 2 months with new-onset seizures, characterized by eye deviation and bilateral limb flexion lasting a few seconds. Initial EEG performed at the time of presentation did not capture any seizures but showed interictal abnormalities consisting of asynchronous sleep spindles and vertex waves (better formed on the right) and decreased complexity of waveforms in the left hemisphere. Seizures continued despite initial treatment with phenobarbital (maximum dose: 5.8 mg/kg/day). Subsequent video-EEG monitoring at 2.5 months of age recorded asymmetric tonic seizures (*figure 1a*). Levetiracetam (maximum dose: 56.1 mg/kg/day) and pyridoxine (dose: 17 mg/kg/day) were added with no added benefit for seizure control. Phenobarbital was discontinued and topiramate was added. Follow-up EEG performed at 7 months of age was similar to that of previous studies. As the dose of topiramate was titrated up (maximum dose: 2.8 mg/kg/day), seizures increased. EEG performed at 9 months of age showed abundant multifocal epileptiform discharges and background slowing with shifting laterality. He was weaned off topiramate and placed on a combination of levetiracetam and lamotrigine. By 15 months of age, seizure semiology had changed to the following: staring, unresponsiveness to verbal or tactile stimulation, hands fisted, and arms flexed. He gradually moved into the foetal position and had slight jerking or tremor. Seizures lasted less than one minute. At this time, divalproex was started and levetiracetam and pyridoxine were weaned off. Presently, at age 3 years, he is on a combination of divalproex (maximum dose: 17.4 mg/kg/day) and lamotrigine (maximum dose: 4.9 mg/kg/day), and remains seizure-free for over a year. The most recent EEG was performed at 3 years old and showed a moderately disorganized background and slow posterior dominant rhythm for age (7 Hz), with multifocal epileptiform discharges consistent with a diffuse epileptogenic encephalopathy (*figure 1b*).

Birth history was remarkable for pregnancy, complicated by advanced maternal age and hypertension. He was born via induced vaginal delivery at 38 weeks. Neonatal course was unremarkable. Developmental history was notable for delayed acquisition of motor and cognitive milestones. From a gross motor standpoint, he sat unassisted at 9 months of age and crawled at 12 months of age. He walked with assistance at age 3 years. Fine motor skills also showed delays. From a cognitive standpoint, he is non-verbal at age 3. He recognizes family members and smiles and laughs appropriately. Family history was negative for neurological disease.

General examination was remarkable for macrocephaly, dysmorphic features including slightly downslanted palpebral fissures, mild hypertelorism, and frontal bossing. On mental status examination, he was visually attentive and made appropriate eye contact. He did not speak, but played with toys. He had frequent stereotyped hand clapping behaviour. Cranial nerve examination was unremarkable. Motor

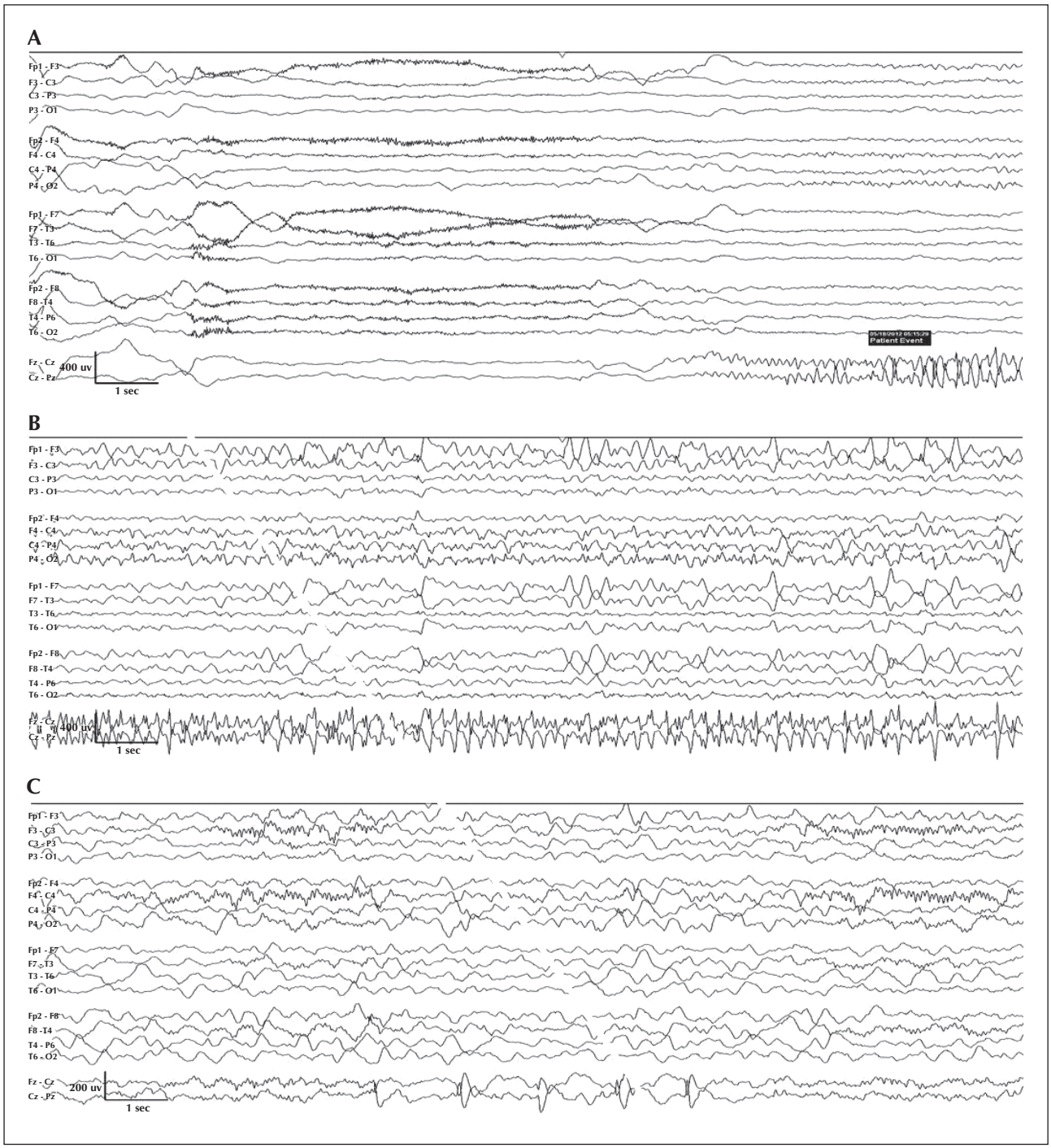

Figure 1a. Serial EEG from initial presentation and the latest follow-up visit. Overnight video-EEG was performed during hospital admission for breakthrough seizures within two weeks of initial epilepsy diagnosis (2.5 months old) and captured an electroclinical tonic seizure characterized by unresponsiveness and staring for six seconds, followed by asymmetric (left>right) limb extension with vibratory stiffening. Electrographically, at onset (A), there was diffuse attenuation, subsequently maximal at the vertex, followed by rhythmic discharges that gradually spread to the bilateral central region; on the right more than the left (B). Sleep background at 2.5 months old (C) was significant for symmetric sleep spindles and intermittent epileptiform discharges maximal in the central vertex region.

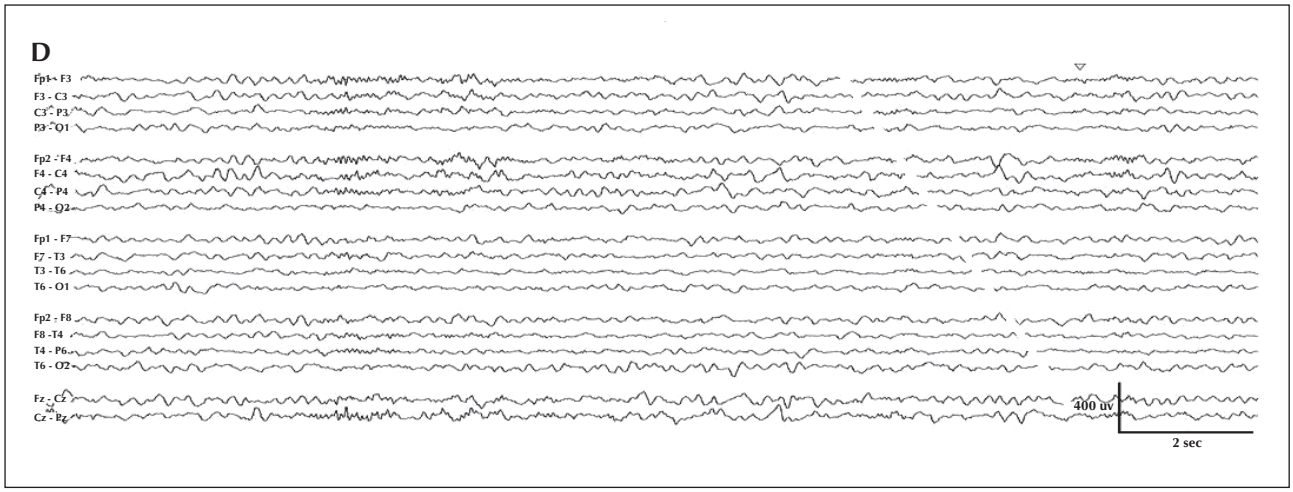

Figure 1b. The most recent EEG was performed at 3 years of age (D) and background activity during sleep was significant for subtle relative left hemispheric attenuation, occasional multifocal epileptiform discharges, and asymmetric sleep spindles and vertex waves, higher in amplitude on the right.

examination showed axial and appendicular hypotonia with normal bulk and fair strength. He had a right hand preference. Assessment of sensation, coordination, gait, and deep tendon reflexes showed no abnormalities. He was able to stand and take steps with assistance.

Initial MRI of the brain at 2 months of age showed subtle hemispheric size discrepancy, with the left cerebrum smaller than the right and a pars intermedia/Rathke's cleft cyst (*figure 2*). Follow-up brain MRI at 11 months of age additionally showed linear radially oriented T2 hyperintensities within the white matter, but hemispheric asymmetries were no longer appreciated (*figure 2*). A third brain MRI study was performed at 2 years of age which showed right periventricular heterotopia and abnormal perisylvian gyral configuration without distinct cortical abnormality (*figure 2*).

He underwent extensive metabolic and genetic evaluation which was unremarkable. This included cerebrospinal fluid evaluation (for cell count, glucose, protein, neurotransmitters, lactate, and pyruvate) and analysis of serum lactate and pyruvate, plasma amino acids, urine organic acids, acylcarnitine profile, serum pipecolic acid, and *ALDHA1* gene sequencing using next-generation sequencing to investigate mutations associated with Fragile X and lysosomal storage diseases. Whole-exome sequencing was performed on patient-parents trio and revealed that he was compound heterozygous for a paternally inherited c.3509_3512delCAGA (p.T1170RfsX22) (NM_015284.3) variant and a maternally inherited c.9703 C>T (p.R3235X) (NM_015284.3) variant in the *SZT2* gene. These variants have not been documented in the NHLBI Exome Sequencing Project control database. No *de novo* or other rare variants were identified in this gene or other reported genes associated with the patient's phenotype. Given these findings and reports of other individuals with similar phenotypic features, these variants were interpreted as disease-causing mutations. The paternally inherited variant causes a frameshift and creates a premature stop codon. The maternally inherited variant is predicted to cause loss of normal protein function, either through protein truncation or nonsense-mediated mRNA decay.

Discussion

Mutation in the *SZT2* gene was originally identified in a mutagenesis screen in C57BL/6J mice for epilepsy susceptibility (Frankel *et al*., 2009). These mice had both lowered acute seizure threshold and decreased latency to kindling (Frankel *et al*., 2009). *SZT2* encodes a highly conserved protein of unknown function that was shown to have no sequence similarity to any known protein (Frankel *et al*., 2009). Clinical reports show phenotypic diversity as a result of autosomal recessive mutations in this gene. Our patient was compound heterozygous for the paternally inherited c.3509_3512delCAGA (p.T1170RfsX22) variant and maternally inherited c.9703 C>T (p.R3235X) variant in the *SZT2* gene. The paternally inherited variant causes a frameshift and creates a premature stop codon. The maternally inherited variant is predicted to cause loss of normal protein function. Different types of mutations may have different phenotypic effects. For example, in some cases, missense mutations result in an alteration that may have a less severe effect on the protein's structure and function, compared to truncating mutations that may result in little or no protein

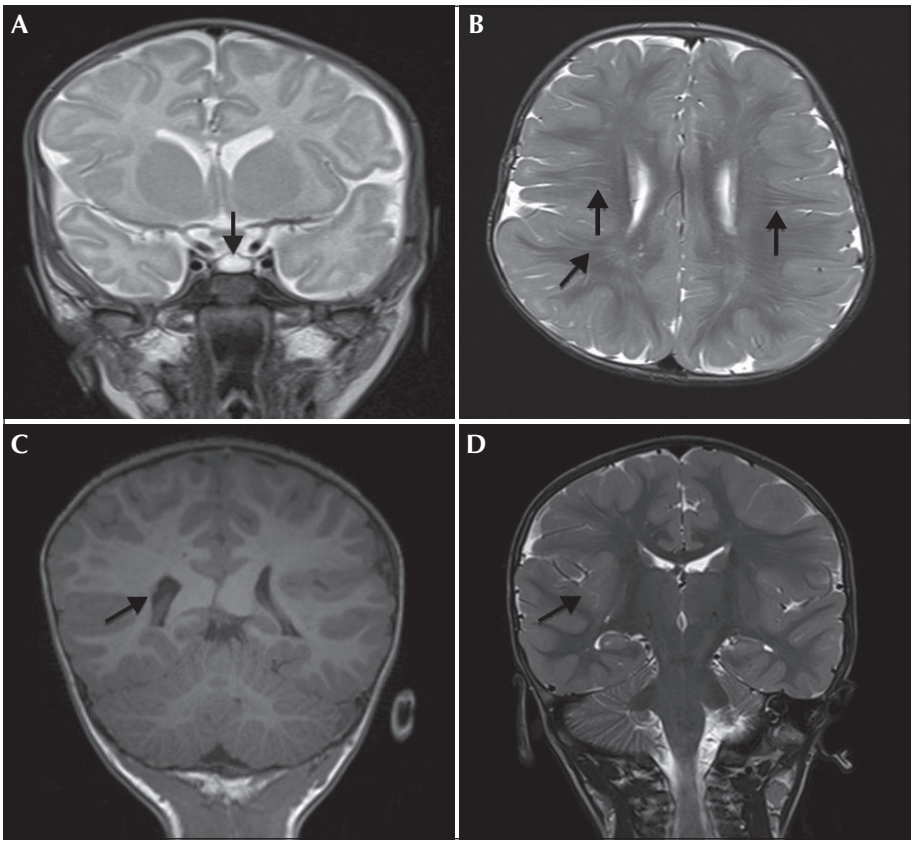

Figure 2. MRI of the patient at different time points. (A) MRI performed at 2 months of age showing Rathke's cleft cyst (arrow) and asymmetric hemisphere size (left cerebrum smaller than right). (B) MRI performed at 11 months of age showing linear, radially-oriented T2 hyperintensities within the white matter with unclear significance (arrows). (C, D) MRI performed at 2 years of age shows right periventricular heterotopia (arrow in C) and abnormal perisylvian gyral configuration (C and arrow in D).

function. It is hypothesized that mutations which may retain some residual SZT2 function may lead to a milder phenotype of mild-to-moderate intellectual disability without seizures (Falcone *et al*., 2013).

Basel-Vanagaite *et al*. described two individuals with *SZT2* mutations who had intractable seizures refractory to multiple drug combinations, although the antiepileptic drugs used in these patients were not discussed (Basel-Vanagaite *et al*., 2013). One patient had a homozygous nonsense mutation c.73C>T (p.Arg25) and the other had a compound heterozygote mutation c.2092C>T (p.Gln698) and c.1496G>T (p.Ser499Ile) (Basel-Vanagaite *et al*., 2013). Seizure onset occurred at two months in one patient, as in our case, and four years in the other. Duration of seizures in the patients was unclear, however, the patient with seizure onset at two months had EEG abnormalities at age 8. We observed a change in seizure semiology over time that included focal and generalized convulsions, tonic seizures, and atypical absences. Seizures in our patient were controlled with a combination of divalproex and lamotrigine. Given the lack of available information about this condition and the relatively short follow-up period, it is unclear whether there is an age-dependent expression of epilepsy in patients with *SZT2* mutation. The family reported by Falcone *et al*. did not have epilepsy and had a milder phenotype characterized by cognitive delay and inattention (Falcone *et al*., 2013). This family had a homozygous mutation leading to deletion of a single phenylalanine residue (c.4202_4204delTTC [p.Phe1401del]) in SZT2 (Falcone *et al*., 2013). Both patients previously reported with epileptic encephalopathy had similar dysmorphic features, including macrocephaly, high forehead, ptosis and down-slanting palpebral fissures, severe developmental delay, hypotonia, and decreased tendon reflexes (Basel-Vanagaite *et al*., 2013). These features are similar to our patient. However, while MRI features reported by Basel-Vanagaite *et al*. showed thick corpus callosum and persistent cavum septum pellucidum, these were not present in our patient. MRI features in our patient of periventricular heterotopia

and abnormal perisylvian gyral configuration suggest cortical migrational abnormality.

In conclusion, patients with *SZT2* gene mutations can have a spectrum of deficits ranging from mild cognitive and behavioural deficiencies to epileptic encephalopathy and profound delays, variable dysmorphic features, and MRI findings. Seizures, when present, can be refractory to AEDs, however, our patient appeared to have the best response to a combination of divalproex and lamotrigine. □

Disclosures.
None of the authors have any conflict of interest to disclose.

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TEST YOURSELF

(1) Which genes are commonly associated with infantile-onset epileptic encephalopathies?

(2) How was the link between the *SZT2* gene and epilepsy first made?

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".