Epilepsy & Behavior 108 (2020) 107097

Contents lists available at ScienceDirect

Epilepsy & Behavior

journal homepage: www.elsevier.com/locate/yebeh

Review

Developmental and epileptic encephalopathy due to *SZT2* genomic variants: Emerging features of a syndromic condition

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ARTICLE INFO

Article history:
Received 1 April 2020
Revised 4 April 2020
Accepted 4 April 2020
Available online 11 May 2020

Keywords:
Developmental and epileptic encephalopathy
SZT2
Macrocephaly
Corpus callosum abnormalities
mTOR

ABSTRACT

Seizure threshold 2 (*SZT2*) gene mutations have been associated with developmental and epileptic encephalopathies (DEEs). Following a literature review, we collected 22 patients and identified the main clinical features related to *SZT2* variants that are epilepsy with onset within the first years of life, intellectual disability (ID), macrocephaly with dysmorphic facial features, corpus callosum (CC) shape abnormalities, and cortical migration disorders. Moreover, we identified the c.7825T>G homozygous missense variant in *SZT2* in two female siblings presenting with focal seizures, mild–moderate ID, behavioral disturbances, and facial dysmorphisms. Interictal Electroencephalogram (EEG) and ictal EEG were both informative and revealed, respectively, temporal bilateral asynchronous slow and epileptiform abnormalities and a focal onset in both of them. Neuroimaging study revealed a thick and abnormally shaped CC.

Seizure threshold 2 has been identified as a component of the KICSTOR complex, a newly recognized protein complex involved in the mammalian target of rapamycin (mTOR) pathway. mTOR signaling dysregulation represents common pathogenetic mechanisms that can explain the presence of both epileptogenesis and ID. Even if few cases had been reported, a new clinical phenotype is emerging, and recent hypothesis of hyperactivation of mTORC1 signaling might also open to targeted treatments, challenging an early diagnosis as of paramount importance.

1. Introduction

Developmental and epileptic encephalopathies (DEEs) in infancy are extremely heterogeneous conditions because of different age at onset, seizure type, developmental and clinical course, and electroencephalographic correlates [1,2]. New genetic tools, and particularly whole exome sequencing (WES), have greatly enhanced the ability to identify pathogenic mutations even in rare epileptic phenotypes [3].

Seizure threshold 2 (*SZT2*) (*MIM 615463*) gene mutations have been recently associated with a neurological condition characterized by epilepsy with infantile onset, intellectual disability (ID), and dysmorphic corpus callosum (CC) [4].

Seizure threshold 2 encodes for a large protein that is widely expressed in the central nervous system (CNS) [5] recently identified as a subunit of KICSTOR complex, a new protein complex involved in mTOR pathway [6]. KICSTOR is supposed to be a negative regulator of the mTORC1; therefore, *SZT2* genetic variants could increase mTORC1 signaling in several tissues, including the brain, leading to possible malformation disorders [6].

The aim of this report was to describe clinical, neurophysiological, and radiological features of all previously reported cases with *SZT2* genetic variants in order to find out a proper clinical phenotype that could be suggestive for the identification of future mutated patients. Moreover, we included the case reports of two sisters with epilepsy

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https://doi.org/10.1016/j.yebeh.2020.107097
1525-5050/

Table 1
Demography and general clinical findings.

Author	Sex	Parental cons	Origin	Pregnancy	Weeks of gestation/delivery/weight at birth	OFC at birth	Age at last follow-up	Development	Dysmorphic features	Additional clinical features
Basel-Vanagaite et al. 2013 (Fam 1; II:6)	F	No	Iraqi Jewish	Polyhydramnios	32w/n.a./1500 g (−1 SD)	31 cm (+1.8 SD)	10 yrs	Severe DD	High forehead, down slanting palpebral fissures, ptosis, arched and laterally extended eyebrows	Scoliosis Hypertrichosis
Basel-Vanagaite et al. 2013 (Fam 2; II:1)	M	No	Spanish	Oligohydramnios	40w/n.a./3280 g (−0.1 SD)	35 cm (+0.6 SD)	9 yrs	Severe DD	High forehead, down slanting palpebral fissures, ptosis, arched and laterally extended eyebrows	Scoliosis Hypotonia
Falcone et al. 2013 (Fam 3; II:1)	M	Yes	Southern Italy	Normal	n.a./C-section with anoxia/n.a.	n.a.	18 yrs	Severe DD, behavioral disturbances	Macrocephaly	None
Falcone et al. 2013 (Fam 3; II:2)	M	Yes	Southern Italy	Normal	n.a./uncomplicated delivery/n.a.	n.a.	10 yrs	Speech delay and inattention IQ: 75 (10 yrs)	Macrocephaly	None
Falcone et al. 2013 (Fam 3; II:3)	M	Yes	Southern Italy	Normal	n.a./uncomplicated delivery/n.a.	n.a.	7 yrs	Speech delay and inattention	Macrocephaly	None
Venkatesan et al. 2016	M	No	n.a.	Normal	38w/uncomplicated delivery/n.a.	n.a.	3 yrs	Severe DD, speech delay, walk with assistance	Macrocephaly, high forehead, down-slanted palpebral fissures	Hypotonia
Tsuchida N et al. 2018	F	No	Japanese	n.a.	32w/n.a./1398 g (−1.3 SD)	29.6 cm (+0.2 SD)	4 yrs	Severe DD, absent speech, walking with support	High forehead, bilateral ptosis, down slanting, palpebral fissures, and arched and extended eyebrows	Hypotonia
Tsuchida N et al. 2018	M	No	Japanese	Maternal HELLP syndrome Neonatal asphyxia at birth	28w/n.a./1492 g (+2.7 SD)	30 cm (+3.2 SD)	2 yrs	Severe DD, bed-ridden, no head control	High forehead, hypospadias, cryptorchidism	Hypotonia, Chorea
Tsuchida N et al. 2018	F	Yes	Malasya	Normal	40w/n.a./3200 g (+0.27 SD)	n. a.	5 yrs.	Severe DD Died at 9 yrs. (aspiration pneumonia)	Microcephaly, right Duane anomaly, high arched palate, right convergent squint	Silent patent ductus arteriosus. Hypotonia, Scoliosis
Nakamura et al. 2018	F	No	Japanese	Maternal pregnancy hypertension	29w/n.a./1060 g (+1.4 SD)	28 cm (+0.6 SD)	4 yrs	Severe DD	Macrocephaly, high forehead, hypertelorism, and macrocephaly Pectus carinatum	Walk unassisted with slightly wide-based gait.
Pizzino et al. 2018	F	n.a.	n. a.	n. a.	n.a./n.a./n.a.	n. a.	8 yrs	Severe DD Died at 8 yrs	Macrocephaly, frontal bossing, hypertelorism, microphthalmia, depressed nasal bridge, long tapered fingers, and hyperextensible joints	Hypotonia Progressive encephalopathy Choreoathetosis areflexia.
Naseer et al. 2018 (Fam 1; IV: 1)	F	Yes	Saudi Arabia	Normal	n.a./uncomplicated delivery/3600 g	37 cm	8 yrs	Floppiness and DD	Macrocephaly	Hypotonia
Naseer et al. 2018 (Fam 1 IV: 2)	F	Yes	Saudi Arabia	Normal	n.a./uncomplicated delivery/3.500 g	36 cm	5 yrs.	Floppiness and DD	Macrocephaly	Hypotonia
Kariminejad et al. 2018	M	No	Iranian	Normal	9 mths/n.a./2650 g (+1.16 SD)	35 cm (+1.1 SD)	5 yrs	DD, absent speech	Prominent forehead, sandal gap in feet	Autistic features
Imaizumi et al. 2018	M	No	Japanese	Normal	39w/n.a./3.310 g (75–90th)	35.5 cm (90–97th)	15 yrs	DD	Macrocephaly, high forehead	Autistic features
Uittenbogaard et al. 2018	M	No	n.a.		Full-term/n.a./n.a.	n.a.	4 yrs	Severe DD, absent speech	n. a.	1 h of life cardiac arrest
Domingues et al. 2019 (Fam 1; II: 1)	M	No	n.a.	Normal	n.a/uncomplicated pregnancy/n. a.	+4.3 SD	29 yrs	Moderate DD	Macrocephaly	Autism spectrum disorder
Domingues et	M	No	n.a.	Normal	n.a/uncomplicated	+4.4 SD	19 yrs	Severe DD,	Macrocephaly	Agenesis of left

Table 1 (*continued*)

Author	Sex	Parental cons	Origin	Pregnancy	Weeks of gestation/delivery/weight at birth	OFC at birth	Age at last follow-up	Development	Dysmorphic features	Additional clinical features
al. 2019 (Fam 1; II.4)					pregnancy/n.a.			absent speech, autistic traits		kidney, paraplegia, bladder dysfunction
Iodice et al. 2019	F	No	Ukrainian	Normal	38w/uncomplicated pregnancy/2100 g	33 cm	4 yrs	DD, absent speech	Macrocephaly, prominent forehead, frontal bossing, down slanting palpebral fissures, prognathism, dental malposition, ligamentous laxity	Severe hypotonia
Sun et al. 2019	M	No	n. a.	Maternal pregnancy induced hypertension.	38w/C-section/3150 g	n. a.	2 yrs. 3 mths	Stagnation at the age 4 months, severe DD Died at 2 yrs 3 mths (SE)	High forehead, flattened nasal bridge, hypertelorism	Hypotonia
Sun et al. 2019	F	No	n. a.	Premature placental abruption	36w/C-section/2350 g	n. a.	2 yrs. 1mths	Stagnation at the age 5 months, absent speech	High forehead, flattened nasal bridge, hypertelorism	Hypotonia
Sun et al. 2019	M	No	Chinese	Premature placental abruption	31w/C-section/2000 g	n. a.	1 yr 9mths	DD/stagnation at the age 5 months, absent speech	High forehead	Hypotonia
Present Case#1 Fam 4; II:1	F	Yes	Southern Italy	Normal	42w/C-section/3035 g (−1.1 SD)	35 cm (+0.5 SD)	11 yrs	Walking (21 mths) First words (3 yrs) IQ: 58 (10 yrs 7 mths)	Macrocephaly, prominent forehead, frontal bossing, down slanting palpebral fissures, ptosis, prognathism, dental malposition, tapering fingers, long toes, ichthyotic skin on the knees	None
Present Case#2 Fam 4; II:2	F	Yes	Southern Italy	Normal	38w/C-section, mild hypoxia/2780 g (−0.8 SD)	36 cm (+2.2 SD)	6 yrs.	Walking (30mths) First words (3 yrs) DQ: 40 (5 yrs 2 mths)	Macrocephaly, prominent forehead, frontal bossing, synophrys of eyebrows, hypertelorism, down slanting palpebral fissures, ptosis, everted simple left ear, persistent finger pads, achromic skin lesion	Bilateral toe syndactyly

F = female, M = male, mths = months, yrs. = years, w = weeks, n.a. = not available, Cons = consanguinity, HELLP = hemolysis, elevated liver enzymes, and a low platelet count, C-section = cesarean section, SD = standard deviation, OFC = occipitofrontal circumference, PD = psychomotor delay, IQ = intelligent quotient, DD = developmental delay, SE = status epilepticus, DQ = developmental quotient.

and ID born from healthy consanguineous parents carrying a homozygous missense mutation in *SZT2* (NM_015284.3) identified through WES.

2. Material and methods

We reviewed clinical, electrophysiological, radiological, and genetic features of 22 previously reported patients with *SZT2* mutations [4,7–18].

We also report two unpublished patients carrying a homozygous c.7825T>G (p.Trp2609Gly) variant in *SZT2*. We reviewed all medical charts, and we performed a follow-up evaluation including clinical, neurophysiological, and neuroradiological investigations.

Neuroimaging studies included brain Magnetic resonance (MR), with Diffusion tensor imaging (DTI) sequences for the two siblings. The CC fiber bundles were reconstructed via Constrained Spherical Deconvolution algorithm by choosing a seed Region of interest (ROI) in the CC. Data from the volumetry of CC were compared with an age- and sex-matched control group. Further details are available in the Supplemental material.

This study was approved by the local institutional ethic committee. Written informed consent was obtained from parents.

3. Results

3.1. Literature review

Relevant clinical information of 22 previously reported patients are summarized in Table 1. Mean age at study was 7,8 years (range: 10

Table 2
Neurological findings and epilepsy.

	Epilepsy onset	Type of seizures	Seizure frequency	Interictal EEG	Ictal EEG	Treatments	Electrophysiological tests	MR findings	SZT2 mutation paternal allele	SZT2 mutation maternal allele
Basel-Vanagaite et al. 2013 (Fam 1; II:6)	4 yrs	Focal with SG	n.a.	Slow BA, R F-C sp-w	n.a.	Multiple ASMs	EMG normal ENG normal	Thick CC Persistent CSP	c.73C>T p.Arg25*	c.73C>T p.Arg25*
Basel-Vanagaite et al. 2013 (Fam 2; II:1)	2 mths	Focal with SG, tonic, atypical absences	Daily	Altered BA, multifocal spikes	n.a.	Multiple ASMs	EMG normal ERG normal	Thick CC Persistent CSP	c.1496G>T p.Gly412Alafs*86	c.2092C>T p.Gln698*
Falcone et al. 2013 (Fam 3; II:1)	–	–	–	Normal	n.a.	ATX ARI, BSP	n.a.	Normal	c.4202_4204delTT (p.Phe1401del)	c.4202_4204delTT (p.Phe1401del)
Falcone et al. 2013 (Fam 3; II:2)	–	–	–	Normal	n.a.	None	n.a.	Normal	c.4202_4204delTT (p.Phe1401del)	c.4202_4204delTT (p.Phe1401del)
Falcone et al. 2013 (Fam 3; II:3)	–	–	–	n.a.	n.a.	None	n.a.	Normal	c.4202_4204delTT (p.Phe1401del)	c.4202_4204delTT (p.Phe1401del)
Venkatesan et al. 2016	2 mths	Focal (staring, unresponsiveness, jerking)	n.a.	Async sleep spindles and vertex waves	Vertex	PB, LEV, PN, TPM, VPA, LTG	n.a.	R periventricular heterotopia, abnormal perisylvian gyral configuration	c.3509_3512delCAGA (p. T1170RfsX22)	c.9703 C>T (p.R3235X)
Tsuchida N et al. 2018	2 mths	Focal with SG	n.a.	Slowed (4–5 Hz) BA with sp-w L F-C	n.a.	Multiple ASMs	n.a.	Shortened and thickened CC	c.3700_3716del (p.Asn1234Alafs*35)	c.5482del (p. Gly1829Valfs*52)
Tsuchida N et al. 2018	3 mths	Cyanosis and stopping motion, adversive seizures	20–100 per day	Rhythmic fast A, rhythmic SW, bursts of Sp-w	n.a.	Multiple ASMs	n.a.	Diffuse brain atrophy, a thinned CC and a persistent CSP	c.3497dup (p.Glu1317Glyfs*4)	c.2929 + 1G>A (p. Leu939Aspfs*19)
Tsuchida N et al. 2018	8 mths	Tonic	Monthly	Intermittent Sp-w posterior quadrants	n.a.	CLZ	n.a.	Dilated lateral and third ventricles, thin CC	c.7303C>T (p.Arg2435Trp)	c.8162C>G (p.Ser2721Cys)
Nakamura et al. 2018	2 yrs. 10 m	Focal with SG	Multiple per month	Slowing BA	n.a.	VPA	n.a.	Short and thick CC	c.4181C>T (p.Pro1394Leu) c.2930-17_2930-3delinsCTCGTG	c.8596dup (p. Tyr2866Leufs*42)
Pizzino et al. 2018	20 mths	Focal motor seizures	n.a.	Multifocal ED	n.a.	Multiple ASMs, KD	n.a.	CC dysgenesis, loss of myelination progressive atrophy	c.5499del (p.Phe1834Serfs*47)	c.6916G>A (p. Gly2306Arg)
Naseer et al. 2018 (Fam 1; IV: 1)	n.a.	n. a.	n.a.	Diffuse slow BA and slowing C4, T4 and O2 multifocal spikes	n.a.	n.a.	n.a.	Prominent extra axial cerebrospinal fluid space with wide sylvian fissure	c.9368G>A (p.Gly3123Glu)	c.9368G>A (p.Gly3123Glu)
Naseer et al. 2018 (Fam 1; IV: 2)	n.a.	n. a.	n.a.	Fast A, slowing over C4, T4 and O2 multifocal spikes	n.a.	n.a.	n.a.	Prominent extra axial cerebrospinal fluid space with wide sylvian fissure	c.9368G>A (p.Gly3123Glu)	c.9368G>A (p.Gly3123Glu)
Kariminejad et al. 2018	3 yrs	Tonic–clonic	Only 3 episodes	Normal	n.a.	PB, VPA	n.a.	Normal	c.7442G>A (p.Cys2481Tyr)	c.7442G>A (p. Cys2481Tyr)
Imaizumi et al. 2018	10 yrs	Focal	n.a.	Multifocal Sp and Sp-w frontal lobes	n.a.	Multiple ASMs	n.a.	Normal	c.6553C>T (p.Arg2185Trp) Uniparental disomy of Chr 1	
Uittenbogaard et al. 2018	1st day	Shaking movements	n.a.	Bilateral frontal sharp waves discharges	n.a.	PB, LEV	n.a.	Increased T2 signal in BG and GP, (neonatal hypoxic–ischemic injury)	(Skin biopsy) c.5949_5951del TGT (p.V1984del) c.818 C>T (p. T273M),	c.5174 CrT (p.A1725V)
Domingues et al. 2019 (Fam 1; II: 1)	8 mths	Focal motor to bilateral TC	n.a.	n. a.	n.a.	VPA, VGB, LCS	n.a.	Normal	c.6553C>T (p.Arg2185Trp)	c.498 G > T. (p. Gln166His)
Domingues et al. 2019 (Fam 1; II.4)	4th day	"Lennox–Gastaut like" features, CM, SE	Daily	Multifocal Sp Sub continuous bifrontal sharp waves	n.a.	Multiple ASMs	n.a.	R frontal polymicrogyria (Normal spinal imaging)	c.6553C > T (p.Arg2185Trp)	c.498 G > T. (p. Gln166His)
Iodice et al. 2019	30 mths	Clonic, Focal (eye deviation, oral automatisms) at times	Rare seizures	Abnormal BA, SW F,T,P	R T	Multiple ASMs	VEP normal EMG-ENG normal	Thick and short CC and bilateral hippocampal asymmetric atrophy	c.3632G>A (p.Arg1211Gln)	c8435delC (p. Ser2812Leufs*41)

		with SG, tonic, SE							
Sun et al. 2019	10 mths	Focal tonic or clonic, SE	Daily	Slow BA, SW R F area	L T	LEV, PB, OXC	n.a.	Low T1, high T2 signal WM Enlarged ventricle and delayed myelination in the terminal zone	c.1626 + 1G>A splicing	c.5772dupA (p.C1924 fs)
Sun et al. 2019	16 mths	Twitching of one side, sialorrhea with or without tonic, SE	Daily to weekly	Slow BA, multi-focal discharges	L T	VPA, NZP, TPM	n.a.	Subependymal cyst and widened CSP	c.1626 + 1G>A splicing	c.5772dupA (p.C1924 fs)
Sun et al. 2019	4 mths	Focal, generalized tonic, SE	Daily to weekly	Slow BA, L parietal, SW complexes in the L F,T,P,O	n.a.	LEV, TPM, CLZ, VPA	n.a.	Cystic signals and widened CSP, Subependymal nodules, shortened CC, enlarged ventricles	c.4209C > A (p. C1403X,1973)	c.7307_7308insG: (p.A2436fs*22)
Present Case#1 (Fam 4; II:1)	5 mths	Focal (eye deviation, oral automatisms) at times with SG	Rare (clustered)	Normal BA, bilateral T theta waves and rare Sp R T	L/R T	PB, CBZ	VEP normal	Thick CC abnormally shaped	c.7825T>G (p.Trp2609Gly)	c.7825T>G; (p. Trp2609Gly)
Present Case#2 (Fam 4; II:2)	6 mths	Focal at times with SG during sleep	Monthly (clustered)	Altered BA, L T and R C-P Sp and SW	R C-P	CBZ, LEV, VPA, PB, CLB, OXC, RFN, LCM	VEP normal	CC abnormally shaped	c.7825T>G; (p.Trp2609Gly)	c.7825T>G; (p. Trp2609Gly)

mths = months, SG = secondary generalization, TC = tonic–clonic, CM = cortical myoclonus, SE = status epilepticus, n.a. = not available, BA = background activity, R = right, L = left, F = frontal, C-central, sp-w = spikes and waves, Async = asynchronous, F = frontal, C = central, T = temporal, P = parietal, O = occipital, A = activity, Sp = spikes, SW = slow waves, ED = epileptiform discharge, ASMs = antiseizure medications, ATX = atomoxetine, ARI = aripiprazole, BSP = buspirone, PB = phenobarbital, LEV = levetiracetam, PN = pyridoxine, TPM = topiramate, VPA = valproate, LTG = lamotrigine, CLZ = clonazepam, KD = ketogenic diet, LCM = lacosamide, VGB = vigabatrin, OXC = oxcarbazepine, NZP = nitrazepam, CLB = clobazam, RFN = rufinamide, EMG = electromyography, ENG = electroneurography, ERG = electroretinogram, VEP = visual evoked potential, CC = corpus callosum, CSP = cavum septum pellucidum, BG = basal ganglia, GP = globus pallidus, WM = white matter, SP = septum pellucidum.

months–29 years). A slight prevalence of males was observed (59,1% males). Six patients had a history of consanguinity [7,9,12].

A history of prematurity and intercurrence during pregnancy was reported, respectively in six out of 22 patients (27%) (range: 28–36 weeks of gestational age) and 8 out of 22 (36%) (see Table 1).

One patient had a history of neonatal asphyxia at birth [9].

All patients had mild to severe ID (see Table 1). Hypotonia was reported in 54,5% of the patients [4,8,9,11,12,17,18]. In two cases, there was an adjunctive abnormal motor pattern with chorea [9] or choreoathetosis [11].

Four patients had autistic features such as poor eye contact, repetitive behavior, and hand flapping [13], use no meaningful words and seldom use of gestures for communication, poor social interaction and communication [16]. One patient had other significant psychiatric symptoms requiring pharmacological treatment [7].

Fourteen out of 22 patients (63,6%) had different types of dysmorphic features (high forehead, down slanting palpebral fissures, and ptosis were the most commonly reported). More rarely reported dysmorphic features are reported in Table 1.

Macrocephaly was present in 54,5% of patients [7,8,10–12,14,16,17]. One patient had microcephaly [9].

Data regarding neurological findings and epilepsy are summarized in Table 2.

Epilepsy was reported in 86% of patients [4,8–18].

Seizure onset was extremely variable, from the first day of life [15] to 10 years [14] with a mean age of 20 months. However, in 58,8% of patients, epilepsy started within the first year of life [4,8,9,15,16,18].

Seizures had a common pattern, and focal to bilateral tonic–clonic seizures were the most frequently reported. More rarely, tonic seizures [9,17], tonic–clonic seizures [13,15], atypical absences [4], among others have been reported as well. Seizure frequency was highly variable among different patients, even in patients from the same family and with the same genetic variant [16]. Five patients also experienced status epilepticus (SE) [16–18]. Thirteen patients were treated with multiple antiseizure medications (ASMs) (three or more).

Electroencephalographic features were reported in twenty patients [4,7–18]. Eight patients had a slow background activity. Seven patients had multifocal epileptiform abnormalities: they were mainly localized in the frontal region (n = 4), frontal, temporal, and parietal regions (n = 2), and more rarely in occipital (n = 1). Ictal EEG was reported in 4 out of 22 patients: tonic seizure with diffuse onset in one patient [8], focal seizure with temporal onset in three patients [17,18], evolving into a SE in one of them [17].

Concerning neuroradiological features, only six patients (27,2%) had a normal brain MR [7,13,14,16]. Sixteen patients had different types of brain abnormalities (Table 2).

Three patients died: one during a SE [18], one for aspiration pneumonia [9], and the last for other complications of a prolonged hospitalization [11].

3.2. Case reports

We report two female siblings (II:1 and II:2; Fig. 1A) born from consanguineous parents (first-degree cousins). No familial history for epilepsy or febrile seizures was reported. Both patients were born at term with C-section delivery. They had apparently normal development before epilepsy onset that occurred at the age of 5 (II-1) and 6 months (II-2). They presented with focal to bilateral tonic–clonic seizures characterized by loss of contact, eye deviation, cyanosis, oral automatisms, limb hypertonia followed by bilateral clonic jerks. The elder patient had rare seizures, which were often clustered, and are currently well controlled with carbamazepine. The youngest one had monthly seizures resistant to ASMs, often evolving into SE. Both of them developed mild to moderate ID, and behavioral disturbances. They also presented with facial dysmorphisms (Fig. 1B–C). Interictal EEG revealed temporal bilateral asynchronous slow and epileptiform abnormalities, ictal EEG

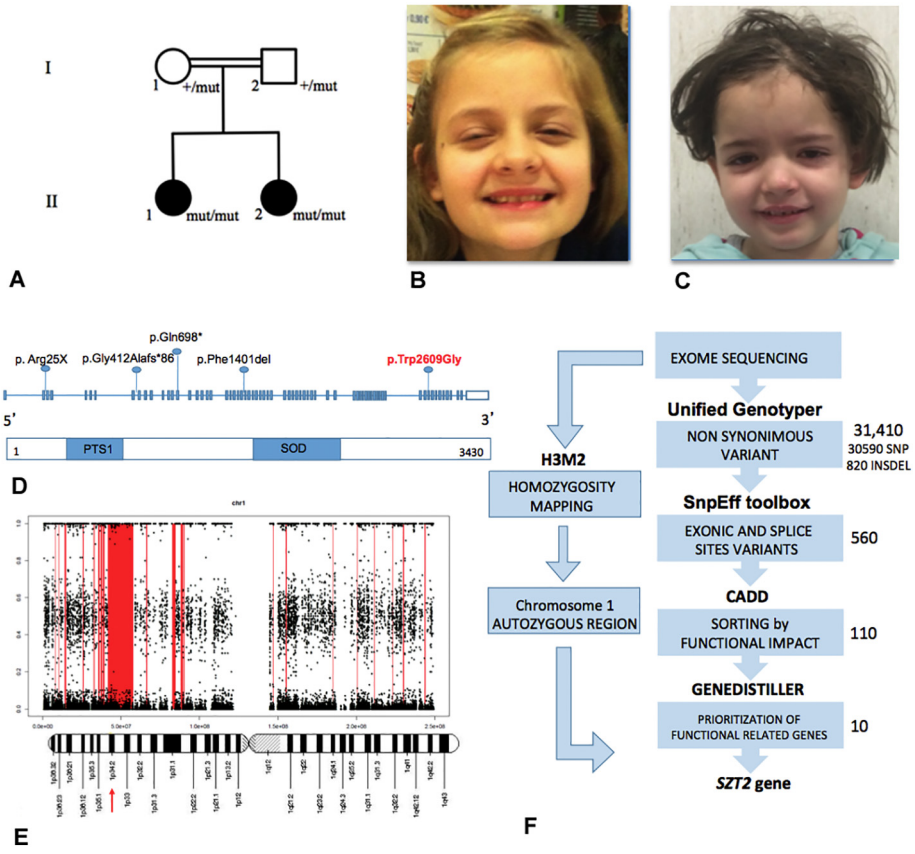

Fig. 1. A. Pedigree of the reported family with SZT2 gene mutation. Affected individuals are represented by filled black symbols. The plus sign (+) denotes the reference sequence, and "mut" indicates the missense mutation c.7825T>G; p.(Trp2609Gly) in SZT2 gene. B and C: individual II-1 and II-2 at the age of 4 and 9 years, respectively. Common facial dysmorphic features include frontal bossing, down slanting palpebral fissures, and palpebral ptosis. D. Schematic representation of SZT2 gene structure and localization of the putative conserved domains: superoxide dismutase (SOD) and peroxisomal target sequence (PTS1) on the protein. The mutation here described is shown in bold. Mutations previously reported, as indicated in Table 1, are also displayed on the gene structure. E. Representation of the H3M2 results. The longest shared autozygous region of 16 mb in two sisters discovered by whole exome sequencing on chromosome 1. Red area indicates homozygous genotypes defining autozygous region spanning SZT2 gene. Ideogram of chromosome 1, the red arrow indicates the SZT2 gene location. F. Exome sequencing workflow: the total high-quality variants were 31,410 (30,590 SNPs and 820 small INDELs). Variant filtering retained 110 novel and clinically associated variants located in exons and splice sites with any functional effect. Based on the occurrence of consanguinity of parents, an autosomal recessive transmission model was hypothesized as the most likely event underlying the trait, the occurrence of genomic regions characterized by homozygous haplotype were supposed. A list of 10 variants in 10 candidate genes was compiled. The resulting variants were sorted based on their predicted functional impact using CADD score. We used Genedistiller, as data warehouse, in order to prioritize the resulting candidate genes based on their functional relevance to epileptic encephalopathy and their expression in central nervous system. Parallel homozygosity mapping analysis was performed on WES data, leading to the identification of a large autozygous region on chromosome 1.

showed a long-lasting bilateral seizure with onset over the right central and parietal regions (Fig. 2A–F). Brain MR showed a dysmorphic CC (Fig. 2G–J).

3.2.1. Genetic results

Whole exome sequencing identified a homozygous c.7825T>G; p.(Trp2609Gly) change in *SZT2* in both patients. By experimental data [19], only a putative superoxide dismutase domain (SOD) (1724–1730) and a peroxisomal targeting site (PTS1) (497–508) were identified (Fig. 1D). We disclosed a 16Mb autozygous region on chromosome 1, shared by both siblings (Fig. 1E), and spanning *SZT2*. No other shared autozygous region >1 Mb was disclosed. The bioinformatics workflow is summarized in Fig. 1F. Sanger sequencing confirmed the variant, and sequencing of parental DeoxyriboNucleic Acids (DNAs) revealed the same variant in heterozygosity. The mutation identified in the two sisters, predicted as pathogenic (Combined Annotation-Dependent Depletion (CADD) score 19,65), is not reported in Exome Aggregation Consortium (ExAC) Browser (see supplementary data).

3.2.2. Neuroimaging results

In order to clarify the role of CC abnormalities in *SZT2*-mutated patients, we compared the brain-scaled CC volumetry of our two patients with a control group. We performed a small case–control study with sex- and age-matched healthy subjects. The analysis ascertained a significant thickening of the CC in one of them. Results from the brain-scaled CC volumetry showed that the younger patient had CC volume similar to the control group, whereas the older slightly larger (Fig. 3).

4. Discussion

Seizure threshold 2 encodes for a large protein that is widely expressed in CNS, and mainly in the parietal and frontal cortex, hippocampus, cerebellum, and dorsal root ganglia [4]. Even if *SZT2* function is not yet completely elucidated, it has been hypothesized to have a protective role against oxidative glutamate toxicity and H_2O_2-induced oxidative stress in neuronal cell model. Therefore, both oxidative glutamate toxicity and excitotoxicity have been considered one of the possible underlying mechanisms for epileptogenesis [19]. More recently, *SZT2* has been identified as a component of the KICSTOR complex, a newly recognized protein complex involved in mTOR pathway and consisting of four proteins KPTN, ITFG2, C12orf66, and *SZT2* [6]. Among them, *SZT2* seems to have a role as the link between the three other proteins [20]. KICSTOR is supposed to be a negative regulator of the mTORC1 pathway binding GATOR1 to the lysosomal surface [6]. As a consequence of *SZT2* variants, KICSTOR is not able to interact more with GATOR1, and to inhibit mTORC1 activity [21]. In animal models,

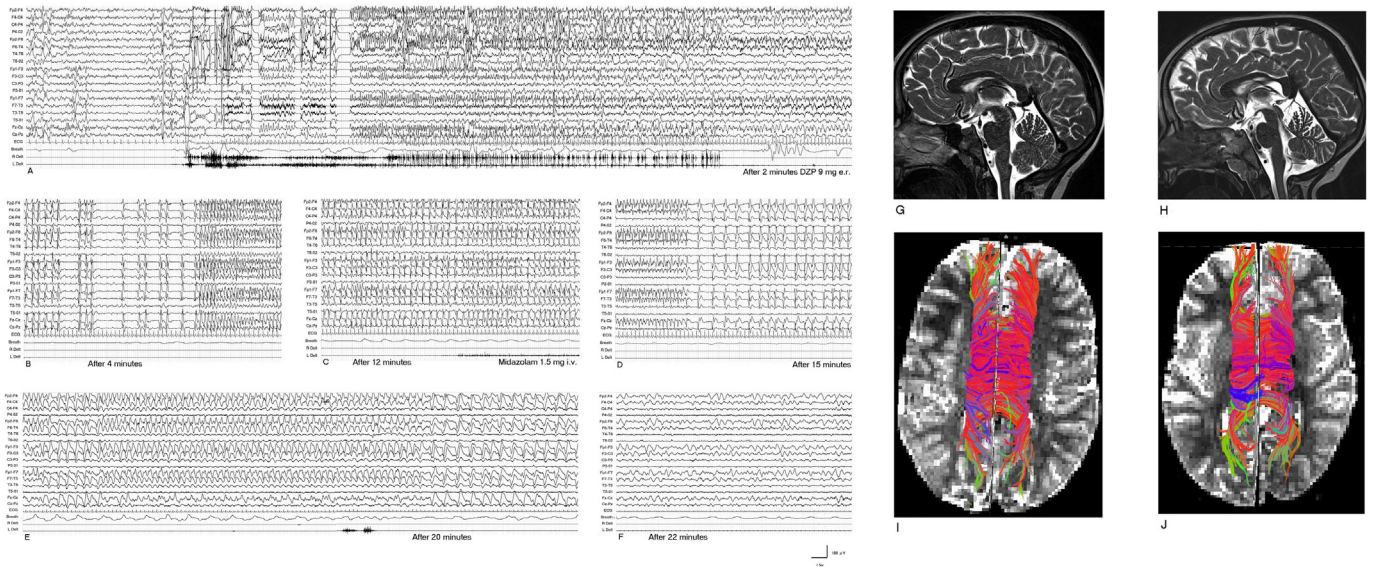

Fig. 2. A–F (ictal EEG). Ictal recording (video/EEG) of the younger sister. (II,2). A. The ictal discharge starts during sleep with a diffuse low-voltage fast activity, increasing in amplitude and decreasing in frequency involving both hemispheres since the onset. After 2 min, administration of intrarectal diazepam 9 mg without effects. B. After 4 min from the onset, spikes and spikes and waves diffuse intermingled with low voltage activity, the discharge becomes more sustained. C. After 12 min from the onset diffuse spikes and wave discharge, administration of midazolam 1.5 mg i.v. D. After 15 min from the onset, the discharge becomes less rhythmic. E. After 20 min from the onset, rhythmic slow waves over bifrontal regions. F. After 22 min, seizure ends. G–J (brain MR). Brain MR: sagittal TSE T2-weighted image and DTI corpus callosum tractography of case 1 (G–I), 11 years old, and case 2 (H–J), 6 years old. The corpus callosum is thin and shows a volume reduction mainly interesting in its posterior part with an abnormal splenium shape. The isthmus is more pronounced than normal, and the splenium is squared and verticalized.

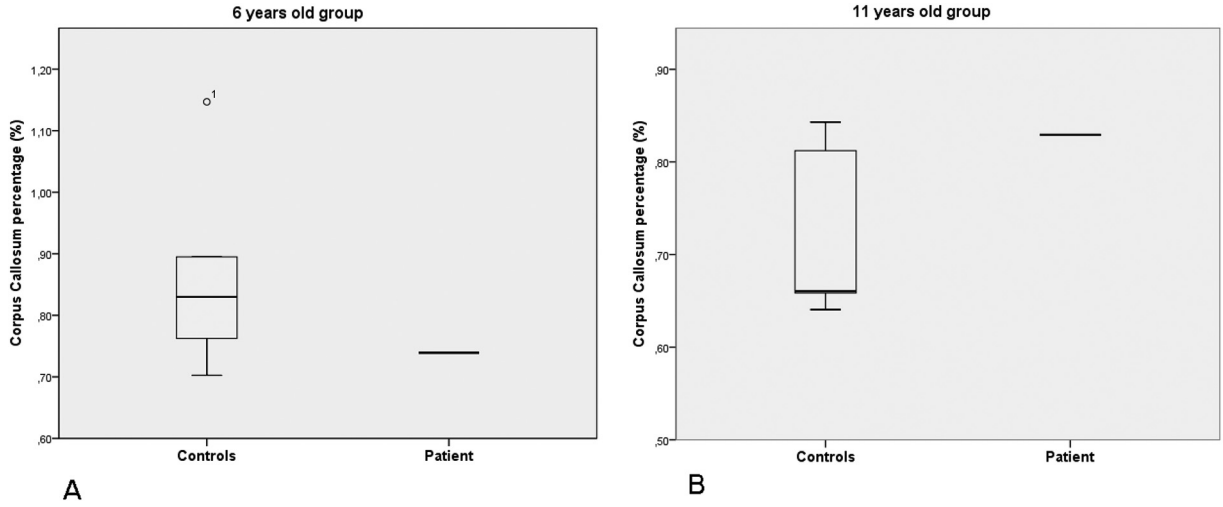

Fig. 3. A. The figure shows the boxplot of corpus callosum volume of the 6-year old patient compared with those from the age-matched healthy subjects. The patient CC volume falls within the 25th and 75th percentile of the healthy subjects. B. The figure shows the boxplot of corpus callosum volume of the 11-year old patient compared with those from the age-matched healthy subjects. The patient CC volume falls over 75th percentile of the healthy subjects.

SZT2 led to an increase of mTORC1 signaling in several tissues, including neurons, possibly causing malformation in brain development [22].

mammalian target of rapamycin signaling dysregulation represents a common pathogenetic mechanisms that can explain the presence of both epileptogenesis and ID [23].

An overall number of 24 affected subjects (including our two patients) have been reported so far with DEE due to *SZT2* genetic variants [4,7–18].

Despite the limited number of patients reported so far, a clinical phenotype is emerging. The main clinical features related to *SZT2* genetic variants are epilepsy, ID, macrocephaly, and dysmorphic features. Phenotypic spectrum is reported to be a high variable, ranging from mild ID without epilepsy to DEE.

Epilepsy starts within the first year of life in 58% of reported patients, and it is mainly characterized by recurrent focal to bilateral tonic–clonic seizures. Seizures are resistant to common ASMs, and 22% of patients experienced a SE. Comparing with other early onset genetic DEEs, epileptic spasms nor myoclonic seizures were reported, and EEG pattern did not show hypsarrhythmia or suppression-burst pattern. However, specific EEG findings for SZT2-DEE did not emerge except for a predominant onset of ictal discharge from temporal regions. Focal epilepsy tends to persist in most of patients during the follow-up and, when present, might be difficult to treat.

Because of hyperactivation of mTORC1 signaling, it is reasonable that this condition includes neurodevelopmental disorders, such as ID and autism spectrum disorders [23]. Two cases have been also reported with the occurrence of movement disorder, such as chorea and choreoathetosis [9,11].

Because of very limited knowledge about the structure and function of the *SZT2* protein, a genotype–phenotype correlation is still

challenging. Although not all reported variants have been functionally characterized, it has been hypothesized that the variability of phenotypic spectrum, ranging from DEE to mild ID without epilepsy, might depend on the residual activity of the *SZT2* protein [21].

Death occurred in three out 22 previously reported patients, but none was caused by SUDEP. The mortality rate (13%) is similar to other severe DEEs [24] and probably reflects the severity of epilepsy and neurological condition, rendering these patients susceptible to pulmonary infections and respiratory distress that ultimately can be fatal.

Macrocephaly is a frequent feature reported in literature, and this was also confirmed in our cases. Moreover, in some patients, high forehead or frontal bossing are reported. We might speculate that a spectrum across a continuum from high forehead to macrocephaly can be the result of mTOR hyperactivation induced by *SZT2* genetic variants.

Patients also present highly variable neuroradiological findings from CC shape abnormalities and persistent/widening of cavum septum pellucidum (CSP) to different disorders of cortical migration, such as periventricular heterotopia [8], subependimal nodules [18], and polymicrogyria [16]. Since the first reports, CC abnormalities were considered radiological findings suggestive for diagnosis, and our radiological study with DTI analysis confirmed these data. Nevertheless, CC abnormalities, which are present in 56% of patients, are often variable and not specific. What is emerging is the recurrence of neuronal migration abnormalities, and *SZT2* defect resulting in hyperactivation of mTORC1 signaling might be crucial in pathogenesis of such cerebral malformations; moreover, recent hypothesis of hyperactivation of mTORC1 signaling might also open to targeted treatments such as rapamycin [25].

The involvement of *SZT2* in the mTOR pathway makes the *SZT2*-related phenotype different from other genetic DEEs because of channelopathies such as SCN2A, KCNQ2, and SCN8A. Dysmorphisms and macrocephaly are not reported in other DEEs, most probably because such findings are the result of mTOR hyperactivation. Corpus callosum abnormalities and other radiological features seem to be constant findings in *SZT2*-related phenotype and can be seen rarely in other DEEs because of channelopathies. Epilepsy onset in SZT2-DEE is mostly within the first year of life; however, it is extremely variable. In DEEs due to channelopathies, age at onset is consistently early in life, being within the first three months of life [26–28]. Seizures in SZT2-DEE are mostly focal to bilateral tonic–clonic, while in other DEEs, they are mostly tonic [29]. Intellectual disability and autism are common features in both conditions. Electroencephalogram does not show peculiar patterns, even if burst suppression, which can be seen in other DEEs, has never been reported in SZT2-DEE.

5. Conclusions

Despite the limited number of patients reported so far, a clinical phenotype is emerging. Seizure threshold 2 variants cause a broad phenotypic spectrum from mild ID without epilepsy to DEE. Other relevant features are macrocephaly and radiological findings such as CC abnormalities and neuronal migration disorders, which might be due to the hyperactivation of mTORC1 signaling. The early diagnosis might improve the outcome and might give higher chances for a targeted treatment; however, more studies are still needed in order to confirm this hypothesis.

Declaration of competing interest

None of the authors has any conflict of interest to disclose.

Acknowledgments

We would like to thank the patients and family for their participation and involvement in the study.

Bambino Gesù Children's Hospital is part of the European Reference Network on Rare and Complex Epilepsies (EpiCare).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.yebeh.2020.107097.

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