Seizure: European Journal of Epilepsy 66 (2019) 81–85

Contents lists available at ScienceDirect

Seizure: European Journal of Epilepsy

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

Short communication

Compound heterozygous *SZT2* mutations in two siblings with early-onset epilepsy, intellectual disability and macrocephaly

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ARTICLE INFO

Keywords:
Epileptic and developmental encephalopathies
Intellectual disability
SZT2
mTORopathies
Whole exome sequencing

ABSTRACT

Purpose: Mutations in *SZT2* have been previously reported in several cases of early onset epilepsy and intellectual disability. In this study we investigate potential causal mutations in two male siblings affected by early onset epilepsy, intellectual disability and macrocephaly.
Methods: We use family-based whole-exome sequencing to identify candidate variants.
Results: We report the identification of two potential causal *SZT2* mutations in compound heterozygous state. We observe considerable differences in the clinical phenotype severity of the two affected individuals. The cerebral MRI revealed no abnormalities in the older affected brother, while in the youngest one it revealed a right frontal polymicrogiria. Moreover, while good seizure control was achieved in the older affected individual the younger brother is affected by pharmacoresistant epilepsy, progressive spastic paraplegia, cortical myoclonus and a more severe intellectual disability. We also analyzed the relative location of the reported pathogenic mutations in the SZT2 protein.
Conclusion: Variable phenotypic expressivity is observed for this condition, while the location and type of mutations in SZT2 also has a potential impact on epilepsy severity. These findings extend our knowledge of epileptogenic conditions related to *SZT2* and mTOR signaling.

1. Introduction

Epileptic and developmental encephalopathies are conditions characterized by epilepsy and developmental impairment of variable severity, many of them with a genetic etiology. Recent advances in genomics technology have enabled the identification of new causal genes and variants, nevertheless many patients remain without a precise genetic diagnosis and genotype-phenotype relationships are not always clear [1,2]. Notably, the hyperactivation of the mammalian target of rapamycin (mTOR) signaling pathway has been related to different epileptogenic conditions (mTORopathies) such as tuberous sclerosis (caused by *TSC1* or *TSC2* mutations), autosomal dominant nocturnal frontal lobe epilepsy (caused by *DEPDC5* mutations), *PIK3CA*-related overgrowth spectrum (PROS), megalencephaly-polymicrogyria-polydactyly-hydrocephalus syndrome-2 (caused by *AKT3* mutations), Smith-Kingsmore syndrome (caused by *MTOR* mutations), focal cortical dysplasia type II (caused by *MTOR*, *TSC1* or *TSC2* mutations) or familial focal epilepsy with variable foci (caused by *DEPDC5*, *NPRL2* or *NPRL3* mutations) [2,3].

In 2009 the *SZT2* gene was shown to influence seizure threshold and

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https://doi.org/10.1016/j.seizure.2018.12.021
Received 28 September 2018; Received in revised form 2 December 2018; Accepted 22 December 2018

epileptogenesis in mice [4], and the protein has been implicated in resistance to oxidative stress [5]. Recently, a role of SZT2 protein in mTOR signaling has been demonstrated in human [6]. So far six independent reports have identified 16 different biallelic *SZT2* mutations (in compound heterozygous or homozygous state) in eleven individuals with epilepsy and/or intellectual disability (ID) [7–12] (Table 1). These reports describe epilepsy of varying severity and ID in eight unrelated children, as well as three brothers with moderate ID without epilepsy. Moreover, in these reports seven patients also present macrocephaly, which appears to be the most significant physical sign of the phenotype.

Here, we report two mutations in *SZT2* in compound heterozygous state in two young adult siblings with early onset seizures, ID and macrocephaly. We compare the different *SZT2* mutations and clinical phenotypes previously reported and investigate their relative location in the protein and their relation to phenotype severity.

2. Patients, materials and methods

2.1. Patients

Three brothers were born to healthy, Caucasian, non-consanguineous parents in the study family (Fig. 1A). Both the oldest (II.1) and the youngest brother (II.4) present early onset epilepsy, ID and macrocephaly. The second pregnancy (II.2) ended in miscarriage at nine weeks of gestation. It was not possible to perform a post-mortem examination of the fetus. The third pregnancy resulted in a healthy individual (II.3). This study was performed after approval by a local ethics committee and with informed consent from all individual participants or their respective legal guardians.

2.2. Sequencing and analysis

DNA was extracted from peripheral blood. Whole exome sequencing (WES) and Sanger sequencing of the candidate variants were performed as described in the supplement.

Candidate variants were selected from the whole exome based on a combination of predefined criteria (see supplement). Notably, the variant must segregate in the family in a recessive mode of inheritance either in homozygous or in compound heterozygous state, or alternatively as X-linked recessive. A potential scenario of parental mosaicism was also investigated.

3. Results

3.1. Clinical phenotype

Individual II.1 was born at term after an uneventful pregnancy. An absolute and relative macrocephaly (head circumference: +4.3 SD; normal values in both parents) was noted at birth. Developmental delay was observed early in life and at 8 months of age he developed focal motor to bilateral tonic-clonic seizures. Sodium valproate and vigabatrin were started with good control of the seizures, and then seizures weaned off, leading to a long-lasting seizure-free period until the age of 16 years. Since then he experienced few focal seizures, controlled at 22 years with valproate and lacosamide. Although a good seizure control was achieved, the patient developed a moderate ID and autism spectrum disorder features such as deficits in social interaction and communication. Now he is 29 years old, is able to walk unassisted and has sufficient autonomy in daily life, the macrocephaly persists. His neurological examination is normal, without evidence of spasticity or cranial nerve or cerebellar dysfunction. Brain magnetic resonance imaging (MRI) at the ages of 3, 6 and 17 years were normal. Genetic testing included standard karyotype analysis, *FMR1* gene testing for fragile X syndrome, and mutation screening of *TSC1*, *TSC2* and *PTEN* genes, all of which gave normal results.

Individual II.4 was born at term after an uneventful pregnancy. At birth he was diagnosed with absolute and relative macrocephaly (head circumference: +4.4 SD) and agenesis of the left kidney. Focal seizures began 4 days after birth and were controlled with phenytoin and later with carbamazepine, which was weaned off at 8 months. Early interictal EEG showed multifocal fronto-temporal spikes, but was normal at the age of 14 months. Focal to bilateral tonic seizure reappeared at 4 years. Between 4 and 9 years seizures were sporadic, treated with carbamazepine and clobazam. Seizures then increased in frequency, leading to several episodes of *status epilepticus*. He currently presents a focal epilepsy with "Lennox-Gastaut-like" features and cortical myoclonus. He experiences daily focal and focal-to-generalized tonic seizures, unresponsive to drugs (topiramate, phenytoin, zonisamide, rufinamide, lacosamide, adrenocorticotropic hormone therapy). EEG shows subcontinuous bifrontal sharp-waves and multifocal epileptiform discharges. Brain MRI at 4 years revealed right frontal polymicrogyria. He was able to walk at 30 months but developed severe ID with no language and autistic traits. During adolescence the patient experienced progressive paraplegia, with spasticity involving the lower limbs; tendon reflexes became brisk, and he developed bilateral extensor plantar response alongside ankle clonus. No deficits in cranial nerve or cerebellar function were observed. These neurological findings associated with the development of neurological bladder brought us to consider a possible diagnosis of tethered cord that was initially not confirmed by neuroimaging. The patient has neither a deep pilonidal dimple, nor a tuft of hair or a cutaneous discoloration in the sacral region. Moreover, the spinal imaging did not show a neural tube defect, nor lipomeningocele, nor other anomalies of the body sacral structures. However, due to the strong clinical suspicion and the progressive course of the symptoms, surgical treatment was offered and performed, with transient benefit. At the last follow-up (age 19), despite the surgical treatment, he was unable to walk unassisted and maintained a clear spastic paraparesis and bladder dysfunction that requires daily catheterism. Metabolic investigations in blood and urine (plasma levels of long chain fatty acids, amino acids and urine organic acids) were normal. Conventional chromosome analysis, array-CGH and a search for mutations in the *MECP2* and *PTEN* genes were performed, all of which gave normal results.

3.2. Genetic and computational analysis

After filtering the variants as described in the supplementary material, only one candidate scenario under a recessive mode of inheritance remained, in which the affected individuals carry compound heterozygous variants in the *SZT2* gene (ENSG00000198198). The paternally inherited variant (Mut1: GRCh38:1:g.43439025C > T, NM_015284.3:c.6553C > T, NP_056099.3:p.Arg2185Trp, rs765848129) is rare (ExAC AF 8.2×10^{-6}, gnomAD AF 2.4×10^{-5}), while the maternally inherited variant (Mut2: GRCh38:1:g.43404550 G > T, NM_015284.3:c.498 G > T, NP_056099.3:p.Gln166His) is not reported in ExAC or gnomAD. Both Mut1 and Mut2 were predicted to be deleterious by different methods (see supplement), and the affected amino acid residues are highly conserved on 56 SZT2 orthologous proteins (see supplement). Mut2 locates at the end of exon 4 and does not disrupt the splicing donor site but was predicted to affect splicing by two methods (see supplement). Splicing could be altered in different ways, including cassette exon skipping (in-frame deletion of exon 4), alternatively the mutation could abolish the splicing donor site at the end of exon 4 leading to a premature stop codon and a truncated protein (only 205 residues length) and/or to nonsense-mediated mRNA decay (NMD).

The relative locations of Mut1 and Mut2 in the SZT2 protein are provided in Fig. 1B. Their predicted effect on protein stability is described in the supplement. Table 1 provides a summary of the reported clinical features of all known patients with *SZT2* mutations.

Table 1
Overview of clinical features of individuals with *SZT2* mutations[a].

Report	Cases	Zygosity	Mutations[b]	Epilepsy severity	Delayed psychomotor development or intellectual disability	Motor function	Speech and social interaction
This study	Siblings II.1: Male 29 years II.4: Male 19 years	Compound heterozygous	Paternal Mut1: c.6553C > T (p.Arg2185Trp) Maternal Mut2: c.498 G > T (p.Gln166His)	II.1: Controlled II.4: Pharmacoresistant	yes	II.1: Able to walk without assistance II.4: Unable to walk without assistance	II.1: Poor speech, autistic features II.4: No speech, autistic features
[7]	Individual 1: Female 10 years	Homozygous	Mut 3: c.73C > T (p.Arg25*)	Pharmacoresistant	yes	Bedridden	No speech
	Individual 2: Male 9 years	Compound heterozygous	Paternal Mut4: c.1496 G > T (p.Gly412Alafs*86) Maternal Mut5: c.2092C > T (p.Gln698*)	Pharmacoresistant	yes	Bedridden	No speech
[8]	Siblings II.1: Male 18 years II.2: Male 10 years II.3: Male 7 years	Homozygous	Mut6: c.4202_4204del (p.Phe1401del)	None	yes	II.1: motor delay II.2 and II.3: normal	Speech delay
[9]	Female 8 years	Compound heterozygous	Mut7: c.5499delC (p.Phe1834Serfs* 47) Mut8: c.6916 G > A (p.Gly2306Arg)	Pharmacoresistant	yes	Bedridden	No speech
[10]	Male 3 years	Compound heterozygous	Paternal Mut9: c.3509_3512delCAGA (p.Thr1170Argfs*22) Maternal Mut10: c.9703 C > T (p.Arg3235*)	Controlled	yes	Unable to walk without assistance	No speech
[11]	Case 1: Female 4 years	Compound heterozygous	Paternal Mut11: c.3700_3716del (p.Asn1234Alafs*35) Maternal: Mut12: c.5482del (p.Gly1829Valfs*52)	Pharmacoresistant	yes	Bedridden	No speech
	Case 2: Male 2 years	Compound heterozygous	Paternal Mut13: c.3947dup (p.Glu1317Glyfs*4) Maternal Mut14: c.2929 + 1G > A (p.Leu939Aspfs*19)	Pharmacoresistant	yes	Bedridden	No speech
	Case 3: Female 5 years	Compound heterozygous	Paternal Mut15: c.7303C > T (p.Arg2435Trp) Maternal Mut16: c.8162C > G (p.Ser2721Cys)	Controlled	yes	Bedridden	No speech
[12]	Female 4 years	Compound heterozygous	Paternal Mut17: c.2930-17_2930-3delinsCTCGTG Maternal Mut18: c.8596dup (p.Tyr2866Leufs*42)	Controlled	yes	Able to walk without assistance	No speech

[a] An extended version is provided in the supplementary material.
[b] Mutation positions are relative to reference transcript sequence NM_015284.3 and protein sequence NP_056099.3.

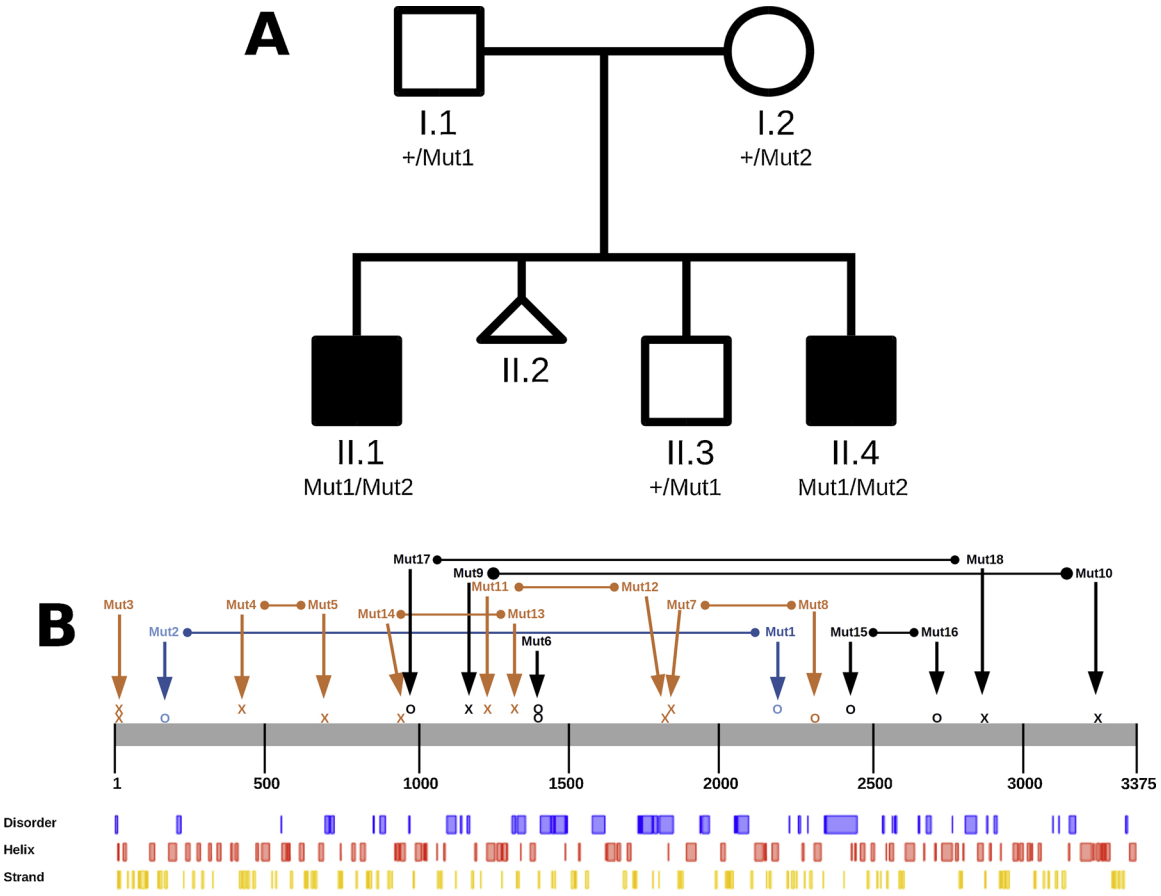

Fig. 1. A: Family pedigree. Individuals affected with epilepsy are represented by filled black symbols. The (+) sign indicates the reference allele, Mut1 indicates mutation c.6553C > T (p.Arg2185Trp), and Mut2 indicates mutation c.498 G > T (p.Gln166His). The affected individuals carry both Mut1 and Mut2. According to an autosomal recessive inheritance mode of the observed clinical phenotype, the healthy brother is carrier of only one *SZT2* variant (the paternal one) and each healthy parent is heterozygous for one of the two identified gene variants. **B:** Overview of *SZT2* mutations and their location in the protein. Mutations were labeled according to Table 1. Mutations related to drug-resistant epilepsy are shown in brown, the two mutations reported in this study (Mut1, Mut2) are in blue, all other mutations are in black. Nonsense mutations are marked with 'X', other nonsynonymous substitutions are marked with 'O', the mutation marks are placed in two rows representing the proteins encoded by the two gene copies. Mutations in compound heterozygosity are connected. The protein secondary structure (helix, strand) and disorder annotations are described in the supplement (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

4. Discussion

We have identified and characterized two compound heterozygous variants in *SZT2* in two patients with epilepsy, intellectual disability, and macrocephaly. Both variants are rare and one has not been previously reported. They are predicted to be deleterious and destabilizing, and one might affect splicing. This study is also the first to report candidate *SZT2* pathogenic mutations in two related individuals affected with early-onset epilepsy and provides the longest follow up period.

Comparison of the relative location of the reported pathogenic mutations in *SZT2* indicates that cases with severe drug-resistant epilepsy tend to have truncating (nonsense) mutations at the protein N-terminus (Fig. 1B). The more severe phenotypes could result from a severely truncated protein, or from NMD and haploinsufficiency. The molecular function and activity of the SZT2 protein is largely unknown and a proper molecular characterization is needed, involving structure determination and identification of functional sites.

The two affected individuals show considerable differences in clinical phenotype severity: the cerebral MRI revealed no abnormalities in II.1, while in II.4 it revealed a right frontal polymicrogiria; moreover II.4 presents a drug-resistant epileptic encephalopathy associated with severe cognitive ID and progressive spastic paraplegia, while a milder form of epilepsy and cognitive impairment without clear neurological deficits is observed in the brother II.1. The clinical variability in *SZT2*-related conditions has already been described in the eleven previously reported patients, whose clinical features are summarized in Table 1 and in the Supplement 2 table. Hypotonia was the more frequent neurological sign, reported in seven patients; in two cases there was an adjunctive abnormal motor pattern with chorea [11] or Choreoathetosis [9]; contractures were reported in two other children [7,11]. There are no reports of tethered-cord in the published cases. Although this small patient group does not reveal a constant and characteristic phenotype, we observe that the association of epilepsy, psychomotor retardation/intellectual disability and macrocephaly is a recurrent clinical pattern in the presence of *SZT2* mutations.

To investigate the determinants of this variable expressivity one could examine the potential impact of Mut2 in splicing by reverse transcription polymerase chain reaction (RT-PCR) and western blot analysis, and use cell models, including iPS derived cells, to explore whether different proportions of aberrant splicing products or haploinsufficiency in II.1 and II.4 lead to measurable differences in mTOR signaling that could influence phenotype severity. Cell models can also play a key role in clarifying the relation between mTOR signaling and

epileptogenesis in mTORopathies. The findings also have therapeutic implications, as rapamycin analogs (rapalogs) counteract mTOR hyperactivation in mTORopathies and are under investigation for epilepsy treatment [3]. In fact everolimus (a rapalog) has already been approved for the treatment of patients with tuberous sclerosis where it shows a beneficial effect in reduction of seizure frequency.

Funding

This work was supported by the Department of Innovation, Research and University of the Autonomous Province of Bozen/Bolzano. The authors also thank the Department of Innovation, Research and University of the Autonomous Province of Bozen/Bolzano for covering the Open Access publication costs.

Declarations of interest

None.

Compliance with ethical standards

This study was performed after approval by a local ethics committee and with informed consent from all individual participants or their respective legal guardians.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.seizure.2018.12.021.

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