Original article

Epileptic Disord 2019; 21 (2): 185-91

SCN1A mutations in focal epilepsy with auditory features: widening the spectrum of GEFS *plus*

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Received July 28, 2018; Accepted February 09, 2019

ABSTRACT – *Aims*. Epilepsy with auditory features (EAF) is a focal epilepsy syndrome characterized by prominent auditory ictal manifestations. Two main genes, *LGI1* and *RELN*, have been implicated in EAF, but the genetic aetiology remains unknown in half of families and most sporadic cases. We previously described a pathogenic *SCN1A* missense variant (p.Thr956Met) segregating in a large family in which the proband and her affected daughter had EAF, thus satisfying the minimum requirement for diagnosis of autosomal dominant EAF (ADEAF). However, the remaining eight affected family members had clinical manifestations typically found in families with genetic epilepsy with febrile seizures *plus* (GEFS+). We aimed to investigate the role/impact of *SCN1A* mutations in EAF.
Methods. We detailed the phenotype of this family and report on *SCN1A* screening in a cohort of 29 familial and 52 sporadic *LGI1* variant-negative EAF patients.
Results. We identified two possibly pathogenic missense variants (p.Tyr790Phe and p.Thr140Ile) in sporadic patients (3.8%) showing typical EAF and no antecedent febrile seizures. Both p.Thr956Met and p.Tyr790Phe were previously described in unrelated patients with epilepsies within the GEFS+ spectrum.
Conclusion. *SCN1A* mutations may be involved in EAF within the GEFS+ spectrum, however, the role of *SCN1A* in EAF without features that lead to a suspicion of underlying GEFS+ remains unclear and should be elucidated in future studies.

Key words: genetics, lateral temporal epilepsy, ADLTE, ADEAF, GEFS *plus*

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doi:10.1684/epd.2019.1046

Epilepsy with auditory features (EAF) is an epileptic syndrome characterized by focal seizures with auditory auras and/or symptoms suggesting a lateral temporal onset, such as aphasia, occasionally evolving to bilateral tonic-clonic seizures (Ottman, 2015). EAF can occur either in sporadic patients (Bisulli *et al.*, 2004) or in families (Ottman, 2015). In families, it is inherited in an autosomal dominant (AD) pattern and is therefore referred to as ADEAF, otherwise known as autosomal dominant lateral temporal lobe epilepsy (ADLTE). Two family members with EAF are considered sufficient to establish a diagnosis of ADEAF, regardless of their degree of kinship and the total number of individuals with epilepsy in the family (Ottman *et al.*, 2004). However, EAF patients usually constitute most or at least half of the total number of clinically ascertainable subjects with epilepsy in families with variants in *LGI1* (leucine-rich, glioma inactivated 1, MIM 604619) or *RELN* (reelin, MIM 600514), the main genes associated with the disorder to date. Variants in *LGI1* account for less than 50% of familial (Kalachikov *et al.*, 2002; Ottman *et al.*, 2004; Michelucci *et al.*, 2013) and approximately 2% of sporadic EAF cases (Bisulli *et al.*, 2004). *RELN* has been implicated in an additional 17% of typical ADEAF families (Dazzo *et al.*, 2015). Recently, a new gene, *MICAL1* (Microtubule-associated monooxygenase, calponin and lim domain containing 1, MIM 607129), was found to be mutated in two ADEAF families (Dazzo *et al.*, 2018). Finally, other established focal epilepsy genes have been associated with EAF. Pathogenic or possibly pathogenic events associated with *DEPDC5* (DEP domain containing protein 5, MIM 614191), *CNTNAP2* (contactin associated protein like 2, MIM 604569), and *SCN1A* (sodium voltage-gated channel alpha subunit 1, MIM 182389) have been described in a few families with heterogeneous phenotypes and apparently complex inheritance (Pippucci *et al.*, 2015). In particular, a pathogenic *SCN1A* variant (p.Met956Thr) was described to segregate in a large family in which the proband and her affected daughter had EAF, fulfilling the minimal criteria for diagnosis of ADEAF, however, all the other eight affected members disclosed a phenotypic range consistent with the diagnosis of generalized epilepsy with febrile seizures plus (GEFS+) (Pippucci *et al.*, 2015). To date, this is the only family in which lateral temporal lobe semiology with auditory symptoms has been specifically associated with *SCN1A* pathogenic variants.

Here, we report the clinical and electro-physiological details of the *SCN1A* variant carriers in this latter family. Moreover, to confirm the role of *SCN1A* variants in EAF, we screened 81 additional EAF cases without *LGI1* variants.

Methods

The local ethical committee approved the study and written informed consent was obtained from all participants or their legal representatives. All consenting subjects underwent a comprehensive clinical assessment, including medical history, physical examination, and review of medical records and instrumental examinations (EEG, video-EEG monitoring, and neuroimaging). Molecular genetic analysis of the family has been already described (Pippucci *et al.*, 2015).

Targeted *SCN1A* screening was performed in a cohort of 81 *LGI1* variant-negative EAF cases: 52 isolated cases, 29 probands either from ADEAF families (9 cases) or with a family history of other epilepsies (20 cases). Genetic analysis was performed using different techniques. One patient was tested based on Sanger sequencing of the coding exons of the gene (RefSeq ID: NM_001165963) following standard protocols, 27 patients underwent whole-exome sequencing (WES) analysis, and 11 patients were tested with a diagnostic targeted next-generation sequencing (NGS) epilepsy panel, including *SCN1A*, based on a custom Nextera Rapid Capture enrichment kit (Illumina Inc., Santa Clara, CA, USA). A molecular inversion probe (MIPs) assay was used in 42 patients to specifically target *SCN1A* coding exons and intronic exon-flanking 5-bp sequences (*see supplementary data*).

Results

Clinical features of the family

The family (F1) (*figure 1*) came to our attention through the proband (III:1), a 66-year-old woman with a history of drug-resistant EAF starting at ten years of age with focal seizures which still recur monthly, despite antiepileptic drug polytherapy (currently lacosamide, valproate, and clonazepam). Seizures were characterized by bilateral auditory auras (sensation of plugged ears and bilateral buzzing with decreasing intensity), followed by rising heat sensation, speech arrest, and staring. Rare focal to bilateral tonic-clonic seizures occurred. At the age of 49 years, concomitant with the administration of lamotrigine, she started having upper limb postural and action tremor (right>left), with severe impairment of daily activities. Lamotrigine also worsened seizures. The proband's 35-year-old daughter (IV:1) had a history of febrile seizures plus (FS+) during childhood, with convulsions that ceased at six years of age but also occurred without fever. After ten years of seizure freedom, she developed focal to bilateral tonic-clonic seizures with auditory aura

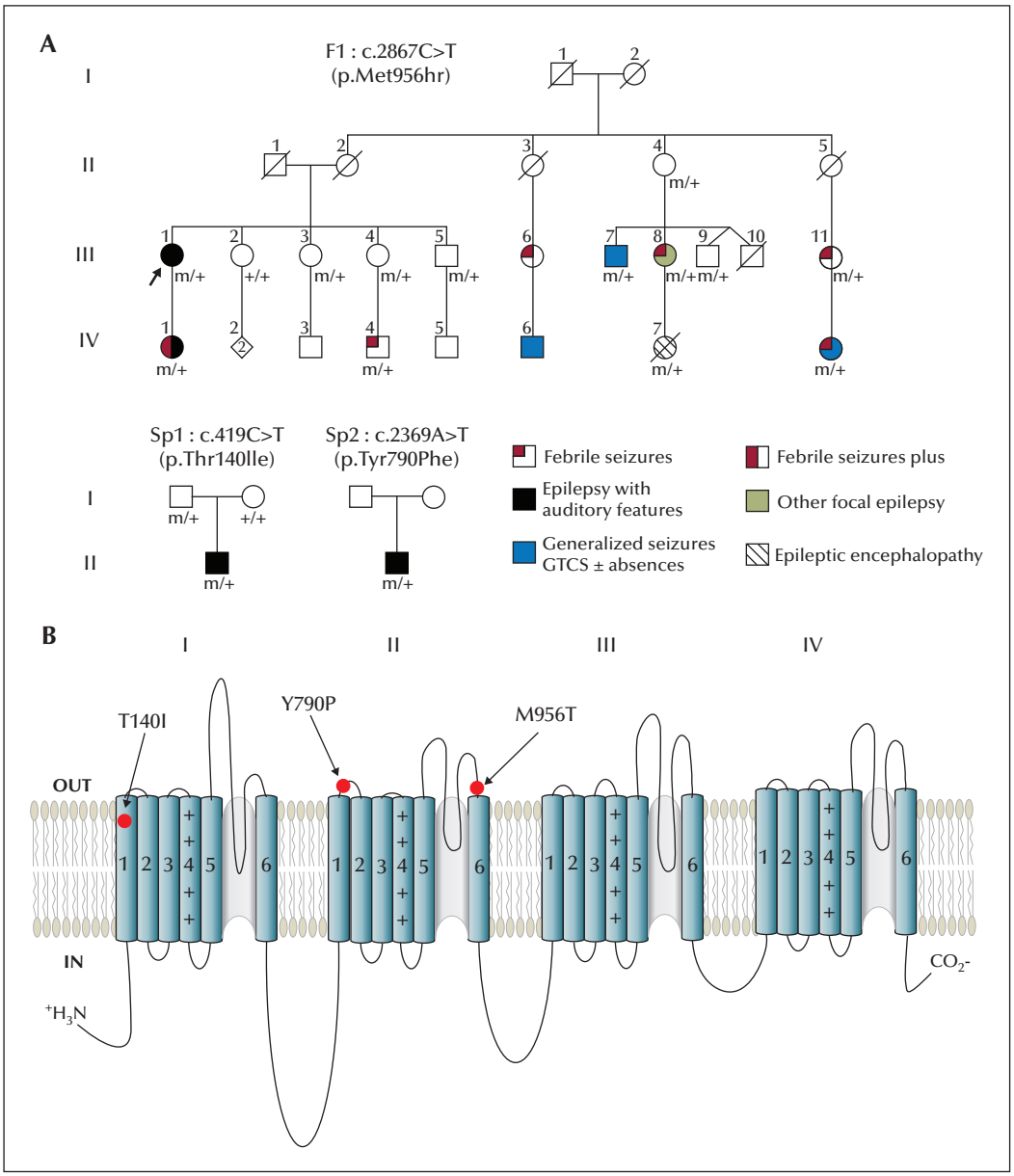

Figure 1. *SCN1A* missense variants. (A) Pedigrees of cases with segregation of *SCN1A* variants. The F1 pedigree has been modified from Pippucci *et al.* (2015). m/+: individuals heterozygous for a *SCN1A* variant, +/+: individuals tested for the variants and found to be negative. Symbols with slashes represent deceased individuals. GTCS: generalized tonic-clonic seizures. (B) Location of identified missense variants in the sodium channel α subunit. The α subunit consists of four homologous domains (I-IV), each containing six transmembrane segments represented by cylinders (1-6). The voltage-sensing segment (4) has multiple positively charged amino acids (+). The identified missense variants are indicated by filled red circles.

(buzzing and voices with increasing and subsequently decreasing intensity). These were initially well controlled by valproate, although its efficacy decreased with time. Seizures ceased at 20 years of age, after the switching of valproate to levetiracetam. Interictal EEG disclosed focal epileptiform abnormalities over the temporal region (III:1, IV:1), associated with generalized spike-and-wave discharges in III:1 (*figure 2*). MRI was unremarkable in both subjects.

Since the phenotype of III:1 and IV:1 was consistent with EAF, this family was classified as ADEAF according to widely recognized criteria (Ottman *et al.*, 2004). After identification of a pathogenic *SCN1A* variant (p.Met956Thr) in these two subjects (*table 1*), reconstruction of the extended pedigree disclosed low penetrance of the variant (61.5%) and six out of eight tested distant relatives (third degree of kinship or higher) who carried the variant displayed vast clinical

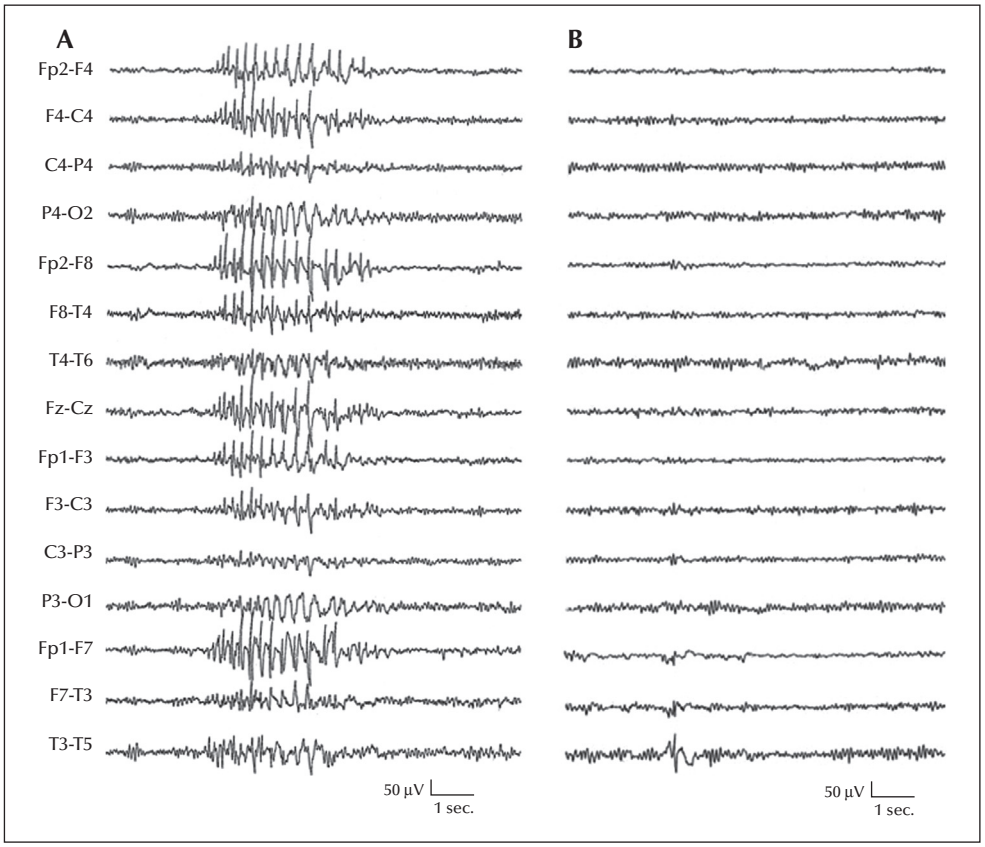

Figure 2. Interictal EEG of the proband of family F1 (III:1), showing generalized spike-and-wave discharges at 3.5 Hz (A) and focal spike and wave abnormalities over the left temporal leads (B).

variability, as previously reported (Pippucci *et al*., 2015). Three family members (III:6, III:11, and IV:4) presented only FS without epilepsy. The other seven affected members (including the proband and her daughter) had epilepsy with a mean age at onset of 4.5 years (range: 2-12). Three patients (III:7, IV:6, and IV:8) experienced rare generalized tonic-clonic seizures in childhood without clear-cut focal origin, and typical absence seizures were also documented in one of them (IV:8). One individual had focal seizures (III:8). At the severe end of the spectrum, the individual IV:7 had features suggestive of Dravet syndrome with hemiclonic seizures, recurrent episodes of febrile status epilepticus, and focal seizures, but later onset at 23 months. Other features of intellectual disability and behavioural problems were also consistent with an epileptic encephalopathy along the Dravet spectrum. Seizures were drug-resistant. Lamotrigine worsened both seizure frequency and psychiatric comorbidities. The patient died at 25 years of age after a drowning event, possibly related to a seizure. EEG showed a slow background activity with diffuse epileptic discharges. Overall, six out of ten affected family members had FS/FS+.

SCN1A screening

We identified possibly pathogenic *SCN1A* variants in 2.5% of patients overall (2/81) and in 3.8% of sporadic cases (2/52). Both sporadic patients presented with typical EAF and no antecedent FS (*figure 1*).

The first patient (Sp1) was a 29-year-old man who experienced, from 19 to 21 years of age, seven seizures characterized by: auditory sensation (left ear buzzing), forced head deviation towards the left, aphasia, loss of awareness, and evolution to bilateral tonic-clonic seizures. He was started on valproate at 19 years of age, then levetiracetam was added, without seizure control. At 21 years of age, he started carbamazepine therapy. No further seizures have occurred since. Both valproate and levetiracetam were withdrawn at 23 years of age and he has been seizure-free for eight years.

The second patient (Sp2) was a 55-year-old man who experienced, from 25 to 40 years of age, monthly episodes characterized by auditory aura (right ear whistle or plugged ear sensation) and motor aphasia, occasionally evolving to bilateral tonic-clonic seizures. He commenced treatment with carbamazepine at low doses at 25 years of age. Seizures were not adequately

controlled until the dose was increased over time, resulting in seizure freedom at 40 years of age. The patient has now been seizure-free for 15 years but is not keen to withdraw therapy.

In both patients, interictal EEG disclosed bursts of sharp theta activity over the left temporal regions and brain MRI was unremarkable.

Patient Sp1 carried the missense variant c.419C>T (p.Thr140Ile), absent in ExAC and gnomAD databases, predicted to be deleterious based on *in silico* tools (*table 1*). Segregation analysis revealed that the variant was inherited from his asymptomatic father, compatible with incomplete penetrance of *SCN1A* variants.

In Patient Sp2, we identified the missense variant c.2369A>T (p.Tyr790Phe), present in a few alleles in ExAC and gnomAD, and predicted to be deleterious based on *in silico* tools (*table 1*). The DNA of the parents was not available to assess the origin of the variant. The affected residue is within the voltage-sensing module (between S1 and S2 segments of domain II). The same change has already been reported as a *de novo* event responsible for an atypical presentation of Panayiotopoulos syndrome (Grosso *et al*., 2007), now considered part of the GEFS+ spectrum (Kivity *et al*., 2017). A different variant affecting the same residue (p.Tyr790Cys) was found to segregate in a GEFS+ family (Annesi *et al*., 2003) and was associated with a remarkable reduction of current density and of Nav1.1 cell surface expression (Bechi *et al*., 2015).

Discussion

SCN1A is arguably the most clinically relevant epilepsy gene, associated with a large number of seizure-related disorders of variable severity, including febrile seizures, GEFS+, and Dravet syndrome, as well as temporal lobe epilepsy (Abou-Khalil *et al*., 2001; Brunklaus *et al*., 2014). Our findings indicate that *SCN1A* missense variants may also be involved in EAF within the GEFS+ spectrum. EAF has been historically related to *LGI1* pathogenic variants and only recently associated with variants in other genes (Dazzo *et al*., 2015, 2018; Pippucci *et al*., 2015).

In the presented large family F1, the proband and her daughter had clinical features resembling the typical EAF phenotype, despite the difficulty in gaining seizure control in the proband and the concomitant EEG traits of genetic generalized epilepsy with clear-cut temporal epileptiform abnormalities. In fact, despite unusual, these features have been reported anecdotally in EAF patients with *LGI1* pathogenic variants (Ottman *et al*., 2004). However, the remaining eight affected members showed marked intra-familial phenotypic variability, with a high recurrence of FS and/or generalized seizures in multiple individuals and infantile epileptic encephalopathy in one, consistent with GEFS+. Accordingly, molecular analysis led to identification of the *SCN1A* p.Met956Thr variant, confirming the clinical diagnosis. This variant, observed only once in gnomAD, was identified in an independent GEFS+ family and was proven to affect Nav1.1 function (Bechi *et al*., 2015). While temporal lobe epilepsy with mesial temporal semiology is considered part of the GEFS+ spectrum, EAF was not reported as a GEFS+ phenotype in a large recent series including 409 GEFS+ pedigrees (Zhang *et al*., 2017), nor in any other family. The evidence that *SCN1A* causes EAF with or without a history of FS/FS+ in our family with prominent features of GEFS+ makes EAF likely to be a rare phenotype of GEFS+.

The p.Tyr790Phe variant, identified in the sporadic Patient Sp2, also affects a functionally relevant highly conserved residue of the voltage-sensing module

Table 1. *SCN1A* missense variants identified in Epilepsy with Auditory Features.

	***SCN1A* variants**		
	F1	Sp 1	Sp 2
Nucleotide change*	c.2867T>C	c.419C>T	c.2369A>T
Protein change†	p.Met956Thr	p.Thr140Ile	p.Tyr790Phe
Protein region	DIIS5-S6 (pore)	DIS1	DIIS1-S2
Inheritance	Autosomal dominant	Paternally inherited	Not available
M-CAP prediction	Possibly damaging	Possibly damaging	Possibly damaging
ExAC/gnomAD allele count	0/1	0/0	1/6

F: family; Sp: sporadic case; D: domain; S: segment; M-CAP: Mendelian Clinically Applicable Pathogenicity score (bejerano.stanford.edu/mcap); ExAC: Exome Aggregation Consortium (exac.broadinstitute.com); gnomAD: Genome Aggregation Database (gnomad.broadinstitute.org). *RefSeq ID: NM_001165963 †RefSeq ID: NP_001159436

reported to be changed in sporadic and familial epilepsies within the GEFS+ spectrum (Grosso *et al*., 2007; Annesi *et al*., 2003; Bechi *et al*., 2015), supporting its pathogenicity. As for some of the cases reported, our patient had no features suggesting a GEFS+ diagnosis, presenting with self-limited EAF, without a personal or family history of FS. Moreover, identification of the additional novel p.Thr140Ile variant in Patient Sp1 supports that *SCN1A* missense variants may be implicated in sporadic patients with typical EAF, although this variant lacks segregation evidence and functional data to support its pathogenicity.

These findings showed that *SCN1A* variants may account for up to 4% of isolated EAF. Hence, we can argue that these cases may belong to GEFS+ pedigrees with a very low penetrance.

Based upon the presented data, we suggest that *SCN1A* screening should be considered in cases with EAF and suspicion of underlying GEFS+ syndrome, considering the role of molecular diagnosis in genetic counselling and its impact on treatment (*e.g.* avoidance of sodium channel blockers). In many epilepsy centres, the use of more complete genetic testing approaches rather than single gene screening, such as gene panels or whole exome sequencing, will allow the detection of such variants. The knowledge of a suspected underlying GEFS+ syndrome in EAF patients may be helpful to correctly interpret the role of disclosed *SCN1A* variants of uncertain significance in these cases.

In conclusion, *SCN1A* variants may be involved in EAF within the GEFS+ spectrum, while their role in EAF without features that lead to a suspicion of underlying GEFS+ remains unclear and should be elucidated in future studies. □

Supplementary data.

Supplementary material and summary didactic slides are available on the www.epilepticdisorders.com website**.**

Acknowledgements and disclosures.

The authors are grateful to the family for participation in the study, especially to the proband of family F1 for her valuable assistance during *in loco* assessment of the family. The authors acknowledge Dr Francesca Pittau, Dr Sara Muscho Ilbeh, and Dr Ibrahim Muscho Ilbeh for their help collecting blood samples from some family members. The authors acknowledge the genetic commission of the Italian League Against Epilepsy, especially Dr Amedeo Bianchi, for discussion of the pedigree of family F1 and valuable suggestions for the study design. The authors are grateful to Prof. Bernardo Dalla Bernardina, Dr Francesca Darra, Dr Elena Fontana, and Dr Carlotta Stipa for their valuable collaboration to collect clinical data. We are particularly grateful to Prof. Ingrid Scheffer for her relevant suggestions.

This work was supported by the Italian Ministry of Health Young Investigators project (GR-2009-1574072).

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

References

Abou-Khalil B, Ge Q, Desai R, *et al*. Partial and generalized epilepsy with febrile seizures plus and a novel *SCN1A* mutation. *Neurology* 2001; 57: 2265-72.

Annesi G, Gambardella A, Carrideo S, *et al*. Two novel *SCN1A* missense mutations in generalized epilepsy with febrile seizures plus. *Epilepsia* 2003; 44: 1257-8.

Bechi G, Rusconi R, Cestèle S, Striano P, Franceschetti S, Mantegazza M. Rescuable folding defective NaV1.1 (*SCN1A*) mutants in epilepsy: properties, occurrence, and novel rescuing strategy with peptides targeted to the endoplasmic reticulum. *Neurobiol Dis* 2015; 75: 100-14.

Bisulli F, Tinuper P, Avoni P, *et al*. Idiopathic partial epilepsy with auditory features (IPEAF): a clinical and genetic study of 53 sporadic cases. *Brain* 2004; 127: 1343-52.

Brunklaus A, Ellis R, Reavey E, Semsarian C, Zuberi SM. Genotype phenotype associations across the voltage-gated sodium channel family. *J Med Genet* 2014; 51: 650-8.

Dazzo E, Fanciulli M, Serioli E, *et al*. Heterozygous reelin mutations cause autosomal-dominant lateral temporal epilepsy. *Am J Hum Genet* 2015; 96: 992-1000.

Dazzo E, Rehberg K, Michelucci R, *et al*. Mutations in *MICAL-1* cause autosomal-dominant lateral temporal epilepsy. *Ann Neurol* 2018; 83: 483-93.

Grosso S, Orrico A, Galli L, Di Bartolo R, Sorrentino V, Balestri P. *SCN1A* mutation associated with atypical Panayiotopoulos syndrome. *Neurology* 2007; 69: 609-11.

Kalachikov S, Evgrafov O, Ross B, *et al*. Mutations in *LGI1* cause autosomal-dominant partial epilepsy with auditory features. *Nature Genet* 2002; 30: 335-41.

Kivity S, Oliver KL, Afawi Z, *et al*. *SCN1A* clinical spectrum includes the self-limited focal epilepsies of childhood. *Epilepsy Res* 2017; 131: 9-14.

Michelucci R, Pasini E, Malacrida S, *et al*. Low penetrance of autosomal dominant lateral temporal epilepsy in Italian families without *LGI1* mutations. *Epilepsia* 2013; 54: 1288-97.

Ottman R. *Autosomal dominant partial epilepsy with auditory features*. University of Washington, Seattle, 2015.

Ottman R, Winawer MR, Kalachikov S, *et al*. *LGI1* mutations in autosomal dominant partial epilepsy with auditory features. *Neurology* 2004; 62: 1120-6.

Pippucci T, Licchetta L, Baldassari S, *et al*. Epilepsy with auditory features: a heterogeneous clinico-molecular disease. *Neurol Genet* 2015; 1: e5.

Zhang YH, Burgess R, Malone JP, *et al*. Genetic epilepsy with febrile seizures plus: refining the spectrum. *Neurology* 2017; 89: 1210-9.

TEST YOURSELF

(1) Which of the following genes has never been associated with Epilepsy with Auditory Features?
A. LGI1
B. EPM2B
C. RELN
D. DEPDC5

(2) Which antiepileptic drug should be avoided or used with caution in patients carrying *SCN1A* mutations?
A. Carbamazepine
B. Valproate
C. Levetiracetam
D. Stiripentol

(3) What percentage cases of Epilepsy with Auditory Features has a documented genetic aetiology in 2019?
A. Up to 10%
B. 50-60%
C. Less than 50% of familial cases and less than 2% of isolated cases
D. 60-80% of familial cases and less than 30% of isolated cases

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".