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DEPDC5 mutations in families presenting as autosomal dominant nocturnal frontal lobe epilepsy

Fabienne Picard, MD[1]; Periklis Makrythanasis, MD, PhD[2]; Vincent Navarro, MD, PhD[3,4,5,6]; Saeko Ishida, PhD[3,4,5]; Julitta de Bellescize, MD[7]; Dorothée Ville, MD[8]; Sarah Weckhuysen, MD[9,10,11]; Erwin Fosselle, MD[12]; Arvid Suls, PhD[9,10]; Peter De Jonghe, MD, PhD[9,10,13]; Maryline Vasselon Raina, MD[14]; Gaetan Lesca, MD, PhD[15,16,17]; Christel Depienne, PhD[3,4,5,18]; Isabelle An-Gourfinkel, MD, PhD[3,4,5,6,19]; Mihaela Vlaicu, MD[3,4,5,6]; Michel Baulac, MD[3,4,5,6,19]; Emeline Mundwiller[20]; Philippe Couarch[21]; Romina Combi, PhD[22]; Luigi Ferini-Strambi, MD[23]; Antonio Gambardella, MD[24]; Stylianos E. Antonarakis, MD, PhD[2,25,26] ; Eric Leguern, MD, PhD[3,4,5,18]; Ortrud Steinlein, MD, PhD[27] and Stéphanie Baulac, PhD[3,4,5].

[1] Department of Neurology, University Hospitals of Geneva (HUG), CH-1211 Geneva 14, Switzerland. [2] Department of Genetic Medicine and Development, University of Geneva, Geneva, Switzerland. [3] Institut national de la santé et de la recherche médicale (INSERM), UMR_S975, Hôpital Pitié-Salpêtrière, F-75013 Paris, France. [4] Université Pierre et Marie Curie-Paris-6 (UPMC), F-75013 Paris, France. [5] CNRS, UMR7225, Hôpital de la Pitié-Salpêtrière, Paris F-75013, France. [6] Epilepsy unit, AP-HP Groupe hospitalier Pitié-Salpêtrière, F-75013 Paris, France. [7] Epilepsy, Sleep and Pediatric Neurophysiology, University Hospitals of Lyon (HCL), Lyon, France. [8] Hospices Civils de Lyon, HFME, centre de référence "déficiences intellectuelles de causes rares" et "sclérose tubéreuse de Bourneville", F-69677, Bron, France. [9] Neurogenetics group, Department of Molecular Genetics, VIB, Antwerp, Belgium. [10] Laboratory of Neurogenetics, Institute Born-Bunge, University of Antwerp, Antwerp, Belgium. [11] Epilepsy Centre Kempenhaeghe, Oosterhout, the Netherlands. [12] Algemeen Stedelijk Ziekenhuis, Aalst, Belgium. [13] Division of Neurology, Antwerp University Hospital, Antwerp University, Antwerp, Belgium. [14] Centre hospitalier général de Valence, F-26953 Valence, France. [15] Department of Medical Genetics, Hospices Civils de Lyon, F-69002 Lyon, France. [16] Claude Bernard Lyon I University, F-69100, Lyon, France. [17] CRNL, CNRS UMR 5292, INSERM U1028, F-69100, Lyon, France. [18] Département de Génétique et de Cytogénétique, AP-HP Groupe hospitalier Pitié-Salpêtrière, F-75013 Paris, France. [19] Centre de référence épilepsies rares et Sclérose tubéreuse de Bourneville. [20] Genotyping and Sequencing Platform, ICM, Hôpital Pitié-Salpêtrière, F-75013 Paris, France. [21] DNA and cell bank, Hôpital Pitié-Salpêtrière, F-75013 Paris, France. [22] Department of Surgery and Interdisciplinary medicine, University of Milano-Bicocca, Monza, Italy. [23] Sleep Disorders Center, University Vita-Salute San Raffaele, Milan, Italy. [24] Institute of Neurological Sciences, National ResearchCouncil, Mangone-Cosenza, Italy. [25] Service of Genetic Medicine, University Hospitals of Geneva, Geneva, Switzerland. [26] iGE3, Institute of Genetics and Genomics of Geneva, University of Geneva, Switzerland. [27] Institute of Human Genetics, University Hospital, University of Munich, Germany.

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Correspondence should be addressed to: Dr. Stéphanie Baulac, Institut du Cerveau et de la Moelle / Brain and Spine Institute (ICM), Hôpital de la Pitié-Salpêtrière, 47 bd de l'hôpital, Paris F-75013, France. Tel +331-5727-4339, Email: stephanie.baulac@upmc.fr

AUTHOR CONTRIBUTION

FP interpreted the clinical data and wrote the manuscript; PM, SI, CD, PC, EM, SEA, AS, OS analyzed the data; VN, MVR, MB, IGA, MV, DV, GL, JD, SW, EF, PDJ, RC, LFS, AG phenotyped the patients; OS wrote the manuscript; EL and SB conceptualized the study, wrote the manuscript and supervised the study.

DISCLOSURE STATEMENT

JDB, DV, EF, MVR, GL, CD, IAG, EM, PC, RC, LFS, AG report no disclosure.

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ABSTRACT

Objective. To study the prevalence of *DEPDC5* mutations in a series of 30 small European families with a phenotype compatible with autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE).

Methods. Thirty unrelated families referred with ADNFLE were recruited in France, Italy, Germany, Belgium and Norway. Whole exome sequencing was performed in ten probands and direct sequencing of the *DEPDC5* coding sequence in 20 probands. Testing for nonsense-mediated mRNA decay (NMD) was performed in lymphoblastic cells.

Results. Exome sequencing revealed a splice acceptor mutation (c.2355-2A>G) in *DEPDC5* in the proband of a German family. Additionally, three nonsense *DEPDC5* mutations (p.Arg487*, p.Arg1087* and p.Trp1369*) were detected in the probands of two French and one Belgian family. The nonsense mutations p.Arg487* and p.Arg1087* were targeted by NMD, leading to the degradation of the mutated transcripts. At the clinical level, seventy-eight % of the patients with *DEPDC5* mutations were drug resistant.

Conclusions. *DEPDC5* loss-of-function mutations were found in 13 % of the families with a presentation of ADNFLE. The rate of drug resistance was high in patients with *DEPDC5* mutations. Small pedigrees with *DEPDC5* mutations might actually represent a part of the broader familial focal epilepsy with variable foci (FFEVF) phenotype.

Keywords: ADNFLE, FFEVF, nicotinic receptor, focal epilepsies, autosomal dominant, mTOR.

INTRODUCTION

Autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE) is a syndrome characterized by clusters of sleep-related motor seizures and an average age of onset between 8 and 12 years. [1] Seizures are of short duration and consist of tonic, dystonic, or hyperkinetic manifestations. Drug resistance is reported in ~30% of the patients. [2-4] ADNFLE was the first familial epilepsy for which a causative gene, *CHRNA4*, was discovered. [5] To date, mutations in three subunit genes (α2, α4 and β2) of the nicotinic acetylcholine receptor (nAChR) have been reported. [4] Furthermore, the potassium channel *KCNT1* gene is causing a more severe form of ADNFLE with intellectual disability and psychiatric features. [6] However, these genes collectively account only for a minority of ADNFLE families (10-15 %), suggesting that additional causative genes exist. [4] Recently, *DEPDC5* (Dishevelled, Egl-10 and Pleckstrin Domain Containing protein 5) mutations were reported in 7/8 families with familial focal epilepsy with variable foci (FFEVF) and linked to the 22q12 locus. [7] *DEPDC5* mutations were also found in smaller families of focal epilepsies including familial temporal lobe epilepsy and ADNFLE. [7-9] DEPDC5 encodes a protein that is expressed ubiquitously in developing and adult brain. It was recently shown that DEPDC5 has a GAP (GTPase-activating protein) activity [10] and is part of a complex that negatively regulates mTORC1. [11] We have here evaluated the importance of *DEPDC5* in ADNFLE by assessing the mutation prevalence in 30 families.

METHODS

Patients

Families were recruited in different European Epilepsy centers (Pitié-Salpêtrière hospital, Paris; Femme-Mère-Enfant hospital, Lyon; University Hospital, Lyon; Institute of Human Genetics, University Hospital Ludwig-Maximilians, Münich; Sleep Disorders Center, Università Vita-Salute San Raffaele, Milan; Institute of Neurological Sciences, Mangone-Cosenza; Algemeen Stedelijk Ziekenhuis Aalst; University Hospital Gasthuisberg, Leuven; University Hospitals of Geneva). Criteria for inclusion in our cohort were 1) the presence of at least two family members with nocturnal frontal lobe epilepsy (NFLE), 2) a transmission compatible with an autosomal dominant inheritance, and 3) the absence of family members with seizures obviously arising from the temporal, occipital or parietal lobe. NFLE was defined by the occurrence of clusters of short-duration (less than one minute) hyperkinetic, tonic or dystonic seizures, arising predominantly during sleep.

Standard protocol approvals, registrations, and patient consents

Written informed consent was obtained from all participants (or the parents of minors). The study was approved by the local ethics committee (CCPPRB of Pitié-Salpêtrière Hospital, Paris, No. 69-03, 25/9/2003).

Genetic analysis

In 10 probands, exome sequencing was performed at the University of Geneva, Switzerland. Exome was captured using the SureSelect Human All Exons v4 reagents (Agilent Inc®). Sequencing was performed in an Illumina HiSeq 2000 instrument. Each exome library was indexed, separated into two equal halves and sequenced in two different lanes. Four half-libraries were sequenced in each HiSeq lane. The raw results were analyzed using a custom pipeline utilizing published algorithms in a sequential manner (BWA for mapping the reads, SAMtools for detection of variants, Pindel for the detection of indels, ANNOVAR for the annotation). *DEPDC5* variant was verified by Sanger sequencing. In 20 probands, all 42 coding exons and intron-exon junctions of *DEPDC5* (except exon 2 which was analyzed by Sanger sequencing) were analyzed by universal tailed amplicon sequencing (454 Sequencing Technology, Roche) as previously described at the

ICM (Pitié-Salpêtrière hospital, Paris, France). [8] *DEPDC5* cDNA refers to NCBI Reference Sequence # NM_001242896.

Cell culture and mRNA experiments

Lymphoblastic cells from individuals of families 2 and 3 were treated overnight with 10 mg/ml emetine to inhibit nonsense-mediated mRNA decay (NMD). Total RNA was extracted with the Qiagen RNeasy Mini kit and reverse-transcribed with the ThermoScript™ RT-PCR System (Invitrogen). *DEPDC5* cDNA was amplified and sequenced using specific primers located in exons 16 and 21 (p.Arg487*), and exons 31 and 35 (p.Arg1087*).

RESULTS

Clinical characterization

DEPDC5 mutations were identified in the probands of four previously undescribed ADNFLE families. Familial segregation study identified a total of 11 mutation carriers: 9 affected and 2 non-affected (Figure 1). Seizures were predominantly nocturnal in all patients. Mean age at onset was 8.7 years (ranged 1.5 to 17, median: 8.5). All nocturnal seizures had hyperkinetic (1/III-1, 1/III-3, 2/III-1, 4/III-4, 4/IV-1), tonic (2/IV-2) or dystonic (2/III-1, 3/II-3, 3/III-4, 4/III-2) components. Seizure duration was < 50 seconds in the nine patients (1/III-1, 1/III-3, 2/III-1, 2/IV-2, 3/II-3, 3/III-4, 4/III-2, 4/III-4, 4/IV-1). Two patients (4/III-2 and 4/IV-1) had vegetative symptoms consisting of sweating and palpitations preceding the motor symptoms. One patient (2/IV-2) had flushing during his tonic seizures. Patient 3/III-4 additionally had rare auras consisting of numbness in one upper limb. Rare generalized tonic-clonic seizures were reported in 5 out of 8 patients (2/IV-2, 3/II-3, 3/III-4, 4-III-2, 4/IV-1). Somnambulism was reported in 3 patients (2/III-1, 3/II-3, 3/III-4).

Three patients (1/III-1, 1/III-3, 4/IV-1) had exclusively nocturnal seizures, while six (2/III-1, 2/IV-2, 3/II-3, 3/III-4, 4/III-2, 4/III-4) also had rare diurnal seizures in the setting of a drug resistant nocturnal epilepsy. In three patients (3/II-3, 3/III-4, 4/III-2), diurnal seizures only consisted of vegetative symptoms (a breathless feeling with hyperventilation in patients 3/II-3 and 3/III-4, associated with a feeling of irregular heart rate in patient 3/III-4). Diurnal seizures were favored by stress and fatigue (3/II-3, 3/III-4, 4/III-4). Patients 4/III-2 and 4/III-4 only had two and one diurnal seizures through life respectively. Individual 2/III-1 experienced diurnal seizures only during

the first months after epilepsy onset, characterized by an alteration of consciousness with hypotonia and pallor. Patients presented 1 to 80 seizures per night, and focal status epilepticus occurred occasionally (2/IV-2 and 3/III-4). Seven out of 9 patients (78 %) did not achieve sustained seizure remission with administration of at least two tolerated antiepileptic drugs (whether as monotherapies or in combination) (Table e-1) and were thus considered as drug resistant. [12] Four patients (2/III-1, 2/IV-2, 3/III-4, 4/III-2) underwent presurgical evaluation due to drug-resistant seizures. Mild intellectual disability or psychiatric disorders were reported in four patients: learning difficulties with dyslexia and dysorthographia (2/III-1), mild language delay and attention deficit-hyperactivity disorder (ADHD) (2/IV-2), mild depression and a history of anorexia nervosa (3/III-4) and learning disability (4/III-4). Brain magnetic resonance imaging (MRI) was normal in all patients when available (Table 1). Interictal EEG was normal in 3 patients (1/III-1, 3/III-4, 4/IV-1), while it showed rare frontal spikes possibly extending to the central or temporal region, only visible during sleep recordings, in 3 individuals (2/III-1, 2/IV-2, 4/III-2). Ictal EEG in patient 2/III-1 showed bilateral frontal rhythmic delta waves while it did not show any clear epileptic discharges in individuals 2/IV-2, 3/III-4 and 4/III-2.

Molecular studies

Twenty-nine patients were negative for mutations in *CHRNA4*, *CHRNA2* and *CHRNB2*. Proband of family 4 (4/IV-1) was only recently diagnosed with epilepsy and was not screened for these genes. Exome sequencing identified a *DEPDC5* splice-acceptor mutation (c.2355-2A>G) in patient 1/III-1 (family 1). This mutation is predicted to cause an in-frame deletion of 55 amino acids (p.Arg785_Gly839del). Sanger sequencing in the remaining probands identified 3 additional mutations, c.1459C>T/p.Arg487* in patient 2/IV-2 (family 2), c.3259C>T/p.Arg1087* in patient 3/III-4 (family 3) and c.4107G>A/p.Trp1369* in patient 4/IV-1 (family 4) (Figure 2A). Mutations p.Arg487* and p.Trp1369* were previously reported in an Australian family with NFLE and a Spanish family with FFEVF respectively [7]. None of the mutations were present in the dbSNP135, 1000 Genomes project database or the 6,503 exome variant server (which lists only one non-sense mutation p.Gln63* at a frequency 1/11,813 in *DEPDC5*). All mutations co-segregated with the seizure phenotype. We failed to detect mRNA with c.1459C>T and c.3259C>T mutations in lymphoblast cells from patients, suggesting that the mutated transcripts are degraded by the nonsense-

mediated mRNA decay (NMD). Emetine treatment, an NMD inhibitor, rescued the transcript expression (Figure 2B).

DISCUSSION

DEPDC5 gene has recently been reported to be the causal gene for FFEVF linked to chromosome 22q12 [7] as well as other syndromes of familial focal epilepsies. [7-9] To evaluate the role of *DEPDC5* in ADNFLE, we have now studied a larger cohort of 30 small families diagnosed with this rare seizure disorder. We found that *DEPDC5* mutations are a frequent cause, accounting for 13 % in our series of families. Clinical features of this series of *DEPDC5*-positive families were comparable to those of ADNFLE patients from the literature: onset in childhood, clusters of brief motor seizures with hyperkinetic, tonic or dystonic manifestations, nocturnal predominance of seizures; rare (secondarily) generalized tonic-clonic seizures; breathless feeling experienced by some of the patients; co-occurrence of intellectual disability and/or psychiatric features in some family members [3, 13]; very few abnormalities on interictal and ictal EEG; incomplete clinical penetrance and association with somnambulism. [14] One notable difference was a high rate of drug resistance (78 %) in the patients with *DEPDC5* mutation in comparison with the one third classically reported in previous cohorts of ADNFLE families. [2-4] The two NFLE patients of our previously described *DEPDC5*-positive ADNFLE family (referred as family B) were also drug resistant. [3, 8] Among the four *DEPDC5*-positive ADNFLE families of the present study, 60% of the patients, all of whom were drug resistant, experienced rare diurnal seizures. This is in accordance with previous studies reporting that more than one third of the patients with ADNFLE had infrequent seizures during daytime wakefulness. [15-17] Despite the fact that the semiology of diurnal seizures lacked motor components in four out of six individuals, they were still concordant with a phenotype of NFLE: one patient had the same vegetative aura as during the nocturnal seizures (4/III-2), two (3/II-3 and 3/III-4) reported a breathless feeling with hyperventilation, a classical symptom of NFLE seizures [1, 2, 15, 18-20], and one (2/III-1, aged 3 years) reported an atonic component, concordant with the semiology described during daytime seizures of several ADNFLE patients during childhood. [17] Another study reported that NFLE seizures, at onset, are accompanied in nearly all patients by major autonomic manifestations involving heart rate, breathing, vasomotor tone and sympathetic skin response. [2] ADNFLE and FFEVF have phenotypical overlap and are sometimes difficult to distinguish. [21-23].

The occurrence of both diurnal and nocturnal seizures recalls frontal lobe seizures in FFEVF families, in which seizures emanate from different cortical regions among family members. [24] However, in our series, nocturnal seizures were clearly predominant in all patients, favoring a diagnosis of ADNFLE in clinical practice. Yet owing to the limited number of affected individuals (between 2 and 5) in the families reported here, it cannot be excluded that small ADNFLE pedigrees with *DEPDC5* mutation may actually represent a part of the broader phenotype of FFEVF, with the currently recognized affected family members suffering only from NFLE. [7]

So far, 23 *DEPDC5* mutations have been reported in familial focal epilepsies, including the four mutations found in this study, among which two are recurrent (Figure 2A). Two-thirds of these mutations are nonsense or frameshift, and we were able to show that two nonsense mutations described here were targeted by NMD. These findings suggest that *DEPDC5*-related epilepsy is likely to result from haploinsufficiency. Further studies are necessary to investigate the pathophysiological mechanisms causing *DEPDC5*-related epilepsies. However, it is first necessary to better understand the role of *DEPDC5* in brain development and functioning. A role of *DEPDC5* in the inhibition of the mTORC1 complex has recently been reported [11] providing new pathways, other than channelopathies, underlying focal epilepsies. It will be interesting to find out if the by now five ADNFLE genes share any biological pathways. Deciphering the function might help us to understand why mutations in nAChR subunit genes are only found in patients with ADNFLE while *DEPDC5* is obviously able to cause other types of focal epilepsy. A possible explanation would be that DEPDC5 is responsible for the development of an epileptic focus, and that other genetic or environmental factors might determine the localization of this focus.

FIGURE LEGENDS

Figure 1: **Pedigrees with *DEPDC5* mutations**. Patients in whom a *DEPDC5* mutation has been identified are indicated as m/+ and individuals tested for mutation and found to be negative are indicated by +/+. One subject (2/III-5), without epilepsy, had severe intellectual disability but did not participate in the study. Question mark indicates subjects with no information on the status affected or not.

Figure 2: **Schematic representation of *DEPDC5* mutations**. (A) Position of *DEPDC5* mutations previously described [7-9] and found in this study. The four mutations described in this study, c.2355-2A>G, c.1459C>T (p.Arg487*), c.3259C>T (p.Arg1087*) and c.4107G>A (p.Trp1369*) are boxed. Mutations p.Arg487* and p.Trp1369* were previously reported in other pedigrees. [7] (B) Degradation of mutated transcripts carrying c.1459C>T (p.Arg487*) and c.3259C>T (p.Arg1087*) mutations by the nonsense-mediated mRNA decay.

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N°	*DEPDC5* mutation	Sex	Age at the study (years)	Age at onset (years)	Somnambulism	Diurnal seizures	Intellectual disability	Psychiatric disorder	Drug Resistance*	Interictal EEG	MRI
Family 1											
III-1	c.2355-2A>G	M	41	8	No	No	No	No	Yes	N (awake recording)	N
III-3	c.2355-2A>G	M	39	11	No	No	No	No	No	NA	NA
Family 2											
III-1	c.1459C>T	M	12	3	Yes	Rare	Yes	No	Yes¶	Rare frontal spikes (sleep recording)	N
IV-2	c.1459C>T	M	5	1,5	No	Rare	Yes	Yes	Yes	Right fronto-central spikes (sleep recording)	N
Family 3											
II-3	c.3259C>T	M	76	17	Yes	Rare	No	No	Yes	NA	NA
III-4	c.3259C>T	F	32	14	Yes	Rare	No	Yes	Yes	N (sleep and awake recording)	N
Family 4											
III-2	c.4107G>A	F	40	8	No	2	No	No	Yes	Right fronto-temporal spikes (sleep recording)	N
III-4	c.4107G>A	M	56	2,5	No	1	Yes	No	Yes	NA	NA
IV-1	c.4107G>A	F	14	13	No	No	No	No	NA §	N (awake recording)	N

Table 1. Main clinical features in families with *DEPDC5* mutation. EEG, electroencephalogram; MRI, magnetic resonance imaging; M, male; F, female; N, normal; NA, not available. * Table e-1 provides further details on patient's prior treatment with AEDs. ¶ Patient

2/III-1 was drug resistant during the first 5 years after epilepsy onset and has been seizure-free for 4 years while treated with three antiepileptic drugs (vigabatrin, topiramate and valproate). § Patient 4/IV-1 has only recently been diagnosed with epilepsy.

Figure 1

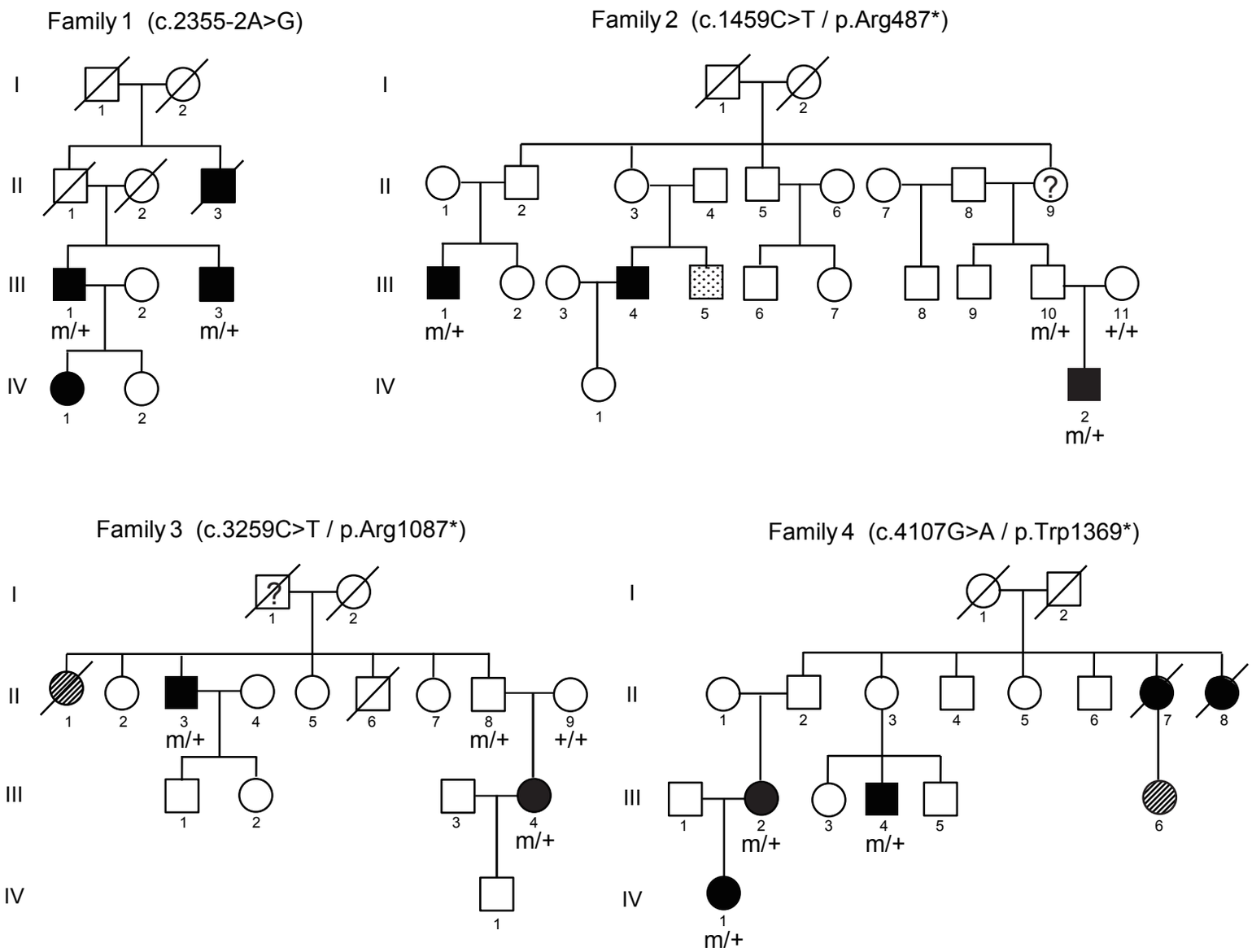
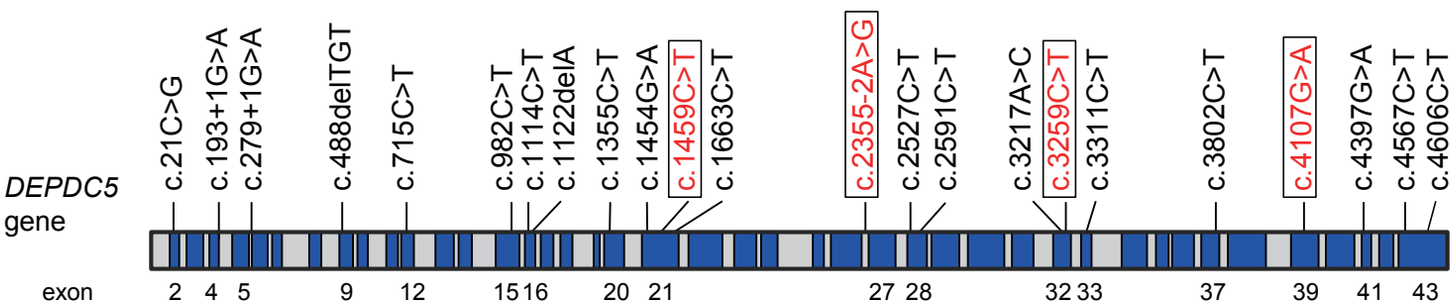
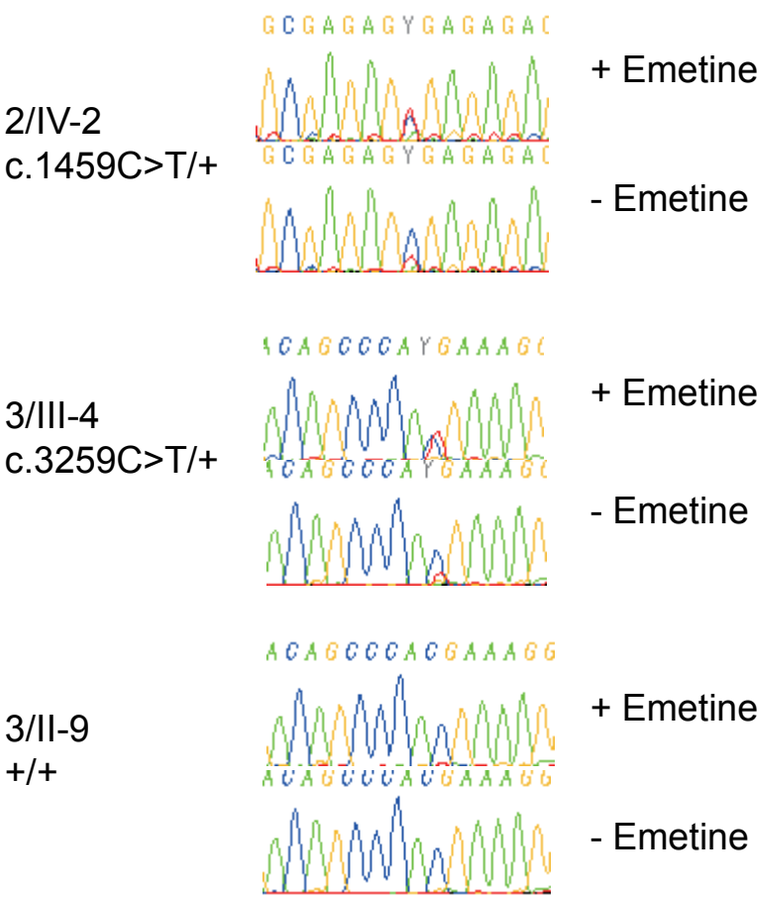

Family 1 (c.2355-2A>G)
Family 2 (c.1459C>T / p.Arg487*)
Family 3 (c.3259C>T / p.Arg1087*)
Family 4 (c.4107G>A / p.Trp1369*)
I
II
III
IV
m/+
+/+
Nocturnal Frontal Lobe Epilepsy
Unknown Epilepsy
Intellectual Disability

Figure 2

A


DEPDC5
gene
c.21C>G
c.193+1G>A
c.279+1G>A
c.488delTGT
c.715C>T
c.982C>T
c.1114C>T
c.1122delA
c.1355C>T
c.1454G>A
c.1459C>T
c.1663C>T
c.2355-2A>G
c.2527C>T
c.2591C>T
c.3217A>C
c.3259C>T
c.3311C>T
c.3802C>T
c.4107G>A
c.4397G>A
c.4567C>T
c.4606C>T
exon
2 4 5 9 12 15 16 20 21 27 28 32 33 37 39 41 43

B


2/IV-2
c.1459C>T/+
+ Emetine
- Emetine
3/III-4
c.3259C>T/+
+ Emetine
- Emetine
3/II-9
+/+
+ Emetine
- Emetine