ORIGINAL COMMUNICATION

The phenotypic spectrum of *KCNT1*: a new family with variable epilepsy syndromes including mild focal epilepsy

Christina Cherian[1] · Juan P. Appendino[2] · Setareh Ashtiani[3] · Paolo Federico[1,4,5,6] · Christine P. Molnar[5] · Marina Kerr[3,7] · Aneal Khan[3,7] · Ping Yee Billie Au[3,7] · Karl Martin Klein[1,3,4,7,8,9,10]

Abstract

Background and objective Pathogenic variants in *KCNT1* have been associated with severe forms of epilepsy, typically sleep-related hypermotor epilepsy or epilepsy of infancy with migrating focal seizures. To show that pathogenic variants in *KCNT1* can be associated with mild extra-frontal epilepsy, we report a *KCNT1* family with a wide spectrum of phenotypes ranging from developmental and epileptic encephalopathy to mild focal epilepsy without cognitive regression and not consistent with sleep-related hypermotor epilepsy.

Methods A large Canadian family of Caucasian descent including 9 affected family members was recruited. Family members were phenotyped by direct interview and review of existing medical records. Clinical epilepsy gene panel analysis and exome sequencing were performed.

Results Phenotypic information was available for five family members of which two had developmental and epileptic encephalopathy and three had normal development and focal epilepsy with presumed extra-frontal onset. All three had predominantly nocturnal seizures that did not show hyperkinetic features. All three reported clusters of seizures at night with a feeling of being unable to breathe associated with gasping for air, choking and/or repetitive swallowing possibly suggesting insular or opercular involvement. Genetic analysis identified a heterozygous *KCNT1* c.2882G > A, p.Arg961His variant that was predicted to be deleterious.

Discussion This family demonstrates that the phenotypic spectrum associated with *KCNT1* pathogenic variants is broader than previously assumed. Our findings indicate that variants in *KCNT1* can be associated with mild focal epilepsy and should not be excluded during variant interpretation in such patients based solely on gene–disease validity.

Keywords Epilepsy · Genetics · Phenotype · *KCNT1* · Focal epilepsy · Extra-frontal

✉ Karl Martin Klein
karl.klein@ucalgary.ca

1. Department of Clinical Neurosciences, Cumming School of Medicine, Foothills Medical Centre, University of Calgary, 1403 29 Street NW, Calgary, AB T2N 2T9, Canada

2. Division of Clinical Neuroscience, Department of Pediatrics, Cumming School of Medicine, Alberta Children's Hospital, University of Calgary, Calgary, AB, Canada

3. Department of Medical Genetics, Cumming School of Medicine, University of Calgary, Calgary, AB, Canada

4. Cumming School of Medicine, Hotchkiss Brain Institute, University of Calgary, Calgary, AB, Canada

5. Department of Radiology, Cumming School of Medicine, University of Calgary, Calgary, AB, Canada

6. Seaman Family MR Research Centre, Cumming School of Medicine, University of Calgary, Calgary, AB, Canada

7. Alberta Children's Hospital Research Institute, University of Calgary, Calgary, AB, Canada

8. Department of Community Health Sciences, Cumming School of Medicine, University of Calgary, Calgary, AB, Canada

9. Epilepsy Center Frankfurt Rhine-Main, Department of Neurology, Center of Neurology and Neurosurgery, University Hospital, Goethe-University, Frankfurt, Germany

10. Center for Personalized Translational Epilepsy Research (CePTER), Goethe University, Frankfurt, Germany

Introduction

Pathogenic variants in *KCNT1*, which encodes a sodium-activated potassium channel, have been described to be mainly associated with two distinct phenotypic presentations, a severe form of sleep-related hypermotor epilepsy (SHE, previously autosomal dominant nocturnal frontal lobe epilepsy) [1] and a developmental and epileptic encephalopathy (DEE) [2]. The latter often fulfills diagnostic criteria for epilepsy of infancy with migrating focal seizures (EIMFS) with seizure onset in the first 6 months of life and random and consecutive involvement of multiple cortical regions during a single seizure as well as significant developmental delay [2]. However, myoclonic atonic epilepsy and other developmental and epileptic

encephalopathies such as Ohtahara syndrome have been observed as well [3–7]. This more severe phenotype is typically due to de novo variants [2, 3].

Sleep-related hypermotor epilepsy shows different phenotypic features. This is a focal epilepsy syndrome that is characterized by clusters of seizures occurring at night, typically with hyperkinetic seizures consistent with a frontal origin. SHE due to *KCNT1* variants shows autosomal dominant inheritance and is often associated with intellectual disability and/or psychiatric features [1, 8] but the cognitive impact is less severe than in DEE/EIMFS.

Many patients with DEE/EIMFS due to *KCNT1* variants have been reported in the literature, but the familial SHE phenotype is less clear. We identified seven families reported in the literature, including at least one individual with SHE [1, 8–11] (Table 1). In five families, the phenotype of all

Table 1 Epilepsy type in published *KCNT1* families

Publication	Designation in publication	*KCNT1* variant	Epilepsy type			Additional features of individuals with focal epilepsy
			SHE	DEE/EIMFS	Other	
Heron et al. [1]	Family A	c.2782C > T;p.Arg-928Cys	6	0	0	Psychiatric or behavioral problems (*n*=5), ID (*n*=3)
	Family B[a]	c.2386 T > C; p.Tyr796His	4	0	0	Severe psychosis (*n*=2), ID (*n*=3), precocious puberty, FCD type Ib (*n*=1)
	Family C	c.1193G > A; p.Arg398Gln	4	0	0	Psychiatric or behavioral problems (*n*=2)
Moller et al. [8]	Family 1	c.2782C > T; p.Arg928Cys	8[b]	0	0	Psychiatric features (*n*=3), ID (*n*=4), cardiac arrhythmia (*n*=1), SUDEP (*n*=1)
	Family 2	c.1193G > A; p.Arg398Gln	2	2	1 (unspecified focal epilepsy)	Intellectual disability (*n*=1)
Rubboli et al. [9]	Family A[c]	c.2849G > A; p.Arg950Gln	3	0	1 (unspecified epilepsy)	Behavioral disturbance, neuropsychological deficits (*n*=1), FCD type Ib (*n*=1), N/A for other family members)
Cataldi et al. [11]	N/A	c. 2896 G > A; p.A966T	3	0	1 (SHE/EIMFS overlap)	Mild developmental delay (*n*=1), psychiatric disorder (*n*=1)
Barcia et al. [10]	Patient 2 and family	c.1193G > A; p.Arg398Gln	2	1	1 (single seizure)	Psychiatric disorder (*n*=1)
Cherian et al. (this study)	N/A	c.2882G > A, p.Arg961His	0	2	3 (extra-frontal focal epilepsy)	None

[a]Also reported in[9]

[b]Details reported on *n*=5

[c]Family B published in Heron et al. [1]

DEE developmental and epileptic encephalopathy, *EIMFS* epilepsy of infancy with migrating focal seizures, *FCD* focal cortical dysplasia, *ID* intellectual disability, *SHE* sleep-related hypermotor epilepsy, *SUDEP* sudden unexpected death in epilepsy

affected family members was consistent with SHE, typically associated with psychiatric features or intellectual disability. However, two families included family members consistent with SHE and others with EIMFS due to the same familial *KCNT1* pathogenic variants [8, 10]. This suggests that additional factors modify the expression of the phenotype. Furthermore, multiple sporadic cases with a SHE phenotype have been reported [1, 7–9, 12].

Knowledge about the phenotypic spectrum that is associated with a particular gene is of utmost importance during variant interpretation. Only variants in genes that are associated with the particular disease phenotype of the patient, i.e., for which gene–disease validity exists, can be interpreted as causative. Knowledge of the level of variable expressivity possible for genes and associated variants is also critical, as inheritance of variants is often used to determine variant pathogenicity. Previous studies reported cognitive regression as a consistent phenotypic feature in *KCNT1*-related epilepsy [1, 7, 8]. Here, we report a *KCNT1* family with a wide spectrum of phenotypes, including individuals with DEE but also individuals with milder focal epilepsy without psychiatric features, normal intelligence and not consistent with SHE.

Methods

A large Canadian family of Caucasian descent including 9 affected family members was recruited. Family members were phenotyped by direct interview and review of existing medical records. EEG reports were available in three patients. Two patients had additionally undergone video-EEG monitoring which were reviewed for this study. MRI was performed in three family members.

Clinical epilepsy gene panel analysis was performed in individuals III-14 and IV-5 via Prevention Genetics. Individual IV-5 also underwent exome sequencing using the MITO-FIND research study protocol [13]. The Nextera Flex for Enrichment Kit in combination with IDT xGen® Exome Research Panel (v1.0) probes was used for library preparation. Paired-end sequencing was performed using the NextSeq 500/550 High Output Kit v2.5 (300 Cycles). The Illumina Enrichment (v3.1.0) app workflow was used to generate variant call files using the GRCh37/hg19 assembly. The variant call file was imported into VarSeq™ 2.2.0 (Golden Helix®, Bozeman, United States of America) for tertiary analysis. The variant filtering process utilized databases and *in silico* predictors to annotate and evaluate the impact of the variants in the context of human disease, and variants were filtered based on quality (read depth and genotype quality), biological impact, clinical significance and minor allele frequency less than 5%. An internal assessment catalog and American College of Medical Genetics (ACMG) [14] classifier filter and Human Phenotype Ontology (HPO) terms [15] were used to assist in prioritizing variants (Phevor algorithm [16]). Variants matching HPO terms were then manually reviewed. Clinical segregation analysis was performed by Sanger sequencing in three other family members, two affected (III-11, II-5) and one unaffected (II-6).

Results

The family included 9 affected individuals over 3 generations (Fig. 1, Table 2). Phenotypic information was available for five family members of which two had DEE and three had focal epilepsy with normal development. Age of seizure onset in individuals with DEE was early (3–7 months), whereas family members with focal epilepsy had later onset (10–18 years). Individuals with DEE had significant developmental delay, whereas family members with focal epilepsy had normal development and showed no associated clinical features of psychiatric or learning disabilities. Neuropsychological assessment in individual III-14 with severe drug-resistant focal epilepsy described impaired to borderline scores for general intellect. However, the results were difficult to interpret due to considerable variability within and between cognitive domains and concomitant intake of topiramate at the time of testing. They were inconsistent with the patient's presentation of normal intellect (she completed secondary school in a normal program with low average grades and maintains stable employment, Table 2).

Seizure semiology and EEG recordings in individual IV-5 with DEE were consistent with EIMFS with multifocal epileptiform discharges, focal and generalized onset seizures and propagation to different cortical areas in the same seizure (Online Resource 1). Her MRI did not show an epileptogenic lesion. No acquired epilepsy risk factors for IV-5 were recognized. Her mother was on carbamazepine and had two seizures but no status epilepticus during pregnancy. The pregnancy was otherwise unremarkable. Individual IV-5 was delivered at term without requiring resuscitation and discharged at two days of life. There was no history of brain injury or infection. The other individual with DEE (III-13) had passed away at age 28 years. She had been diagnosed with Lennox–Gastaut syndrome, but no further details on seizure semiology were available.

The three individuals with focal epilepsy (II-5, III-11, III-14) were most consistent with extra-frontal onset. All had predominantly nocturnal seizures but did not show hyperkinetic features associated with their seizures. All three reported episodes they had termed "breathing spells" which typically occurred in clusters at night. These were characterized by the feeling of being unable to breathe associated with gasping for air, choking and/or repetitive swallowing. Additional seizure types in these individuals were consistent with a temporal or insular/opercular onset (Table 1). Individual
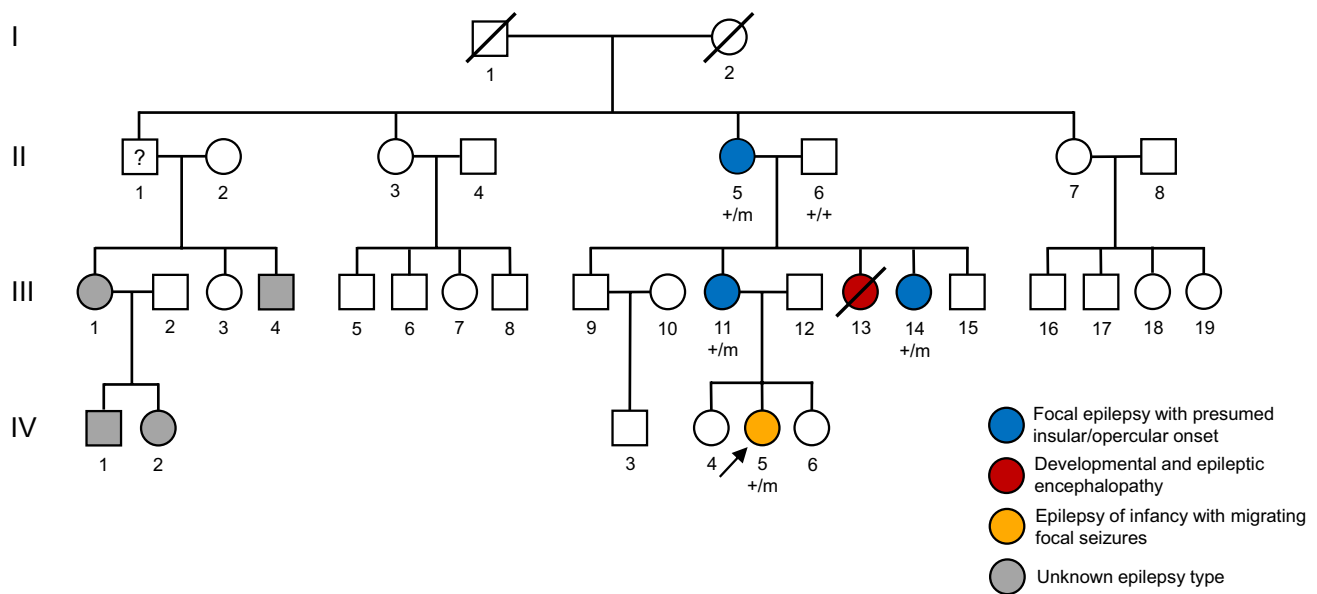
Fig. 1 Pedigree of the family showing segregation of the *KCNT1* c.2882G > A variant. The arrow indicates the proband. ?: uncertain affected status, m: *KCNT1* c.2882G > A variant, +: wild-type allele

III-14 had undergone pre-surgical work-up including ictal subtraction SPECT and PET which was most consistent with a left temporal onset (Online Resource 2). A routine EEG in her sister III-11 had shown a single right temporal sharp transient that was not definitively epileptiform (Online Resource 3). MRI in both patients did not reveal an epileptogenic lesion.

Epilepsy severity was variable in the family. The two individuals with DEE and individual III-14 with focal epilepsy had severe drug-resistant epilepsy. The latter had frequent focal aware and impaired awareness seizures and rare focal to bilateral tonic–clonic seizures despite a combination of three anti-seizure medications. Individual III-11 had less severe epilepsy with focal aware seizures and focal to bilateral tonic–clonic seizures only from sleep on a monotherapy with carbamazepine. Individual II-5 had not been diagnosed with epilepsy previously. She experienced an episode with loss of awareness at age 14 years of uncertain etiology but reported similar "breathing spells" as her daughters which were considered focal aware seizures. She had been treated with phenobarbital for two years after the event at age 14 years but had not taken anti-seizure medication since. The family members with focal epilepsy did not show significant changes in seizure semiology, severity and frequency over time.

Four additional family members had been reported to have epileptic seizures but were unavailable for direct study (Fig. 1). No cardiac or respiratory comorbidities had been identified in the family.

Genetic analysis identified a heterozygous conserved (PhyloP and GERP + +) missense variant with a z-score > 1 (3.52) exhibiting an autosomal dominant inheritance pattern in *KCNT1* (g.138675910; NM_020822.2:c.2882G > A; pArg961His). The variant was predicted to be damaging (MSA-SIFT and MSA-PolyPhen2) but has not, to our knowledge, been functionally evaluated yet. The variant was not present in control cohorts including the Genome Aggregation Database (gnomAD) [17] and had been reported previously in seven patients with DEE and SHE [3, 7, 18, 19]. Based on clinical and family history, previous reports, allele frequency and in silico predictors, we classified the variant as likely pathogenic (PS1, PM2, PP1, PP3) using the ACMG recommendations [14]. This is consistent with previous ClinVar reports.

A review of variants of uncertain significance identified no other candidate gene that was related to the phenotype using ACMG criteria [14]. Particularly, there was no evidence for a second contributing variant in individual IV-5 as an explanation for the more severe phenotype.

Discussion

This multigenerational family segregating a *KCNT1* pathogenic variant shows significant variability in epilepsy severity and epilepsy syndrome. Intra-familial variability has been well described for other genes such as *SCN1A* ranging from simple febrile seizures to Dravet syndrome [20]. However, only two *KCNT1* families have been described previously with differing epilepsy severity between family members, ranging from the more severe EIMFS to the generally less severe SHE [8, 10]. Specific single variants may also be

Table 2 Phenotypic information of the five available affected family members

Family member	Age of seizure onset	Seizure semiology and frequency	Diagnostic tests	Comorbidities	Anti-seizure medication
II-5 (64 yrs)	14 yrs	Focal aware non-motor onset sensory seizures: "breathing spells" feeling of being unable to breathe and gasping for air (from sleep, clusters over 3–4 nights with 2–3/ night every 3 months) Single event with loss of awareness at 14 years, PB for 2 years afterwards	EEG: N/A (normal according to patient) Brain imaging: N/A	None	Current: none Previous: PB
III-11 (40 years)	10 yrs	Focal aware non-motor onset sensory seizures: "breathing spells" sensation of choking and frequent swallowing (after waking from sleep, clusters of 2–3 days with 1 seizure per night every 2 weeks to 3 months) FBTCS: from sleep (5/year) Questionable auditory aura (from wakefulness) (1/year)	EEG 12 yrs: normal 18 yrs: mild left temporal slowing 20 yrs: single right mid-temporal sharp transient (Online Resource 3), mild bitemporal slowing MRI 19, 24, 37 yrs: normal	Migraine without aura, neuro exam normal, no ID	Current: CBZ Previous: none
III-13	6–7 mths	No details available. Diagnosed with Lennox–Gastaut syndrome	EEG: N/A Brain imaging: N/A	Developmental regression at age 11 years, deceased at age 28 years	VPA, PHT, LEV

Table 2 (continued)

Family member	Age of seizure onset	Seizure semiology and frequency	Diagnostic tests	Comorbidities	Anti-seizure medication
III-14 (30 yrs)	18 yrs	Focal aware non-motor onset sensory seizures: (1) Epigastric aura (from wakefulness, none currently) (2) Nausea with urge to vomit, repetitive swallowing (from wakefulness and sleep) (multiple seizures on 1 day/month) (3) "Breathing spells" sensation of being unable to breathe, gasping for air (from sleep, one cluster per month of 4–5 nights with multiple per night) FIAS: tonic contraction of arms, stiffness, facial grimacing (1/2 month during day, nocturnal frequency unknown) FBTCS: from sleep (1/year)	EEG 25 yrs: single IED right anterior temporal VEM 26 yrs: Interictal: rare bifrontal slowing, no IED Ictal: FIAS with subtle hand and foot automatisms, often from sleep, EEG onset left temporal > frontal or no clear correlate (Online Resource 2) FBTCS with arousal from sleep, blank staring, oral automatisms, followed by BTCS. EEG onset more diffuse, max left frontotemporal (Online Resource 2) MRI 24 yrs: normal Ictal subtraction SPECT 26 yrs: hyper-perfusion anterior left temporal lobe, left insula and small region of left peri-insular frontal operculum (injection 31 s after clin onset, Online Resource 2) PET (26 yrs): extensive hypometabolism in cerebral gray matter and thalamic nuclei (Online Resource 2) EKG: normal sinus rhythm	Migraine without aura, neuro exam normal, no ID, Montreal Cognitive Assessment normal (27 points at 27 yrs) Neuropsychological assessment (26 yrs): Cognitive profile marked by considerable variability within and between cognitive domains which was inexplicable and partly inconsistent with her presentation. The patient did not present as having reduced intellectual functioning (completed secondary school with low average grades, maintains employment) but scores for general intellect were impaired to borderline. Patient on TPM at the time of testing	Current: PHT, BRV, TPM Previous: LTG, LEV

Table 2 (continued)

Family member	Age of seizure onset	Seizure semiology and frequency	Diagnostic tests	Comorbidities	Anti-seizure medication
IV-5 (7 years)	3 mths	3 mths: rare focal motor tonic seizures: left face and arm, eyelid fluttering, eye rolling (none currently) 6 mths: additional focal impaired awareness non-motor onset behavior arrest seizures, may include desaturation (~70%), or mild head deviation with contralateral tonic arm extension (none currently) 4 yrs: focal motor tonic seizures: right head and left eye deviation followed by bilateral tonic seizures (clusters every 2–4 month) Later: generalized myoclonic seizures (none currently) Focal impaired awareness non-motor onset behavioral arrest seizures (1 every 1–2 weeks) Unknown onset bilateral tonic–clonic seizures (rare, none currently)	EEG 4 mths: sleep-activated right central spikes. No ictal EEG available 6 mths: multifocal IED during sleep. Seizure right > left centrotemporal focal onset (Online Resource 1) 4 yrs: background slowing, multifocal IED awake and sleep. Focal onset either left or right posterior quadrant, then shift to contralateral, then to bilateral tonic seizures (Online Resource 1). Subclinical left or right posterior quadrant seizures MRI: normal (5 months) CT: mild bilateral frontal atrophy (5 years) EKG 4, 6, 7 yrs: normal sinus rhythm Chromosomal microarray negative, Angelman testing negative	Significant developmental delay after seizure onset: no purposeful words, not ambulatory, GT tube fed Examination: diffuse hypotonia, able to partially hold head. DTR present 2 + in four limbs, no clonus, no upgoing toes, localizing soft touch in four limbs. Distinctive facial features: longer palpebral fissures and epicanthal folds, lateral eyebrow flare, broader nasal tip and thicker alae nasi, tented mouth with open mouth posture, prominent cheeks and broader face. Many missing teeth (including secondary dentition, recently lost), hypertrophic swollen appearing gums	Current: quinidine (since age 7 years, > 50% seizure reduction, improved QOL), TPM, PB, BRV, CLB, IVIG, ketogenic diet Previous: OXC, LEV, nitrazepam

BRV brivaracetam, *BTCS* bilateral tonic–clonic seizure, *CBZ* carbamazepine, *CLB* clobazam, *FBTCS* focal to bilateral tonic–clonic seizure, *FIAS* focal impaired awareness seizure, *IED* interictal epileptiform discharge/s, *IVIG* intravenous immunoglobulin, *LEV* levetiracetam, *LTG* lamotrigine, *N/A* not available, *OXC* oxcarbazepine, *PB* phenobarbital, *PHT* phenytoin, *QOL* quality of life, *TPM* topiramate, *VEM* video-EEG monitoring, *VPA* valproate

associated with either EIMFS or SHE in unrelated individuals [6, 7, 12].

Most affected individuals with *KCNT1* pathogenic variants reported so far have been either consistent with DEE/EIMFS or SHE. One patient was reported with overlapping features of both conditions [11]. A recent study described a patient with drug-resistant bilateral temporal lobe epilepsy confirmed by invasive pre-surgical evaluation [7]. In others, the available phenotypic information was insufficient to assign a clear phenotype. The latter includes a patient with nocturnal focal epilepsy without further published details on seizure semiology and EEG findings [8], a patient with multifocal epilepsy who also had anti-GAD65 antibodies [8] and a patient with diurnal bilateral tonic–clonic seizures with normal EEG and MRI [7]. Additional described phenotypes are myoclonic atonic epilepsy [6] and temporal lobe epilepsy with intellectual disability and ataxia [21]. However, in the last case, pathogenicity of the underlying variant is questionable as 23 heterozygotes are reported in gnomAD. A consistent feature was cognitive regression after seizure onset [7].

Our family is unique as it includes one individual (II-5) who had mild epilepsy with only focal aware seizures and one single event with loss of awareness. All three individuals with focal epilepsy had seizures that were not consistent with SHE or nocturnal frontal lobe epilepsy based on seizure semiology, routine EEG, video-EEG monitoring and functional imaging. In addition, none of the family members with focal epilepsy had intellectual disability, although we cannot exclude subtle cognitive dysfunction based on the neuropsychological testing in individual III-14 with severe drug-resistant epilepsy.

Our findings therefore expand the phenotypic spectrum associated with *KCNT1* pathogenic variants and show that *KCNT1* can also cause mild focal epilepsy without cognitive regression. The strength of evidence that variation in a particular gene is causative for a specific disease or seizure type (i.e., the concept of gene–disease validity) is critical for variant interpretation [22]. Therefore, our finding that *KCNT1* variants are causative for mild focal epilepsy means that *KCNT1* variants that may have previously been disregarded in this context should now be considered. Although we cannot exclude that the association with mild extra-frontal epilepsy may be a feature specific for the p.Arg961His variant, we suspect that other *KCNT1* variants may show similar features. It is possible that preferential genetic workup in more severe epilepsies has precluded the identification of milder phenotypes in the past. It is also possible that inherited variants that may have been disregarded previously should be reconsidered given further evidence that intra-familial variability can be quite striking for *KCNT1*-related epilepsy.

The identified *KCNT1* c.2882G > A, p.Arg961His variant has been reported previously in seven patients [3, 7, 18, 19]. Three of these individuals had DEE (1 de novo, 1 inherited

and 1 unknown inheritance) and four had SHE (3 de novo, 1 inheritance unknown). For two individuals with DEE, detailed phenotypic information was reported, with onset within the first 3 months of life and multiple seizures daily [3, 7]. The phenotype of these reported individuals was comparable to the two family members with DEE in our family (III-13, IV-5). However, individual IV-5 had typical features for EIMFS which had not been described in the previously reported patients. Detailed information was also reported on three patients with SHE due to the Arg961His variant [7]. These individuals had onset between 6 and 11 years and mild to moderate intellectual disability. They were different to the family members with focal epilepsy in our family (II-5, III-11, III-14), who had later seizure onset (10–18 years) with no typical features of SHE and no intellectual disability.

An interesting observation was that the three family members with focal epilepsy all reported focal sensory aware seizures with unique semiology which they had termed "breathing spells". These typically occurred in clusters at night and were associated with a feeling of being unable to breathe associated with gasping for air, choking and/or repetitive swallowing. The described semiology may suggest an involvement of the insular or opercular cortex in the seizures although this could not be substantiated with EEG data. However, it is consistent with a previous report of insular onset of SHE [23] of which one patient was later found to have a *KCNT1* pathogenic variant [11].

The significant variability in severity and epilepsy syndrome within our and other families suggests that additional factors influence the phenotype. This may include modifier genes but also environmental factors. We did not identify a second contributing rare variant in the most severely affected individual, but it may well be possible that common variants, or rare variants in other so far unknown genes, may modify epilepsy severity. Identification of these factors would significantly advance our understanding of the pathophysiology in genetic epilepsies. However, this remains challenging as the few individuals known with similar variants preclude statistical comparisons.

A limitation of our study is that not all affected family members participated in the research study and detailed EEG and MRI data were unavailable in two family members including one deceased individual. Although extra-frontal onset was highly suspected based on semiology and non-invasive data, it could not definitely be confirmed without intracranial EEG evaluation.

In conclusion, we report a new *KCNT1* family with variable phenotype including individuals with severe DEE and mild focal epilepsy suggesting an extra-frontal onset. This family shows that the phenotype associated with *KCNT1* pathogenic variants is broader than previously assumed. This is an important observation for variant interpretation as it indicates that *KCNT1* variants may be responsible for mild

focal epilepsy. Identified *KCNT1* variants in patients with mild focal epilepsy should, therefore, assessed according to ACMG criteria [14] and not excluded based on gene–disease validity. This is particularly important as quinidine may be a precision medicine treatment for *KCNT1*-related epilepsy [24] although the outcome has been variable [25, 26].

Declarations

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