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Bonardi, Claudia M; Heyne, Henrike O; Fiannacca, Martina; Fitzgerald, Mark P; Gardella, Elena; Gunning, Boudewijn; Olofsson, Kern; Lesca, Gaétan; Verbeek, Nienke; Stamberger, Hannah; Striano, Pasquale; Zara, Federico; Mancardi, Maria M; Nava, Caroline [**and 34 more**]

KCNT1-related epilepsies and epileptic encephalopathies: phenotypic and mutational spectrum

Claudia M. Bonardi,[1,2] Henrike O. Heyne,[3,4] Martina Fiannacca,[5] Mark P. Fitzgerald,[6] Elena Gardella,[1,7] Boudewijn Gunning,[8] Kern Olofsson,[9] Gaétan Lesca,[10,11] Nienke Verbeek,[12] Hannah Stamberger,[13,14] Pasquale Striano,[15] Federico Zara,[15] Maria M. Mancardi,[16] Caroline Nava,[17] Steffen Syrbe,[18] Salvatore Buono,[19] Stephanie Baulac,[20] Antonietta Coppola,[21] Sarah Weckhuysen,[13,14] An-Sofie Schoonjans,[22] Berten Ceulemans,[22] Catherine Sarret,[23] Tobias Baumgartner,[24] Hiltrud Muhle,[25] Vincent des Portes,[26] Joseph Toulouse,[27] Marie-Christine Nougues,[28] Massimiliano Rossi,[10,29] Geneviève Demarquay,[30,31] Dorothée Ville,[32] Edouard Hirsch,[33] Hélène Maurey,[34] Marjolaine Willems,[35] Julitta de Bellescize,[36] Cecilia Desmettre Altuzarra,[37] Nathalie Villeneuve,[38] Fabrice Bartolomei,[39] Fabienne Picard,[40] Frauke Hornemann,[41] David A. Koolen,[42] Hester Y. Kroes,[12] Chiara Reale,[1,43] Christina D. Fenger,[1] Wen-Hann Tan,[44] Leanne Dibbens,[45] David R. Bearden,[46] Rikke S.Møller[1,7] and Guido Rubboli[1,47]

Abstract

Variants in *KCNT1,* encoding a sodium-gated potassium channel (subfamily T member 1), have been associated with a spectrum of epilepsies and neurodevelopmental disorders. These range from familial autosomal dominant or sporadic sleep-related hypermotor epilepsy ((AD)SHE) to epilepsy of infancy with migrating focal seizures (EIMFS) and include developmental and epileptic encephalopathies (DEE). This study aims to provide a comprehensive overview of the phenotypic and genotypic spectrum of *KCNT1* mutation-related epileptic disorders in 248 individuals, including 66 unpreviously published and 182 published cases, the largest cohort reported so far. Four phenotypic groups emerged from our analysis: i) EIMFS (152 individuals, 33 previously unpublished); ii) DEE other than EIMFS (non-EIMFS DEE) (37 individuals, 17 unpublished); iii) (AD)SHE (53 patients, 14 unpublished); iv) other phenotypes (6 individuals, 2 unpublished). In our

cohort of 66 new cases, the most common phenotypic features were: a) in EIMFS, heterogeneity of seizure types, including epileptic spasms, epilepsy improvement over time, no epilepsy-related deaths; b) in non-EIMFS DEE, possible onset with West syndrome, occurrence of atypical absences, possible evolution to DEE with SHE features; one case of sudden unexplained death in epilepsy (SUDEP); c) in (AD)SHE, we observed a high prevalence of drug-resistance, although seizure frequency improved with age in some individuals, appearance of cognitive regression after seizure onset in all patients, no reported severe psychiatric disorders, although behavioural/psychiatric comorbidities were reported in about 50% of the patients, SUDEP in one individual; d) other phenotypes in individuals with mutation of *KCNT1* included temporal lobe epilepsy, and epilepsy with tonic-clonic seizures and cognitive regression.

Genotypic analysis of the whole cohort of 248 individuals showed only missense mutations and one inframe deletion in *KCNT1*. Although the *KCNT1* mutations in affected individuals were seen to be distributed among the different domains of the KCNT1 protein, genotype-phenotype considerations showed many of the (AD)SHE-associated mutations to be clustered around the RCK2 domain in the C-terminus, distal to the NADP domain. Mutations associated with EIMFS/non-EIMFS DEE did not show a particular pattern of distribution in the KCNT1 protein. Recurrent *KCNT1* mutations were seen to be associated with both severe and less severe phenotypes. Our study further defines and broadens the phenotypic and genotypic spectrums of *KCNT1*-related epileptic conditions and emphasizes the increasingly important role of this gene in the pathogenesis of early onset DEEs as well as in focal epilepsies, namely (AD)SHE.

Author affiliations

1 Department of Epilepsy Genetics and Precision Medicine, Danish Epilepsy Centre, member of the ERN EpiCARE, 4293 Dianalund, Denmark

2 Department of Woman's and Child's Health, University Hospital of Padua, 35100 Padua, Italy

3 Finnish Institute for Molecular Medicine: FIMM, University of Helsinki, 00290 Helsinki, Finland

4 Program for Medical and Population Genetics, Broad Institute of MIT and Harvard, 02142 Cambridge, MA, USA

5 Radiology Department, University of Genoa, 16126 Genoa, Italy

6 Division of Neurology, Departments of Neurology and Pediatrics, The Children's Hospital of Philadelphia and the Perelman School of Medicine at the University of Pennsylvania, Philadelphia PA, USA

7 Institute of Regional Health Research, University of Southern Denmark, 5230 Odense, Denmark

8 Stichting Epilepsie Instellingen Nederland, Zwolle, 8025 BV, The Netherlands

9 Department of Pediatric Neurology, Danish Epilepsy Center, 4293 Dianalund, Denmark

10 Department of Genetics, Hospices Civils de Lyon, 69002 Bron, France

11 Institut NeuroMyoGène, CNRS UMR 5310 - INSERM U1217, Université Claude Bernard Lyon 1, 69008 Lyon, France

12 Department of Genetics, University Medical Center, 3584 CX Utrecht, Netherlands

13 Neurogenetics Group, VIB-Center for Molecular Neurology, B-2610 Antwerp, Belgium

14 Department of Neurology, University Hospital, 2650 Antwerp, Belgium

15 IRCCS "G. Gaslini" Institute, University of Genoa, 16147 Genoa, Italy

16 Child Neuropsychiatry Unit, IRCCS Istituto Giannina Gaslini, 16147 Genoa, Italy

17 Département de Génétique, APHP, GH Pitié-Salpêtrière, 75013 Paris, France

18 Division of Pediatric Epileptology, Centre for Paediatrics and Adolescent Medicine, University Hospital Heidelberg, 69120 Heidelberg, Germany

19 Neurology Division, Hospital of National Relevance (AORN), Santobono Pausilipon, 80122 Naples, Italy

20 Sorbonne Université, Institut du Cerveau - Paris Brain Institute - ICM, Inserm, CNRS, F-75013, Paris, France

21 Department of Neuroscience and Reproductive and Odontostomatological Sciences, Federico II University, 80138 Naples, Italy

22 Department of Pediatric Neurology, Antwerp University Hospital, University of Antwerp, 2650 Edegem, Belgium

23 Service de Neuropédiatrie, CHU de Clermont-Ferrand, 6310 Clermont-Ferrand, France

24 Department of Epileptology, University of Bonn, D – 53105 Bonn, Germany

25 Department of Neuropediatrics, University Medical Center Schleswig Holstein, 24105 Kiel, Germany

26 Neuropaediatrics Department, Femme Mère Enfant Hospital, 69500 Lyon, France

27 Epileptology, Sleep Disorders and Functional Pediatric Neurology CHU Lyon, 69500 Bron, France

28 Neuropaediatrics Department, Hospital Armand Trousseau, APHP, 75012 Paris, France

29 Lyon Neuroscience Research Center (CRNL), INSERM U1028, CNRS UMR5292, GENDEV Team, Claude Bernard Lyon 1 University, 69675 Bron, France

30 Service de neurologie fonctionnelle et épileptologie, Neurological Hospital, 69677 Bron, France

31 Lyon Neuroscience Research Center (CRNL), INSERM U1028, CNRS UMR5292, NeuroPain, 69677 Bron, France

32 Pediatric Neurology Department, Lyon University Hospital, 69500 Bron, France

33 Epilepsy Unit, Hautepierre Hospital, University of Strasbourg, 67100 Strasbourg, France

34 Department of Pediatric Neurology, Hopital Bicêtre, Le Kremlin-Bicêtre, 94270 Paris, France

35 Department of Clinical Genetics, Arnaud de Villeneuve Hospital, 34090 Montpellier, France

36 Department of Pediatric Clinical Epileptology, Sleep Disorders and Functional Neurology, Hospices Civils de Lyon, 69677 Bron, Lyon, France

37 Department of Pediatrics, St. Jacques Hospital, 25000 Besançon, France

38 Pediatric Neurology Department, Timone Children Hospital, 13005 Marseille, France

39 Epileptology Department, Timone Hospital, Public Assistance Hospitals of Marseille, Aix-Marseille University, 13005 Marseille, France

40 Department of Clinical Neurosciences, University Hospitals and Faculty of Medicine, CH-1211 Geneva, Switzerland

41 Centre of Pediatric Research, Hospital for Children and Adolescents, 04103 Leipzig, Germany

42 Department of Human Genetics, Donders Institute for Brain, Cognition and Behaviour, Radboud university medical center (Radboudumc), 6525 GA Nijmegen, The Netherlands

43 Department of Clinical and Experimental Medicine, University of Messina, 98122 Messina, Italy

44 Division of Genetics and Genomics, Boston Children's Hospital, Harvard Medical School, Boston, MA 02115, USA

45 Epilepsy Research Group, UniSA Clinical and Health Sciences, University of South Australia, and Australian Centre for Precision Health, SA 5001 Adelaide, Australia

46 Division of Child Neurology, Department of Neurology, University of Rochester School of Medicine, Rochester, NY14642 , USA

47 Institute of Clinical Medicine, University of Copenhagen, 2200 Copenhagen, Denmark

Correspondence to: Guido Rubboli

Danish Epilepsy Center, Filadelfia/University of Copenhagen

Kolonivej 1

4293 Dianalund, Denmark

E-mail: guru@filadelfia.dk

Correspondence may also be addressed to: Rikke S. Møller

E-mail: rimo@filadelfia.dk

Introduction

KCNT1 encodes a sodium-gated potassium channel (potassium channel, subfamily T, member 1), previously known as Slack, Slo2.2 or KCa4.1 (OMIM*608167).[1] It is expressed diffusely in the nervous system, mainly in the cerebellum, frontal cortex, brainstem and hippocampus,[2] playing an important role in regulating neuronal excitability. *KCNT1*-channels contribute to slow hyperpolarization following a single action potential or repetitive firings, which regulates the bursting rate and enables variation of the adaptation rate with the intensity of stimulation (Carrasquel-Ursulaez *et al.,* 2018).[2]

Since the first description of *KCNT1* pathogenic variants in a cohort of individuals with epilepsy of infancy with migrating focal seizures (EIMFS),[3] and in cases with autosomal dominant or sporadic sleep-related hypermotor epilepsy ((AD)SHE),[4] the phenotypic spectrum has expanded to include a wide group of heterogeneous developmental and epileptic encephalopathies (DEE). These include distinct phenotypes such as Ohtahara (OS) and West syndromes (WS), as well as unclassified DEEs.[5,6]

In this study we aim to provide a comprehensive overview of the phenotypic and genotypic spectrum of *KCNT1*-related epileptic disorders in 248 individuals by describing a cohort of 66 previously unreported cases, the largest cohort studied to date, and by reviewing 182 published cases.

Materials and Methods

Previously unpublished cohort

Sixty-six individuals with pathogenic *KCNT1* variants were studied through an international network of Epilepsy and Genetic Centers in Europe, North America and Australia. *KCNT1* variants were identified in individuals with intellectual disability (ID) and/or childhood epilepsies/encephalopathies by genomic analysis performed either by using targeted gene panels or whole exome sequencing. Genomic variants and, when possible, familial segregation were confirmed by direct Sanger sequencing. Maternity and paternity has not been confirmed in probands with de novo variants. All missense variants were considered pathogenic if the phenotype of the individual resembled a *KCNT1*-related disorder and they fulfilled at least one of the following conditions: 1) occurred *de novo,*; 2) segregated with the disease in the family; 3) confirmative functional studies had previously been performed; 4) novel missense variant at a location classified as pathogenic according to the above conditions. Only individuals with a *KCNT1* variant classified as pathogenic according to our criteria were included in this study.

Approval from local ethics committees and informed consent were obtained according to local ethics guidelines. Caregivers and clinicians provided anonymized patients' data via online questionnaire and standardized phenotyping sheets. The database was stored at the Danish Epilepsy Centre. Seizures, and, when possible, epileptic syndromes, were classified according to the International League Against Epilepsy (ILAE) proposals.[7,8] ID/developmental delay (DD) were ranked as none, mild, moderate, severe at onset and at last evaluation by the referring physician; formal neuropsychological assessments were available only in few cases. Moderate and severe motor impairment were defined as disabling motor disturbances with preservation or lack of capabilities to perform daily motor activities (f.ex., to eat and to walk autonomously), respectively. Auto- and hetero-aggressive behaviors referred to behaviors tending to harm the patient himself or others. Patients were classified as: *(i)* EIMFS, with onset of focal seizures within six months of age, a specific ictal EEG pattern of 'migrating seizure' and progressive developmental delay[9]; *(ii)* non-EIMFS DEE,

comprising heterogeneous DEEs other than EIMFS, including OS and WS; *(iii)* (AD)SHE; *(iv)* other phenotypes. Antiepileptic treatment data were retrospectively assessed. Referring clinicians identified the treatment which led to the best seizure control. In previously unpublished patients who received quinidine, reduction in seizure frequency was considered sustained if it persisted for at least 3 months.

Literature review

We searched Pubmed (up to 31st August 2020) with the terms "*KCNT1* epilepsy or *KCNT1*" (search strategy and study selection are summarized in Supplementary Fig.1). Search results were evaluated based on title and abstract. Moreover, references of the selected articles were examined, as well as other papers citing the eligible articles. Potentially eligible articles were reviewed, excluding those not reporting individual phenotypic-genotypic patient information or not available in English. After data cross-checking to exclude duplicates, relevant patient-related information were included in the *KCNT1* dataset.

Statistical analysis

R programming language (https://www.r-project.org/) to draw the figures and for statistical analyses. Comparisons between groups were made using Fisher's exact test for dichotomous variables, Cochran Armitage test for categorical data and Wilcoxon rank tests/Spearman correlations for continuous not normally distributed data as indicated in the manuscript.

Data availability

The data supporting the findings of this study are available on request from the corresponding authors.

Results

We analysed the data of 248 individuals with *KCNT1*mutations , including 66 previously unpublished and 182 previously published cases.

Phenotypic features of previously unpublished *KCNT1* patients

Of the 66 unpublished cases, 33 (50.0%) were clinically diagnosed with EIMFS, 17 (25.8%) with non-EIMFS DEE, 14 (21.2%) with (AD)SHE, and 2 (3.0%) presented with other phenotypes (Fig.1A). Sixty cases were unrelated and six cases (i.e. #51-#56) belonged to four unrelated families. The electro-clinical characteristics of individuals are summarized in Tables 1 and 2; phenotypic-genotypic features of each subject are presented in Supplementary Table 1.

EIMFS

Twenty-one/33 (63.6%) subjects were male. Mean age at last evaluation was 6.13 years (range 4 months-22 years). Mean age at seizure onset was 1.8 months (range 0-6 months). Most common seizure types were tonic-clonic, hemiclonic/clonic, focal impaired awareness, myoclonic seizures, gelastic seizures, subclinical seizures. Epileptic spasms were observed in 9/33 (27.3%) subjects. Focal seizures evolving to bilateral tonic-clonic seizures were reported in 17/33 patients. Seizure frequency varied from daily/several per day to weekly. In 23/31 patients, reduction of seizure frequency over time occurred. Three patients/31 at last follow-up had been seizure-free for 2, 3 and 10 years respectively (age range at seizure freedom 5-8 years).

Interictal EEG showed abnormal background activity, focal/multifocal and generalized epileptic abnormalities, hypsarrhythmia, burst suppression (BS). Besides a migrating ictal EEG pattern observed in all patients, ictal EEG showed multifocal seizures, diffuse electrodecremental pattern,

generalized fast rhythmic activity. An EEG pattern of "continuous-spike-waves-during-sleep" (CSWS) was observed in one individual.

No overt neurological signs were detected before seizure onset in 26/31cases, although early seizure onset precluded detailed developmental evaluation in most children. Development was mildly to severely impaired in the remaining five individuals (Fig.2A). Regression after seizure onset occurred in 28/31 cases and at last evaluation, cognitive impairment was observed in all cases (severe in 29/33) (Fig.2B). Ambulation was severely affected in 22/27 individuals who were wheelchair-bound or bedridden.

Thirteen/30 patients presented with abnormal MRI findings such as cerebral atrophy, white matter abnormalities, delayed myelination, and corpus callosum abnormalities. Magnetic Resonance Spectroscopy (MRS) in patients #24 and #26 was unremarkable. PET in case #24 showed hypometabolism in bilateral temporal structures extending to the posterior regions.

Mild to severe respiratory disorders requiring continuous ventilatory support in patients with extremely compromised neurological status, were reported in 12/32 subjects.

Seventeen/30 cases presented with microcephaly.

Non-EIMFS DEE

Seven/17 (41.2%) subjects were male. Mean age at last evaluation was 11,7 years (range 4 months-31.5 years), and mean age at seizure onset was 4.7 months (range 0-24 months). Seizure types included epileptic spasms, tonic-clonic, focal seizures, subclinical, myoclonic seizures and focal evolving to bilateral tonic-clonic seizures. Four individuals had atypical absences and five experienced status epilepticus (SE). Seizure frequency ranged from multiple seizures per day (15/17

patients) to weekly seizures (2/17). Over the years (mean follow-up:11,3 years), a reduction of seizure frequency occurred in 11/14 subjects; case #41 achieved seizure freedom (follow-up: 6 yrs).

Interictal EEG showed abnormal background activity, focal/multifocal epileptic abnormalities, hypsarrhythmia and BS, whereas a multifocal onset was the most common ictal EEG pattern. Subjects #40 and #44 presented epileptic spasms and hypsarrhythmia, consistent with WS diagnosis. Before epilepsy onset, no overt neurological abnormalities were reported in 11/15 individuals (Fig.2A), however all subjects regressed after seizure appearance (Fig.2B). At the last evaluation, 16/17 displayed a severe ID/DD.

Motor function was either moderately or severely impaired in 4/14 and 10/14 individuals, respectively.

Common MRI abnormalities were cerebral atrophy, white matter hyperintensities, and delayed myelination. In patient #42, MRI demonstrated hyperintensities of the lenticular nucleus, thalamus and brainstem, while MRS showed reduction of N-acetylaspartate levels in the central gray nuclei. This individual had brainstem dysfunction and a neurovegetative disorder with episodes of extreme bradycardia and apneas; death occurred at 4 months of age.

Respiratory impairment varied from mild dysfunction with autonomous breathing (individuals #34, #36, #38) to moderate or severe impairment, requiring partial ventilatory support (#35, #37). Individual #37 died at 15 years of age after a progressive worsening to a state of severe neurological compromise.

Additional comorbidities included autism spectrum disorder (ASD) (#41), severe auto/hetero-aggressive behaviour (#40), non-specific facial dysmorphisms (#40, #47), cortical visual impairment (#40, #47), scoliosis (#37, #40, #47), osteopenia and retinal dysfunction with hypometropia (#41),

DEE with later onset and atypical evolution: three individuals had a distinct phenotype characterized by normal (#49, #50) or mildly impaired (#48) neurodevelopment before seizure onset which occurred after the first year of life (age range: 15-24 months). At epilepsy onset, seizure types were myoclonic, focal impaired awareness, tonic-clonic, tonic, atypical absences, spasms. During early infancy, they started to experience sleep-related focal (hypermotor, hemiclonic, gelastic, tonic asymmetric) seizures, coupled with moderate/severe neurodevelopmental regression, and in two individuals, SE. Additional clinical features were kyphosis and hypothyroidism (#48), microcephaly (#49) and chorea and dystonia (#50). Over the years, in these three individuals, sleep-related seizures with frontal semiology became the prominent seizure type, thus displaying a phenotypic evolution toward a DEE with SHE features. Individual #50 died at the age of 23 years of unknown causes.

(AD)SHE

Twelve/14 (85.7%) individuals were male. Mean age at last evaluation was 17.1 years (range 6-37 years). Mean age at seizure onset was 63.1 months (range 10-168 months). Familial occurrence was observed in 6/13 individuals. Seizure types were hypermotor, tonic asymmetric, focal impaired awareness and clonic. Evolution from focal to bilateral tonic-clonic was reported in 5/14 cases. Seizure frequency ranged from multiple episodes per night to monthly nocturnal seizures. During the clinical evolution, exclusively sleep-related seizures were reported in 9/14 (64.3%) cases whereas in 5/14 (35.7%) diurnal seizures occurred. Over time, seizure frequency decreased in 8/13 individuals. Two/13 individuals (#53,#54) achieved seizure freedom with antiseizure treatment (seizure offset at 3 and 16 years respectively; follow-up 2 and 13 years, respectively).

Interictal EEG was normal in 4/12 individuals, with the remainders displaying focal/multifocal epileptic abnormalities. Ictal EEG (reported in 8/14 individuals) featured bilateral independent, multifocal, focal or electrodecremental seizure onsets.

Prior to the onset of seizures, cognition was normal in 12/13 subjects, (Fig.2A) but after seizure onset mild/moderate regression occurred. At the last evaluation, cognitive disturbances, such as IQ below the normal range, learning difficulties, ID, were observed in all individuals (Fig.2B).

Psychiatric and/or behavioural comorbidities, including attention deficit hyperactivity disorder (ADHD), Tourette syndrome, obsessive behaviours, social impairment, ASD, and psychogenic non epileptic seizures (PNES) were reported in 8/14 (57.1%) patients. MRI scanning was unremarkable in all patients.

Other phenotypes

Two patients presented with phenotypes not previously reported. Individual #65 (male, 48 years of age, mutation R928C) presented with drug-resistant temporal lobe epilepsy with onset at 8 years of age. EEG showed bilateral epileptic abnormalities; MRI scanning was unremarkable; invasive presurgical evaluation demonstrated a bitemporal seizure onset. Over the years, the individual developed a severe depressive syndrome and mild cognitive regression. Individual #66 (male, 15 years-old, variant G288S) had epilepsy onset at 3.8 years of age with monthly generalized tonic-clonic seizures during wakefulness. Interictal EEG and MRI scanning were unremarkable. The disease course was characterized by mild cognitive regression and achievement of seizure freedom with antiseizure medicines (ASMs) (follow-up 3 years).

Phenotypic features of previously published *KCNT1* patients

The phenotypic features of 182 previously published *KCNT1* cases collected from 61 papers are summarized in Table 3 and Supplementary Table 2, whereas their individual characteristics are reported in Supplementary Table 3. Sex was reported in 153 individuals, 92 were male.

EIMFS

One-hundred and nineteen individuals (56.8% males) (Fig.1B) presented with EIMFS; mean age at seizure onset was 1.2 months (range 0-5 months). In five cases EIMFS evolved to WS.[10,11] Most frequent seizures types were hemiclonic/clonic, focal, tonic symmetric/asymmetric, focal impaired awareness. Ictal autonomic features were present in 29/100 individuals. SE occurred in 10 individuals. In 64/100 children, interictal EEG showed mainly multifocal discharges. Besides a migrating pattern (in all patients), most frequent ictal EEG manifestation was a diffuse electrodecremental EEG pattern. One child displayed a CSWS EEG pattern.[12]

MRI was abnormal in 48/88 cases, showing primarily delayed myelination and cortical atrophy.

Psychomotor development after seizure onset (previously normal in 30/33 children) was reported in 99 patients as moderately (5/99) or severely (94/99) delayed. Neurological features included hypotonia (64/101), hypertonia (18/101), microcephaly (43/119) and movement disorders (such as limb dystonia or chorea) (9/101).

Comorbidities included systemic-to-pulmonary collateral arteries (SPCA) (n=3),[13] cardiomyopathy,[14] artero-venous fistula (Kuchenbuch et al., 2019),[14] cardiac arrhythmia associated to patent foramen ovale and ventricular septal defect[11] and systemic proliferative vasculopathy of pulmonary and mediastinal vessels (one individual each),[15] the latter appearing after quinidine administration. Death attributed to the *KCNT1*-related disorder occurred in 26/119 patients (21.8%) (mean age at death:3,86 ± 5.5 years). Most frequent additional findings were visual impairment, scoliosis, gut dysmotility and precocious puberty.

Non-EIMFS DEE

Non-EIMFS DEE, reported in 20 cases (13/19 males), included OS (two cases), OS evolving to EIMFS (one), WS (three), DEE evolving to (AD)SHE (one), DEE not further specified (thirteen)

(Fig. 1B). Mean age at seizure onset was 1.6 months (range 0-5 months). Most frequent seizures were tonic, focal, hemiclonic/clonic, tonic-clonic, tonic asymmetric. Most common interictal EEG abnormalities were multifocal discharges and BS; ictal EEG showed mostly multifocal seizure onset and electrodecremental patterns.

Abnormal MRI was reported in 13/17 individuals. Six cases presented with a combination of cortical atrophy, abnormalities of the white matter and of the corpus callosum. Three subjects[16,17] showed a diffuse leukoencephalopathy with severely delayed myelination, associated with cortical atrophy and thin corpus callosum in two of them. Neurodevelopment after seizure onset was either moderately (1/16) or severely affected (15/16). Additional findings included hypotonia (10/16), hypertonia (6/16), microcephaly (9/20), spasticity (5/16), choreoathetosis (2/16), opsoclonus (1/16). One death occurred at the age of 2 years due to unspecified causes.

(AD)SHE

Thirty-nine individuals (23/36 males) were diagnosed with (AD)SHE (Fig. 1B). Mean age at seizure onset was 59,1 months (range 2-216 months). Familial occurrence was reported in 26/35 (74.2%) individuals. Predominant seizure types were frontal hypermotor and focal (including focal impaired awareness). Other seizure types were tonic asymmetric, tonic-clonic, hemiclonic/clonic, SE, tonic, autonomic. Interictal EEG findings showed focal/multifocal discharges; ictal EEG patterns showed more frequently a focal or bilateral onset, or an electrodecremental pattern.

One individual featured an early onset (2 years of age) of sleep-related hypermotor seizures associated with neurodevelopmental regression that evolved after few months to a phenotype with episodes of SE with migrating focal seizures, controlled by phenobarbital.[18]

In 3/26 individuals, MRIs showed cortical atrophy. In one individual, a periventricular nodular heterotopia was detected, while her affected MRI-negative brother and two other unrelated MRI-negative affected individuals had focal cortical dysplasia type I which was histopathologically demonstrated after epilepsy surgery.[19]

Cognitive status after seizure onset was described as normal in 8/33 subjects, whereas mild to severe impairment was observed in the remaining.

Nineteen/33 individuals showed psychiatric/behavioral comorbidities, including personality disorders, depression, anxiety, ADHD, behavioural disturbances, impulsivity, obsessive-compulsive behaviour, ASD, psychosis, and schizophrenia.

Additional findings included precocious puberty (two cases) and microcephaly, asthma, growth failure, dysmorphic features and scoliosis (one case each).

Other phenotypes

One individual displayed multifocal (atypical absences, atonic, focal atonic, tonic-clonic) seizures, which started after an antibody-positive limbic encephalitis.[20] In another individual, epilepsy with a myoclonic-atonic seizures (EMAS) has been reported.[21] Finally, two individuals were diagnosed with no distinctive syndrome.[11]

Mutational landscape of all cases of *KCNT1*-related phenotypes

In the combined cohort of 248 individuals, 64 different mutations in *KCNT1* were identified. All mutations, apart from one inframe deletion Gln550del,[22] were missense. Other than two mutations, one near the amino (NH2) end of the protein and the other near the carboxy (COOH) terminus,[10,11] all other mutations were located between amino acid positions 250 and 1000, ranging from the S5

domain to the middle of the RCK2 domain (Fig. 3A, C and Supplementary Table 4). This finding is consistent with localized depletion of genomic variants seen in population data. The number of variants in the ExAC[23] database was lower than expected by a mutational model in between aminoacid positions 1 to 1068 in the KCNT1 protein (Chi Square statistic 67), but not between positions 1069 to 1236 (Chi Square statistic 0.8)[24] (gnomAD population variants shown in Fig. 3C). The *KCNT1* mutations occurred *de novo* in 51/56 cases in our cohort, based on being absent in either parent, and in 123/159 cases in the previously reported cases (Supplementary Tables 1 and 3). The remaining mutations segregated in an autosomal dominant manner or with an unknown pattern of inheritance, except for one homozygous variant A966T[25] in an individual with OS.

Twenty-four recurrent *KCNT1* mutations where observed, with G288S, A934T, R474H, R428Q, R398Q and R950Q together accounting for about half of all affected individuals (Fig. 4A and Supplementary Table 4).

Late onset syndromes (i.e., (AD)SHE and the single cases with EMAS, TLE, epilepsy with tonic-clonic seizures, epilepsy with multifocal seizures) were more likely to be inherited than EIMFS and non-EIMFS DEE (Fisher's Exact test, *P*-value=10[-13], odds ratio 20, 95%-CI 8-57). In the cohort of 66 individuals, all inherited *KCNT1* mutations, were associated with ADSHE; 2/5 were maternally inherited and 3/5 paternally inherited, one of the latter being mosaic for the mutation. The fathers (not included in this study) of patients #52 and #56 were affected by ADSHE, while the other 3/5 (60.0%) carrier parents were asymptomatic, emphasizing that incomplete penetrance can occur in *KCNT1* families, and this should be considered in genetic counselling. Among the published cases, inherited mutations were associated with ADSHE in 26/35 patients (in 6 unrelated families), with EIMFS in 9/105 individuals (in 7 unrelated families), and with non-EIMFS DEE in 2/16 subjects.

In our novel cohort of 66 individuals, EIMFS-patient #17 harbouring a *de novo* R474H mutation was mosaic with a mutant/wild-type ratio of about 1:2.5, indicating that mosaicism should be considered when testing patients for *KCNT1* variants, as more than one child may be affected. In non-EIMFS patient #37, who had a very severe phenotype and early death, exome sequencing excluded a second pathogenic variant. A small number of subjects in our unpublished cohort had novel clinical phenotypes. R928C and G288S (one of the most recurrent mutations) were detected in individual #65 (TLE) and #66 (epilepsy with tonic-clonic seizures), respectively. No mosaicism was detected in either individual, thus there was no evidence that this contributed to the varied phenotypes. In the published cohort, the mutations V340M and G288S were reported in a patient with multifocal epilepsy,[20] and in a patient with an EMAS phenotype,[21] respectively. Non-EIMFS DEE featuring leukoencephalopathy has been found associated with the mutations F932I, Q906H and G288S.[16,17]

Genotype-phenotype associations in 248 patients with *KCNT1*-related disorders

We found no significant differences in the age of seizure onset (Wilcoxon rank test, *P*-value=0.63), number of inherited versus *de novo* mutations (Fisher's exact test, *P*-value=1) and ID phenotype (Cochran Armitage test ranking ID as normal, mild, moderate and severe, *P*-value=0.8) when comparing individuals with EIMFS (149 patients) and non-EIMFS DEE (28 patients). Compared to the third main phenotype group of (AD)SHE (53 patients), EIMFS and non-EIMFS DEE were associated with earlier seizure onset (Wilcoxon rank test, *P*-value=2x10^{-25}), more severe ID (Cochran Armitage Test, *P*-value=2x10^{-32}) and the *KCNT1* mutation was more frequently *de novo* than inherited (Fisher's Exact test, *P*-value=9x10^{-17}). Similarly, individuals with a *de novo* mutation showed earlier seizure onset (median: age 1 month) than individuals with inherited variants (median: age 24 months), Wilcoxon rank test, *P*-value=3x10^{-12}). Individuals with (AD)SHE had a median

seizure onset of 54 months (interquartile range 23-72 months, 95%-interval 5-176 months); individuals with EIMFS/non-EIMFS DEE had a median seizure onset of 1 month (interquartile range 0-2 months, 95%-interval 0-5 months) (Fig.5). These data distinguished two *KCNT1*-associated phenotypic groups: early onset (i.e., EIMFS and non-EIMFS DEEs) and late onset syndromes ((AD)SHE, other phenotypes). We searched for genotype-phenotype correlations in these two groups within the total cohort of 248 individuals. We found specific amino acid positions that were more often associated with EIMFS/non-EIMFS DEE phenotypes: 23/29 patients with a *KCNT1* mutation at aminoacid position 288 in the protein loop region, 21/21 individuals with a mutation at position 428, 31/33 individuals with a mutation at 474 in the RCK1 domain and 25/30 at position 934 in the RCK2 domain. Mutations that were most often seen in patients with (AD)SHE were: 14/20 at position 398 in the RCK1 domain, 5/5 at site 796 and 14/14 at site 928 in the RCK2 domain (Fig.4A and Supplementary Table 4). These aminoacid positions also corresponded to early/late seizure onset (Fig.4B).

There were no significant differences in mutation location in different domains for EIMFS versus non-EIMFS DEE phenotypes (Fisher's exact tests, Fig. 3A). Two thirds of variants associated with (AD)SHE clustered in the RCK2 domain (66%), significantly more than for EIMFS/non-EIMFS DEE phenotypes (Fisher's Exact test, *P*-value=5×10^{-7}, odds ratio 5.2, 95%-CI 2.6-11), whereas only 3.8% and 26.4% of (AD)SHE pathogenic variants were located in the pore and RCK1 domains, respectively (Fig. 3B, 4A).

Treatment of patients with KCNT1-associated phenotypes

Previously unreported cohort

The majority of individuals had seizures which were drug-resistant (Supplementary Table 1). Effectiveness in reducing seizure frequency was estimated comparing the number of clinicians rating a given treatment as the most efficacious compared with the total number of patients who attempted that particular therapy. The treatment response of 43 individuals from our cohort of 66 novel individuals has been published by Fitzgerald et al.[26]

- In EIMFS-patients, a range of 3-18 different anti-seizure medications (ASMs) were used (median: 9; IQ1-IQ3:7-11). Seizure reduction occurred more frequently with ketogenic diet (KD) (9/27), quinidine (4/16), clobazam (2/19) and carbamazepine (2/9).

- In non-EIMFS DEE-patients, 3 to 17 ASMs were used (median: 9; IQ1-IQ3:6-12). Clobazam (4/8) and vigabatrin (2/8) were most frequently associated with seizure improvement.

- (AD)SHE-patients were treated with a range of 1 to 9 ASMs (median:6; IQ1-IQ3:4.7-7.5). Drug-resistance was more common in our cohort as compared to individuals so far reported in the literature (11/13 vs. 23/33, Fisher's exact test: 0.46). Reduction of seizures occurred more often with oxcarbazepine (2/4), clobazam (3/7) and valproic acid (2/7).

Literature findings

Literature findings confirm the severe refractoriness of *KCNT1*-related epilepsies observed in our cohort (Supplementary Table 3). Indeed, only 10/156 (6.4%) (all presenting with (AD)SHE phenotype) of the published individuals achieved seizure freedom. Our overview of the treatment of previously published cases with *KCNT1*-associated phenotypes showed:

- Three to 16 ASMs were tried in EIMFS-patients (median:8; IQ1-IQ3:7-11). Highest rates of seizure reduction were reported with KD (14/37), quinidine (9/31), phenobarbital (11/44), potassium bromide (5/19) and clobazam (5/24).

-        In non-EIMFS DEE-patients a range of 4 to 12 drugs were administered (median:7; IQ1-IQ3 6-9,25). Seizure improvement to different drugs has been reported in single patients. KD was effective in 3/7 cases.

-        Individuals with (AD)SHE- were treated with 1-14 different ASMs (median:6; IQ1-IQ3 4-7). Seizure improvement was reported most frequently with levetiracetam (3/5) and carbamazepine (3/5). Eight patients tried quinidine, without improvement. Three patients underwent epilepsy surgery; none of them achieved seizure freedom.[19]

Mortality in *KCNT1*-associated phenotypes

Our analysis of the mortality rate according to syndromes in the 248 individuals, showed that non-EIMFS DEE and EIMFS were associated with higher risk of death compared to (AD)SHE (*P*-value 0.02, hazard ratio of 16 and *P*-value 0.004, hazard ratio of 27, respectively; Cox proportional hazard model) (Supplementary Fig.2A). In our previously unpublished EIMFS cohort, no deaths were reported, which varies with the high rate of mortality seen in the published cohort (26 deaths out of 119 subjects) (*P*<0.01) (Supplementary Fig.2B). No statistically significant difference between mean ages were observed between the novel and the literature cohorts (6.13±6.23 years versus 4.28±4.71 years, respectively; *P*=0.31). In our previously unpublished non-EIFMS DEE cohort, three individuals were deceased (two with severely compromised neurological features, the third due to unknown cause). In the literature only one deceased case is reported without specifying the cause.[27] In subjects with (AD)SHE, SUDEP occurred in a 6 year old child (case#59) in our novel cohort and in one patient in the literature with clinically diagnosed ADSHE (not genetically tested) who was part of a large *KCNT1*-ADSHE family.[20]

Discussion

Our comprehensive study investigated the phenotypic and genotypic features of 248 patients with *KCNT1*-related epilepsies, including 66 novel cases.

Phenotypic spectrum

EIMFS represents the largest phenotypic subgroup in our study with the inclusion of 152 individuals (33 novel cases) (Fig.1C). Predominant phenotypic features that defined EIMFS, besides migrating focal seizures observed clinically and/or electrographically in all patients, were: a) lack of overt neurological impairment before seizure onset in most patients; b) neurodevelopmental regression after seizure onset; c) high seizure frequency and heterogeneous seizure types including tonic-clonic, focal impaired awareness, myoclonic, hemiclonic/clonic, tonic, gelastic, and subclinical seizures; d) refractoriness to ASMs; e) various EEG features such as focal/multifocal and generalized interictal epileptic abnormalities, BS, ictal electrodecremental pattern, focal/multifocal ictal onset; f) evolution to severe ID during the course of the disease. Transition from EIMFS to West Syndrome has been described in a few children.[10,11] Additional features observed in our novel cohort were: i) occurrence in 27% of individuals of epileptic spasms (similar to the findings by Burgess et al.)[28] which are considered to occur rarely in EIMFS; ii) reduction of seizure frequency during follow-up in 23/31 cases, with seizure-freedom in childhood in a fraction of them (3/31). Previously, a milder evolution of EIMFS with seizure improvement and mild/moderate ID was documented in rare cases with unknown etiology.[29]

The non-EIMFS DEE subgroup included 37 individuals (Fig.1C) including 17 that were previously unpublished. Our novel cases almost equal the number of previously published *KCNT1*-related non-EIMFS DEE cases, suggesting that this phenotype might not be as rare as previously indicated.

Clinical-EEG findings in non-EIMFS DEE individuals were: a) seizure onset in the neonatal period/early infancy; b) neurodevelopmental delay or regression/stagnation after seizure onset; c) various seizure types (i.e.; focal, epileptic spasms, tonic-clonic, tonic, myoclonic and subclinical seizures; d) EEG patterns including focal/multifocal epileptic abnormalities, hypsarrhythmia, BS, ictal electrodecremental pattern; d) ASMs refractoriness; e) severe disease course with evolution to moderate/profound ID and moderate/severe motor impairment. Peculiar features in our previously unpublished cases were: i) four children with atypical absence seizures, which have not previously been reported to be associated with mutation of *KCNT1*; ii) clinical presentation with WS, which when combined with three published cases[6,11,30] further supports the inclusion of *KCNT1* in the growing list of genes associated with WS or infantile spasms. Other phenotypes reported in our series and in the literature are OS (one case in our novel cohort and two published individuals)[25,31] and OS evolving to EIMFS.[6] Finally, we report three cases with a phenotype not previously described with *KCNT1* mutations, characterized by a DEE with a later onset (between the age of 15 and 24 months), heterogeneous seizure types and moderate/severe neurodevelopmental regression. Over time, drug-resistant frontal hypermotor seizures appeared, becoming the main seizure type, associated with severe cognitive impairment. Interestingly, these cases illustrate the possible evolution of *KCNT1*-related late onset DEE into a DEE with prominent SHE features. A similar transition to ADSHE has been recently reported in a child with DEE with onset at 1 month of age.[11] The opposite course, i.e. from SHE to drug-responsive EIMFS[18], as well as the transition from OS to EIMFS and, at an older age, to a focal epilepsy with exclusively frontal/fronto-temporal seizures have been observed, suggesting a continuum between these three different conditions.[14]

The third phenotypic group observed was (AD)SHE, which included 52 cases (14 novel) (Fig.1C). The main phenotypic characteristics defining *KCNT1*-associated (AD)SHE were: a) familial inheritance (6/13 novel cases and 26/35 subjects in the literature) with incomplete penetrance and

intra-familial phenotypic variability, b) seizure onset in early childhood, c) hypermotor and tonic asymmetric seizures associated with heterogeneous seizures types, d) seizures occurring also during daytime in about one third of patients; e) focal/multifocal ictal EEG onset; f) ASMs refractoriness g) cognitive impairment and psychiatric/behavioral problems. Distinctive features in our novel cohort were: i) reduction of seizure frequency over time with seizure freedom (with/without treatment) achieved in a fraction of subjects; ii) higher incidence of ASMs-refractoriness compared to the literature; iii) cognitive regression after seizure onset in all cases (100% versus 62,5% in the literature), further supporting a role of *KCNT1* in cognitive functions and neurodevelopment[32]; iv) although in our novel cohort behavioral/psychiatric disorders were observed at frequencies comparable to those reported in the literature (57.1% and 57,5% respectively), none of our novel cases were affected with severe psychiatric comorbidities, such as psychosis, schizophrenia, depression or personality disorders.

Finally, other phenotypes besides those reported above were observed in six patients with a *KCNT1* mutation. Two cases in our previously unpublished cohort displayed a clinical picture not yet associated with *KCNT1*: one presented with drug-resistant TLE, mild cognitive regression, and psychiatric comorbidity, the other with epilepsy with generalized tonic-clonic seizures, mild cognitive impairment and seizure-freedom with ASMs. Four additional *KCNT1*-patients with atypical presentations were described in the literature, including drug-resistant multifocal epilepsy (one subject),[20] epilepsy with myoclonic-atonic seizures (one subject),[21] and unclassified epilepsy (two cases).[11] These six cases suggest that the phenotypic spectrum of *KCNT1*-related epilepsies could be broader than currently understood and not yet completely defined.

Genetic landscape and genotype-phenotype associations

Our data show a consistent clinical overlap between *KCNT1*- associated EIMFS and non-EIMFS DEE without striking phenotypic differences between the two groups, besides lack of migrating seizures in non-EIMFS DEE. In fact, it cannot be excluded that some non-EIMFS DEE cases also had migrating seizures during their disease course. In contrast, we report significant phenotypic differences between individuals with early (EIMFS, non-EIMFS DEE) and late onset syndromes (ADSHE) due to mutation of *KCNT1*, supporting the view of two main phenotypic groups with either early or late seizure onset. There is no clear distinction in the protein location of *KCNT1* mutations associated with early or late onset syndromes, again adding to the complexity of providing genetic counselling to individuals with an identified *KCNT1* mutation and their families. The overlap in the positions of mutations may suggest shared pathogenic mechanisms of the early onset (i.e., EIMFS and non-EIMFS DEE) and late onset conditions, but this requires further studies.

Only missense mutations and one inframe deletion[22] have been identified in all *KCNT1* cases identified to date and all functionally tested variants show a gain-of-function effect, leading to an increased K+ current.[33] To date, no clear pathogenic loss-of-function (LOF) variants have been described, and several truncating variants have been seen in controls (pLI=0), indicating that LOF variants may be tolerated and devoid of clinical consequences. All pathogenic variants, besides two, are located from the beginning of S5 to the end of the RCK2 domain. suggesting a direct role of the variants in altering the pore function/permeability or in modulating sensitivity to intracellular sodium levels, leading to regulation of channel gating.[34,35] Since the N-terminal region before the S5 domain, the region between the RCK1 and RCK2 domains, and the C-terminus of the KCNT1 protein are tolerant to genetic variation in the general population, we predict that missense variants in these areas likely have no clinical effects.

Our systematic analyses of the phenotypic and genetic data identified distinct genotype-phenotype correlations. While pathogenic *KCNT1* variants are widely distributed among the different protein

domains, (AD)SHE associated mutations mainly cluster in the RCK2 domain in the C-terminus distal to the NADP domain, whereas EIMFS/non-EIMFSDEE-associated mutations do not show clustering in any part of the protein (Fig. 3A,B). Recurrent *KCNT1* mutations were seen in cases at both the less severe and more severe ends of the phenotypic spectrum. For instance, mutations F346L, R428Q, R474H, R474C, P924L have been seen predominantly in patients with EIMFS or non-EIMFS DEE, whereas variants R928C, R961H or A966T have been mainly observed in SHE patients. However, large phenotypic variability was observed with the other recurrent mutations, making it challenging to provide an individual with a clinical prognosis. For instance, R398Q has been seen in nine families with SHE and five patients with EIMFS as well as in unaffected carriers, indicating that genotype–phenotype relationships are not straightforward. The same *KCNT1* mutation can be associated with different phenotypes, even in individuals within the same family.[20] Additional mechanisms (i.e., effect of modifier genes, environmental factors, or both) are likely to influence the pleiotropy, variable expressivity and incomplete penetrance associated with mutations of *KCNT1*.

Treatment response

Our study confirms previous data showing that the treatments most frequently reported to reduce seizures in EIMFS and non-EIMFS DEE associated with mutation of *KCNT1* were KD, followed by quinidine, phenobarbital, clobazam, whereas (AD)SHE respond more often to levetiracetam, carbamazepine, clobazam, valproic acid, alone or in combination.[26] Recently, cannabidiol (CBD) has shown effectiveness in a fraction of patients with EIMFS.[11] However, inconsistencies in the descriptions in the literature of the responses of individuals to ASMs, the lack of objective evaluations and the small sizes of the cohorts of cases, render difficult to assess the efficacy of ASMs in treating individuals with *KCNT1*-related epilepsies. However, despite these limitations, the available evidences suggest that EIMFS and non-EIMFS DEE respond poorly to current treatment options.

Recent experimental findings in mice models suggest that ASO-based gene silencing might be a promising therapeutic approach in *KCNT1*-associated epilepsies,[36] however validation studies and additional data are necessary before translation in humans. Failure of surgery in three *KCNT1*-(AD)SHE patients has been attributed to insufficient resection of a type I focal cortical dysplasia, histopathologically detected, possibly coupled with a *KCNT1*-sustained widespread epileptogenicity.[19] Current clinical evidences support the view that *KCNT1*-related epilepsis are in general intractable, suggesting that realistic treatment goals should be focused not only to seizure control but also on quality of life measures.

Mortality

No deaths were observed in our novel EIMFS cohort, at variance with data in the literature reporting high risks of premature death due to disease progression and SUDEP.[11,14] Our report might mitigate previous high mortality findings, however our results might be driven by a selection bias and by a limited long term follow-up in the novel cohort in comparison to published patient series. Among the causes of death, SUDEP in *KCNT1*-related EIMFS, may be related to *KCNT1* gain-of-function effects in heart cells.[14,20,33] The observation of a child with EIFMS without cardiac pathologies harbouring a *KCNT1* pathogenic variant which is also involved in Brugada syndrome[10] warrants further investigations on *KCNT1* and cardiac arrhythmias. Regarding (AD)SHE, SUDEP occurred in a 6 year old child in our novel cohort. In the literature, one patient with ADSHE, not genetically investigated, belonging to a *KCNT1*-ADSHE family has been described.[20] The overall incidence of SUDEP in SHE of various etiologies is 0.36 per 1000 person-years, similar to that of the general epilepsy population.[37] However, our findings together with studies showing a susceptibility to cardiac arrhythmias or to SUDEP in *KCNT1*-related disorders[14,33] suggests that we cannot exclude that *KCNT1*-related (AD)SHE might represent a subset of SHE with higher risk of cardiac disturbances or SUDEP.

Limitations and conclusions

Our study has some limitations, mainly due to: a) its retrospective nature; b) heterogeneity of data reporting, especially for the published cases, in particular regarding neuropsychological, psychiatric and drug response assessment which were not performed systematically or missing in a proportion of patients; c) inhomogeneous evaluation of the electroclinical data of the novel cohort by referring physicians (only in a minority of cases we reviewed the EEGs and original medical reports).

In conclusion, this comprehensive study contributes to further define and broaden the phenotypic-genotypic spectrum of *KCNT1*-related epilepsies, confirming the importance of this gene among the pathogenetic causes of DEEs as well as focal, namely (AD)SHE, epilepsies. Moreover, the demonstration of previously unreported phenotypes suggests that the full phenotypic spectrum has not been defined yet. Genotypic characterization has shown that mutations associated with EIMFS/non-EIMFS DEE do not show particular patterns of distribution in the KCNT1 protein whereas (AD)SHE-phenotypes cluster around the C-terminus RCK2 domain. Such genotype-phenotype assessments are important in attempting to inform future clinical diagnoses and prognoses of individuals in whom a *KCNT1* mutation is identified.

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Competing interests

The authors report no competing interests.

Supplementary material

Supplementary material is available at *Brain* online.

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Figure legends

Fig. 1: **Distribution of the phenotypes in our novel cohort (A), in the literature (B) and in the global cohort (C)**

Legend: (AD)SHE: autosomal dominant sleep-related hypermotor epilepsy; EIMFS: epilepsy of infancy with migrating focal seizures; non-EIMFS DEE: developmental and epileptic encephalopathy other than EIMFS; EMAS: epilepsy with myoclonic-atonic seizures; SHE: sleep-related hypermotor epilepsy; OS: Ohtahara syndrome; Sz: seizures; TCS: tonic-clonic seizures; TLE: temporal lobe epilepsy

Fig. 2: Psychomotor development and cognitive status evolution before (A) and after (B) seizure onset for the main *KCNT1-related* phenotypes (EIMFS, non-EIMFS DEE, (AD)SHE).

Comparisons between groups were made using Fisher´s exact test.

Legend: (AD)SHE: Autosomal dominant sleep-related hypermotor epilepsy; EIMFS: Epilepsy of infancy with migrating focal seizures; non-EIMFS DEE: developmental and epileptic encephalopathy other than EIMFS; TCS: tonic-clonic seizures; TLE: temporal lobe epilepsy.

Fig. 3: Mutational landscape and genotype-phenotype associations

A) Graphical representation of the positions of previously published and novel variants in Slack protein (*KCNT1* gene). The Slack protein (formed by 1230 aminoacids), encoded by *KCNT1* gene, is characterized by 6 transmembrane domains (S1-S6) with a pore-forming region between S5 and S6. Intracellular domains in the C-terminus are named RCK1 and RCK2, the latter containing the NADP binding domain. Pathogenic variants from both literature and our cohort are shown: previously published (black dots) and novel variants (red stars, written in bold). Hotspot sites (repeated variants) are highlighted (squared). VUS and likely benign variants published in literature are colored in light grey. B) Genotype-phenotype correlation. Distribution along the protein of the three main phenotypes in our novel cohort. C) Distribution of variants across the *KCNT1* gene. Pathogenic or likely pathogenic missense variants in *KCNT1* (red bars) are clustered in functionally important regions of the protein. In comparison, missense variants in population controls (gnomAD, v2.1, minor allele count $>= 1$) is given as black bars. GnomAD variants are taken from genomes as these show a more even sequencing coverage than exomes.

Legend: (AD)SHE: autosomal dominant sleep-related hypermotor epilepsy; EIMFS: epilepsy of infancy with migrating focal seizures; non-EIMFS DEE: developmental and epileptic encephalopathy other than EIMFS; TLE: temporal lobe epilepsy; VUS: Variant of uncertain significance.

Fig. 4. Recurrent variants and genotype-phenotype associations

A) Each recurrent variant with associated phenotypes, from both literature and novel cohort. B) Variants at different amino acid sites and protein domains are differently associated with age at seizure onset. Seizure onset is given in years (Log10) on the y-axis. For recurrent variants, the distribution of seizure onset is also shown as violin plots. The variants are colored according to their location in different protein domains.

Legend: (AD)SHE: autosomal dominant sleep-related hypermotor epilepsy; EIMFS: epilepsy of infancy with migrating focal seizures; non-EIMFS DEE: developmental and epileptic encephalopathy other than EIMFS; EMAS: epilepsy with myoclonic-atonic seizures; TCS: tonic-clonic seizures; TLE: temporal lobe epilepsy.

Fig. 5. Violin plot showing the distribution of the the different syndromes according to age of onset. EIMFS and non-EIMFS DEE were associated with earlier seizure onset as compared to (AD)SHE (Wilcoxon rank test, p-value = 5x10-23). Individuals with (AD)SHE had a median seizure onset of 60 months (interquartile range 24-72 months, 95%-interval 4.7-180 months). Individuals with EIMFS/DEE had a median seizure onset of 1 month (interquartile range 0-2.1 months, 95%-interval 0-5.3 months).

Legend: (AD)SHE: autosomal dominant sleep-related hypermotor epilepsy; EIMFS: epilepsy of infancy with migrating focal seizures; non-EIMFS DEE: developmental and epileptic encephalopathy other than EIMFS; LO-DEE: late onset DEE with atypical evolution; Others: other phenotypes.
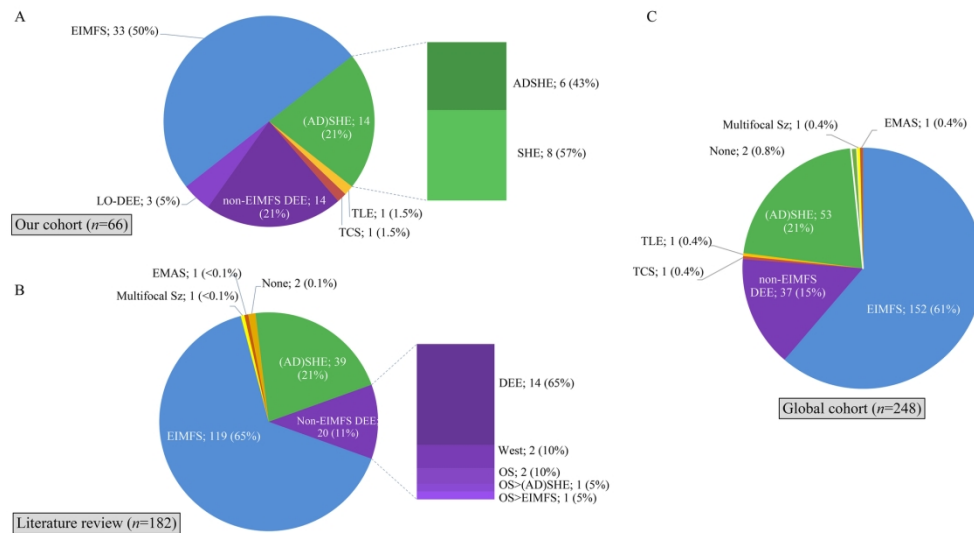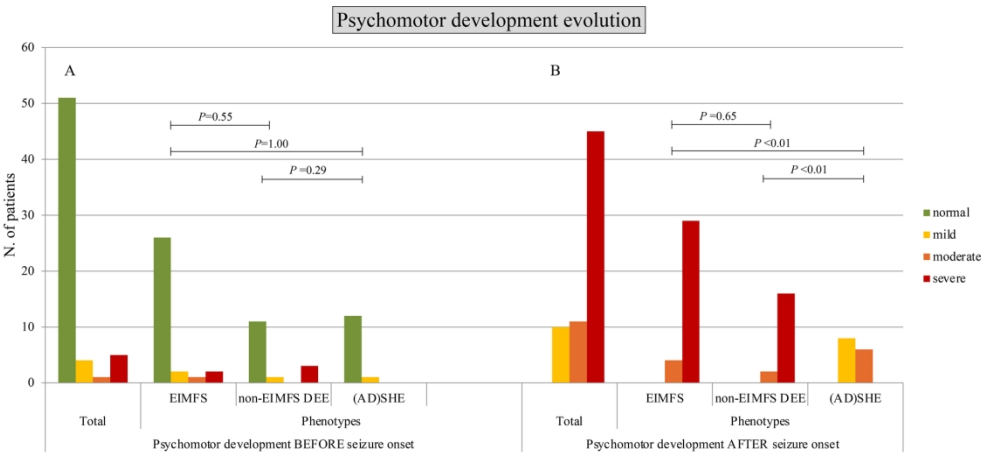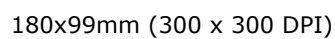
Figure 1

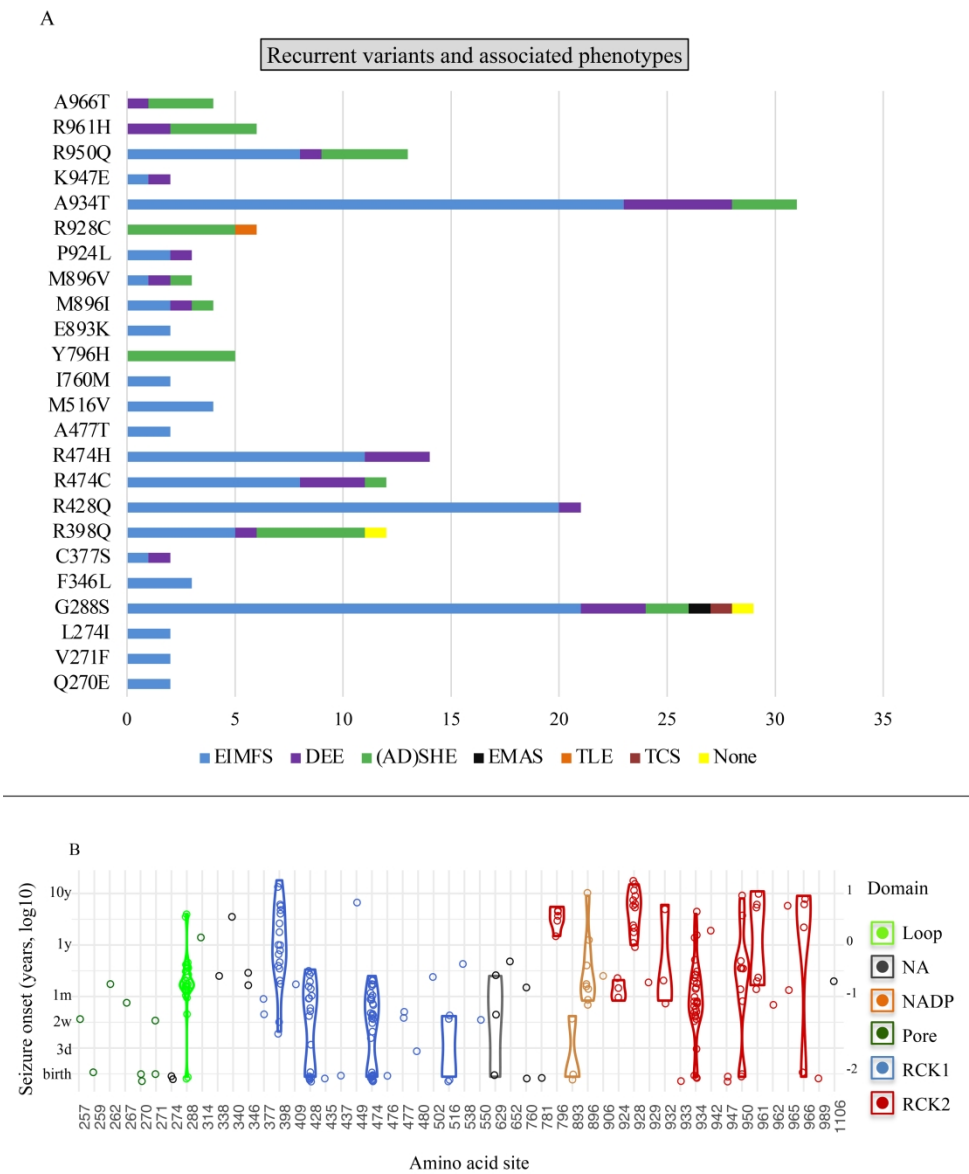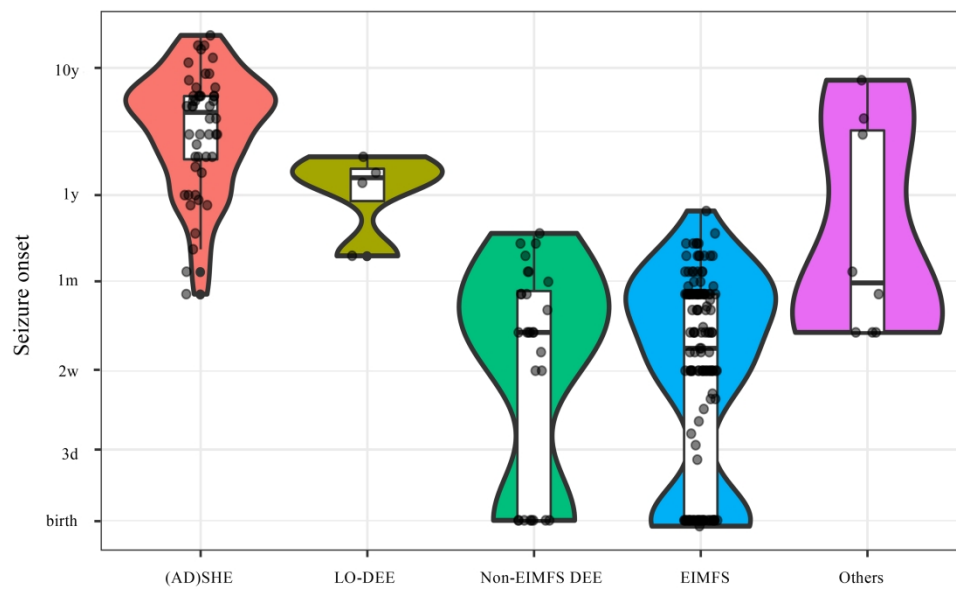180x98mm (300 x 300 DPI)

Figure 2

180x84mm (300 x 300 DPI)

Figure 3

180x99mm (300 x 300 DPI)

A

B

Figure 4

Figure 5

Table 1: Phenotypic features of our novel cohort

FEATURES (%)	PHENOTYPE[§]		
	EIMFS [n=33]	non-EIMFS DEE [n=17]	(AD)SHE [n=14]
Sex	[n=33]	[n=17]	[n=14]
Male	21 (63.6)	7 (41.2)	12 (85.7)
Female	12 (36.4)	10 (58.8)	2 (14.3)
Age (years)	[n=33]	[n=16]	[n=12]
Median (IQR)	4 (1.4-10)	10.8 (2.5-19.9)	16.5 (12.8-18.5)
Mean (range)	6.1 (0.3-22)	11.7 (0.3-31.5)	17.1 (6-37)
ND before seizure onset	[n=31]	[n=15]	[n=13]
Normal ND	26 (83.9)	Normal ND 11 (73.3)	Normal ND 12 (92.3)
Mild NDD	2 (6.5)	Mild NDD 1 (6.7)	Mild NDD 1 (7.7)
Moderate NDD	1 (3.2)	Severe NDD 3 (20.0)	
Severe NDD	2 (6.5)		
ND after seizure onset	[n=33]	[n=17]	[n=14]
Moderate NDD	4 (12.1)	Moderate NDD 1 (5.9)	Mild NDD 8 (57.1)
Severe NDD	29 (87.9)	Severe NDD 16 (94.1)	Moderate NDD 6 (42.9)
Regression after epilepsy onset	[n=31]	[n=15]	[n=13]
	28 (90.3)	11 (73.3)	13 (100)
Psychiatric / behavioral problems	[n=33]	[n=17]	[n=14]
	0	2 (11.8)	8 (57.1)
Neurological features	[n=33]	[n=17]	[n=14]
Hypotonia	5 (15.1)	Hypotonia 5 (29.4)	Hypotonia 2 (14.3)
Hypertonia	17 (51.5)	Hypertonia 8 (47.1)	
Hemiparesis	1 (3.0)	Chorea, dystonia 1 (5.9)	
	[n=27]		
Mild IMF	1 (3.7)		[n=14]
Moderate IMF	4 (14.8)	Moderate IMF 4 (28.6)	
Severe IMF	22 (81.5)	Severe IMF 10 (71.4)	
MRI	[n=30]	[n=12]	[n=14]
Normal	17 (56.7)	Normal 6 (50.0)	Normal 14 (100)
Cerebral atrophy	10 (33.3)	Cerebral atrophy 4 (33.3)	
WM hyperintensities	6 (20.0)	Delayed myelination 3 (25.0)	
Delayed myelination	4 (13.3)	WM hyperintensities 2 (16.7)	
CC abnormalities	4 (13.3)	CC abnormalities 1 (8.3)	
Cbl abnormalities	2 (6.7)	Incomplete rotation Hy 1 (8.3)	
WM loss	1 (3.3)	LN, thalamus, brainstem hyperintensities 1 (8.3)	
Thin brainstem	1 (3.3)		
Chiari malformation	1 (3.3)		
Cardiac problems*	[n=33]	[n=13]	[n=14]
	0	1 (7.7)	0
Respiratory problems	[n=32]	[n=16]	[n=14]
Mild problems	6 (18.8)	Mild problems 3 (18.8)	0
Partial support	3 (9.4)	Partial support 2 (12.6)	
Continuous support	2 (6.3)	Recurrent pneumonias 4 (25.0)	
Recurrent pneumonias	1 (3.1)	Apneas 1 (6.3)	

	[n=33]		[n=17]		[n=14]	
Other features	Microcephaly	17 (51.5)	Scoliosis/Kyphosis	4 (23.5)	Scoliosis	1 (7.1)
	Joint hypermobility	1 (3.0)	Visual impairment	2 (11.8)	Precocious puberty	1 (7.1)
			Facial dysmorphisms	2 (11.8)		
			Hypothyroidism	1 (5.9)		
			NV disorder	1 (5.9)		
			Osteopenia	1 (5.9)		
			Joint contractures	1 (5.9)		
			GERD	1 (5.9)		
			Retinal dysfunction	1 (5.9)		
			Hip dysplasia	1 (5.9)		
			Microcephaly	1 (5.9)		
Death	[n=33]		[n=17]		[n=14]	
		0		3 (17.6)		1 (7.1)

§ Two additional patients with other phenotypes (#65, #66, Supplementary Table 1) are not shown here in the table and described in the text.
* cardiac problems not related to quinidine
Legend: (AD)SHE: Autosomal dominant sleep-related hypermotor epilepsy; Cbl: cerebellar; CC: corpus callosum; EIMFS: epilepsy of infancy with migrating focal seizures; GERD: gastroesophageal reflux disease; Hy: hippocampus; IMF: impairment of motor function; IQR: interquartile range (IQ1-IQ3); LN: lenticular nucleus; MD: movement disorder; MRI: magnetic resonance imaging; non-EIMFS DEE: non-EIMFS Developmental and epileptic encephalopathy; NV: neurovegetative; ND: neurodevelopment; NDD: neurodevelopmental delay; WM: white matter.

Table 2: Electroclinical features of patients of our novel cohort

ELECTROCLINICAL FEATURES	PHENOTYPE[§]		
	EIMFS [n=33]	non-EIMFS DEE [n=17]	(AD)SHE [n=14]
Seizure onset (age in months)	median (IQR) 2 (0.5-2) mean (range) 1.8 (0-9)	median (IQR) 1.5 (0-5) mean (range) 4.7 (0-24)	median (IQR) 69 (25.5-72) mean (range) 63.1 (10-168)
Seizure types	[n=33] Tonic-clonic 23 (69.7) Hemiclonic / clonic 20 (60.6) FIA 18 (54.5) Myoclonic 18 (54.5) Focal eTCS 17 (51.5) Subclinical seizures 17 (51.5) Status epilepticus 13 (39.4) Gelastic 10 (30.3) Spasms 9 (27.3) Focal 8 (24.2) Frontal hypermotor 4 (12.1) Tonic 4 (12.1) Tonic asymmetric 2 (6.1)	[n=17] Spasms 12 (70.6) Tonic-clonic 11 (64.7) Focal 6 (35.3) Subclinical seizures 6 (35.3) Myoclonic 6 (35.3) Status epilepticus 5 (29.4) Gelastic 5 (29.4) Focal eTCS 5 (29.4) FIA 4 (23.5) Frontal hypermotor 4 (23.5) Atypical absences 4 (23.5) Tonic 4 (23.5) Hemiclonic / clonic 3 (17.6) Tonic asymmetric 3 (17.6) Reflex 1 (5.9)	[n=14] Frontal hypermotor 8 (57.1) Tonic asymmetric 7 (50.0) Focal eTCS 5 (35.7) Tonic 5 (35.7) FIA 3 (21.4) Tonic-clonic 3 (21.4) Hemiclonic / clonic 1 (7.1) Focal 1 (7.1) Reflex 1 (7.1) Status epilepticus 1 (7.1)
Interictal EEG recording	[n=23] Multifocal 22 (95.7) Hypsarrhythmia 3 (13.0) Generalized 3 (13.0) Focal 1 (4.4) Burst suppression 1 (4.4) ESES-like pattern 1 (4.4)	[n=14] Hypsarrhythmia 5 (35.7) Multifocal 5 (35.7) Burst suppression 4 (28.6) Focal 4 (28.6)	[n=12] Normal 4 (33.3) Focal 5 (41.7) Multifocal 3 (25.0)
Ictal EEG recording	[n=28] Migrating 28 (100) Multifocal 8 (28.6) Electrodecremental 4 (14.3) Generalized 2 (7.1)	[n=3] Multifocal 3 (100) Generalized 1 (33.3)	[n=8] Multifocal 3 (37.5) Bilateral independent 3 (37.5) Focal 2 (25.0) Electrodecremental 1 (12.5)
Worst seizure frequency	[n=33] Multiple daily 29 (87.9) Daily 2 (6.1) Weekly 2 (6.1)	[n=17] Multiple daily 15 (88.2) Weekly 2 (11.8)	[n=13] Multiple daily 8 (61.5) Daily 3 (23.1) Weekly 1 (7.7) Monthly 1 (7.7)
Seizure frequency at last follow-up	[n=31] Multiple daily 4 (12.9) Daily 14 (45.2) Weekly 5 (16.1) Monthly 4 (12.9) Yearly 1 (3.2) Seizure free 3 (9.7)	[n=14] Multiple daily 4 (28.6) Daily 6 (42.9) Weekly 1 (7.1) Monthly 1 (7.1) Yearly 1 (7.1) Seizure free 1 (7.1)	[n=13] Multiple daily 3 (23.1) Daily 2 (15.4) Weekly 4 (30.8) Monthly 2 (15.4) Seizure free 2 (15.4)
Reduction in seizure frequency	[n=31] 23 (74.2)	[n=14] 11 (78.6)	[n=13] 8 (61.5)

Legend: (AD)SHE: Autosomal dominant sleep-related hypermotor epilepsy; EEG: electroencephalogram; EIMFS: epilepsy of infancy with migrating focal seizures; ESES: encephalopathy with status epilepticus during slow sleep; eTCS: evolving to tonic-clonic seizures; FIA: focal impairment of awareness; IQR: interquartile range (IQ1-IQ3); non-EIMFS DEE: non-EIMFS Developmental and epileptic encephalopathy.
§ Two additional patients with other phenotypes (#65 and #66, Supplementary Table 1) are described in the text and not shown in this table.

Table 3: Phenotypic features of the previously published patients

PHENOTYPIC FEATURES (%)	PHENOTYPE[§]		
	EIMFS [n=119]	non-EIMFS DEE [n=20]	(AD)SHE [n=39]
Sex	[n=95]	[n=19]	[n=36]
Male	54 (56.8)	13 (68.4)	23 (63.9)
Female	41 (43.2)	6 (31.6)	13 (36.1)
ND before seizure onset	[n=33]	[n=5]	[n=15]
	Normal ND 30 (90.9)	Normal ND 3 (60.0)	Normal ND 15 (100)
	Mild NDD 2 (6.1)	Moderate NDD 1 (20.0)	
	Severe NDD 1 (3.0)	Severe NDD 1 (20.0)	
ND after seizure onset	[n=99]	[n=18]	[n=24]
	Moderate NDD 5 (5.1)	3 (16.7)	Normal ND 8 (33.3)
	Severe NDD 94 (94.9)	Moderate NDD 15 (83.3)	Mild NDD 7 (29.2)
		Severe NDD	Moderate NDD 6 (25.0)
			Severe NDD 2 (8.3)
Psychiatric and behavioral problems	[n=103]	[n=20]	[n=39]
	yes 2 (1.9)	yes 3 (15.0)	yes 19 (48.7)
Neurological features	[n=101]	[n=16]	[n=14]
	Severe IMF 71 (70.3)	Severe IMF 15 (93.8)	Severe IMF 1 (7.1)
	Moderate IMF 2 (1.9)	Moderate IMF 1 (6.3)	Moderate IMF 3 (21.4)
	Mild IMF 4 (3.9)	Hypotonia 10 (62.5)	Hypotonia 2 (14.3)
	Hypotonia 64 (63.4)	Hypertonia 6 (37.5)	Hypertonia 1 (7.1)
	Hypertonia 18 (17.8)	Spasticity 5 (31.3)	Pyramidal signs 1 (7.1)
	Pyramidal signs/spasticity 18 (17.8)	MD (choreo-athetosis) 2 (12.5)	Ataxia 1 (7.1)
	MD (chorea. dystonia) 9 (8.9)	Tetraparesis 1 (6.3)	Crouch gait 1 (7.1)
	Tetraparesis 3 (2.9)	Opsoclonus 1 (6.3)	Right-sided neglect 1 (7.1)
			MD 1 (7.1)
MRI	[n=88]	[n=17]	[n=26]
	Normal 40 (45.5)	Normal 4 (23.5)	Normal 17 (65.4)
	Delayed myelination 30 (34.1)	Cerebral atrophy 9 (52.9)	Cerebral atrophy 3 (11.5)
	Cerebral atrophy 27 (30.7)	Delayed myelination 8 (47.1)	Lobes asymmetry 3 (11.5)
	CC abnormalities 20 (22.7)	CC abnormalities 7 (41.2)	Cbl abnormalities 2 (7.7)
	WM loss 5 (5.7)	WM hyperintensities 4 (23.5)	Delayed myelination 2 (7.7)
	Cbl abnormalities 2 (3.4)	WM loss 2 (11.8)	Cavernomas 1 (3.8)
	Subdural hematoma 1 (1.1)	Cbl abnormalities 1 (5.9)	PNH 1 (3.8)
	Subdural hygroma 1 (1.1)	Increased PV spaces 1 (5.9)	WM abnormalities 1 (3.8)
	Subdural effusion 1 (1.1)		
	P/S CC disconnection 1 (1.1)		
	Temporal lobe asymmetry 1 (1.1)		
	Unilateral widening of SF 1 (1.1)		
	Indistinct G/W interface 1 (1.1)		
Cardiovascular* and respiratory problems	[n=119]	[n=20]	[n=39]
	Recurrent pneumonias 10 (8.4)	Pulmonary infections 1 (5.0)	Cardiac arrhythmia 1 (2.6)
	SPCA 3 (2.5)	VSD 1 (5.0)	Asthma 1 (2.6)
	Cardiomyopathy 1 (0.8)		
	AV fistula 1 (0.8)		
	Proliferative vasculopathy[+] 1 (0.8)		
	Sleep apnea 1 (0.8)		
	Arrhythmia, PFO, VSD 1 (0.8)		
Other features	[n=119]	[n=20]	[n=39]
	Microcephaly 52 (43.7)	Microcephaly 9 (45.0)	Precocious puberty 2 (5.1)
	Visual impairment 14 (11.8)	Visual impairment 4 (20.0)	Microcephaly 1 (2.6)
	Scoliosis 12 (10.1)	Gut dysmotility / GERD 4 (20.0)	Facial dysmorphisms 1 (2.6)
	Gut dysmotility / GERD 7 (5.9)	Dysmorphisms 2 (10.0)	Scoliosis 1 (2.6)
	Tube fed 7 (5.9)	Tube fed 2 (10.0)	Growth deficit 1 (2.6)
	Failure to thrive 6 (5.0)	Constipation 2 (10.0)	Constipation 1 (2.6)
	Constipation 6 (5.0)	Metopic synostosis 1 (5.0)	
	Facial dysmorphisms 5 (4.2)	Cushingoid facies 1 (5.0)	
	Precocious puberty 4 (3.4)		
	Hard palate cleft 1 (0.8)		
	Right gaze preference 1 (0.8)		
	Craniosynostosis 1 (0.8)		
	Dolichocephaly 1 (0.8)		
	Erratic eye movements 1 (0.8)		
	Hearing impairment 1 (0.8)		
Death	[n=119]	[n=20]	[n=39]
	26 (21.8)	1 (5.0)	0

[§] Four additional patients with other phenotypes (Møller et al. 2015, Routier et al. 2019, Borlot et al. 2020) are described in the text and not shown in the table.

*cardiac problems not related to quinidine
+occurred soon after a quinidine trial

Legend: (AD)SHE: Autosomal dominant sleep-related hypermotor epilepsy; AV: artero-venous; bilat: bilateral; Cbl: cerebellar; CC: corpus callosum; EIMFS: Epilepsy of infancy with migrating focal seizures; GERD: gastroesophageal reflux disease; G/W: grey-white; IMF: impairment of motor function; MD: movement disorder; MRI: magnetic resonance imaging; ND: neurodevelopment; NDD: neurodevelopmental delay; non-EIMFS DEE: non-EIMFS Developmental and epileptic encephalopathy; PFO: patent foramen ovale; PNH: periventricular nodular heterotopia; P/S: post-surgical; PV: periventricular; SF: sylvian fissure; SPCA: systemic to pulmonary collateral arteries; VSD: ventricular septal defect; WM: white matter.