Mild malformations of cortical development in sleep-related hypermotor epilepsy due to *KCNT1* mutations

RUBBOLI, Guido, *et al*.

**Abstract**

Mutations in the sodium-activated potassium channel gene KCNT1 have been associated with nonlesional sleep-related hypermotor epilepsy (SHE). We report the co-occurrence of mild malformation of cortical development (mMCD) and KCNT1 mutations in four patients with SHE. Focal cortical dysplasia type I was neuropathologically diagnosed after epilepsy surgery in three unrelated MRI-negative patients, periventricular nodular heterotopia was detected in one patient by MRI. Our findings suggest that KCNT1 epileptogenicity may result not only from dysregulated excitability by controlling Na+K+ transport, but also from mMCD. Therefore, pathogenic variants in KCNT1 may encompass both lesional and nonlesional epilepsies.

Reference


DOI: 10.1002/acn3.708
Mild malformations of cortical development in sleep-related hypermotor epilepsy due to KCNT1 mutations

Guido Rubboli1,2, Giuseppe Plazzi3,4, Fabienne Picard5, Lino Nobili6, Edouard Hirsch7, Jamel Chelly8, Richard A. Prayson9, Jean Boutonnat10, Manuela Bramerio11, Philippe Kahane12, Leanne M. Dibbens13, Elena Gardella1,14, Stephanie Baulac15,16,17,* & Rikke S. Møller1,14,*

1Danish Epilepsy Centre, Filadelfia, Dianalund, Denmark
2University of Copenhagen, Copenhagen, Denmark
3Department of Biomedical and Neuromotor Sciences, University of Bologna, Bologna, Italy
4IRCCS Institute of Neurological Sciences, Bologna, Italy
5Department of Clinical Neurosciences, University Hospitals and Medical School of Geneva, Geneva, Switzerland
6Epilepsy Surgery Center, Niguarda Hospital, Milan, Italy
7INSERM Unité 964: Génétique et Physiopathologie des Maladies Neuro Développementales Epileptogènes Epilepsy Unit « Francis Rohmer », Hautepierre Hospital, University Hospital, Strasbourg, France
8Service de Diagnostic Génétique, Hôpital Civil de Strasbourg, Hôpitaux Universitaires de Strasbourg, Strasbourg, France
9Department of Anatomic Pathology, Cleveland Clinic, Cleveland, Ohio
10Département d’Anatomie et de Cytologie Pathologiques Institut de Biologie et de Pathologie CHU de Grenoble, Grenoble, France
11Department of Pathology, Niguarda Hospital, Milan, Italy
12Neurology Department, Grenoble-Alpes University and Hospital, Grenoble, France
13Epilepsy Research Group, School of Pharmacy and Medical Sciences, University of South Australia and Sansom Institute for Health Research, Adelaide, Australia
14Institute for Regional Health Services, University of Southern Denmark, Odense, Denmark
15Institut du Cerveau et de la Moelle, ICM, Inserm, U1127, F-7501 Paris, France
16CNRS, UMR 7225, F-75013 Paris, France
17Sorbonne Université, F-75013 Paris, France

Correspondence
Guido Rubboli, Danish Epilepsy Centre, Filadelfia/University of Copenhagen, Kolenivvej 1, 4293 Dianalund (Denmark).
Tel.: +45 58271648; Fax: +45 58 27 10 50; E-mail: gur@filadelfia.dk

Abstract
Mutations in the sodium-activated potassium channel gene KCNT1 have been associated with nonlesional sleep-related hypermotor epilepsy (SHE). We report the co-occurrence of mild malformation of cortical development (mMCD) and KCNT1 mutations in four patients with SHE. Focal cortical dysplasia type I was neuropathologically diagnosed after epilepsy surgery in three unrelated MRI-negative patients, periventricular nodular heterotopia was detected in one patient by MRI. Our findings suggest that KCNT1 epileptogenicity may result not only from dysregulated excitability by controlling Na+/K+ transport, but also from mMCD. Therefore, pathogenic variants in KCNT1 may encompass both lesional and nonlesional epilepsies.

Introduction
Sleep-related hypermotor epilepsy (SHE), formerly known as nocturnal frontal lobe epilepsy, is characterized by focal seizures with various motor manifestations occurring during sleep.1 Autosomal dominant forms of SHE (ADSHE) have been associated with mutations of genes encoding subunits of the neuronal acetylcholine receptor (CHRNA4, CHRN B2, CHRNA2) or proteins of the mTORC1/GATOR1 complex (DEPDC5, NPRL2/3).1 Heterozygous missense mutations in KCNT1 were reported in a severe form of ADSHE with normal MRI, drug-resistant seizures, intellectual
disability, and psychiatric features. The KCNT1 gene encodes a sodium-activated potassium channel that regulates excitability in neurons by contributing to the slow hyperpolarization that follows repetitive firing.

Malformations of cortical development (MCDs) have previously been documented in SHE, but only few studies have elucidated their genetic etiologies. Here, we report the co-occurrence, not described previously, of mild MCDs (mMCDs) and KCNT1 pathogenic variants in four subjects with SHE.

Patients and Methods

We performed genetic diagnostic testing in one ADSHE patient (patient A.III.1) previously submitted to epilepsy surgery in whom mMCD was detected by histopathological examination, and in his family relatives (Fig. 1A). A cohort of further 36 published and unpublished patients with KCNT1-related SHE, collected through data sharing with Epilepsy and Genetic Centers in Europe and North America, was reviewed for MRI abnormalities and/or surgical treatment. We collected two additional patients with de novo KCNT1 variants, one with ADSHE (patient B.III.2, Fig. 1B) and one with sporadic SHE (patient C). Both patients underwent epilepsy surgery before genetic testing. A fourth patient (B.III.1) belonging to family B (Fig. 1B) showed MCD at the MRI. Electro-clinical phenotyping was performed by detailed clinical history and video-EEG monitoring including seizure recording. All patients had had 1.5 or 3 Tesla brain MRIs. Epilepsy surgery was performed after long-term scalp and intracranial video-EEG monitoring. Histopathological diagnosis on FFPE neuropathological surgical specimens was made according to the classification of the International League Against Epilepsy (ILAE) Diagnostic Methods Commission. Sequencing was performed on genomic blood DNA using targeted gene panels comprising known epilepsy genes including those known to cause MCD-related epilepsy (i.e., DEPDC5, NPRL2/3, MTOR). Informed consent from all participants and approval from the local ethics committees were obtained.

Results

Genetic findings

In family A, genetic analysis revealed a c.2849G>A; p.Arg950Gln KCNT1 (NM_020822.2) missense mutation segregating in subjects A.III.1, A.III.2, inherited from their unaffected father, and found in A.II.3 (Fig. 1A), reflecting

Family A: c.2849G>A; p.Arg950Gln

Family B: c.2386T>C; p.Tyr976His

Figure 1. (A) Pedigree of family A (KCNT1 c.2849G>A; p.Arg950Gln). (B) Pedigree of family B (KCNT1 c.2386T>C; p.Tyr976His). The patient indicated with a dot is an unaffected carrier. Red contours indicate the presence of MCD, dark symbols indicate individuals with focal epilepsy. Individuals with KCNT1 mutations are indicated by m/+, and those negative by +/-FCD, focal cortical dysplasia; PNH, periventricular nodular heterotopia.
the incomplete penetrance seen in KCNT1 families. Family B (c.2386T>G; p.Tyr796His KCNT1) was previously reported. Patient C had a de novo c.2800G>A; p.Ala934Thr mutation. All KCNT1 variants were considered as pathogenic given their: a) recurrence in other published SHE cases; b) absence in control databases (gnomAD); c) familial segregation/de novo occurrence (Fig. 1A), and d) in vitro gain-of-function effect of the Tyr796His and Ala934Thr variants on the current magnitude.

**Electro-clinical phenotyping**

Family A. Patient A.III.1 is a 23-year-old male with drug-resistant sleep-related frontal lobe seizures since 8 years of age (Table 1). Behavioral disturbances and neuropsychological deficits consistent with frontal lobe dysfunction appeared after epilepsy onset. 3T MRI was unremarkable. Epilepsy surgery was performed at age 14 to attempt to control seizures occurring several times per night, almost every night. After 2 years of postoperative seizure freedom, seizures reappeared with partially modified semiology (Table 1) reaching progressively the same frequency as before surgery (Engel class IV). Histopathological examination (NeuN immunostaining) showed cortical dyslamination suggesting focal cortical dysplasia (FCD) type Ib (Fig. 2A). In patient A.III.2, seizures were drug-resistant, whereas in patient A.II.3 they were well controlled by carbamazepine. In both patients, 1.5T MRI was normal. Familial history, clinical, and EEG features were consistent with ADSHE.

Family B. All four affected members of this family were diagnosed with ADSHE. Patient B.III.2 (previously described) suffered from drug-resistant sleep-related seizures since the age of 3 years. Brain MRI (1.5T) was unremarkable. At 21 years of age, he underwent epilepsy surgery with resection of the right mesio-lateral frontal region. Histopathological examination (Nissl staining) displayed cortical laminar disorganization indicative of FCD type Ib (not mentioned in the initial publication) (Fig. 2B). Epilepsy improved and seizure semiology changed after surgery. At 14-years follow up he has only rare diurnal seizures with staring (Engel Class II). Patient B.III.1 is the 37-year-old sister with drug-resistant sleep-related frontal seizures. Brain MRI (1.5T) showed left periventricular nodular heterotopia (PNH) connected by a radial band with the normal-appearing overlying cortex (Fig. 2D). Both patients presented with learning disabilities, severe psychosis, and precocious puberty. Patients B.II.1 and B.I.2 had sleep-related seizures consistent with ADSHE. Brain MRI (1.5T) was normal in patient B.II.1 and not available in patient B.I.2.

Patient C is a 27-year-old female with drug-resistant sleep-related frontal lobe seizures since the age of 20 months (seizures occurred almost every night). Brain MRI (1.5T) showed discrete temporal lobe asymmetry. Two epilepsy surgeries were performed at the age of 6 and 25 years, without improvement of her epilepsy (Engel class IV, follow-up: 2 years). Histopathological analysis (NeuN immunostaining) after the second surgery showed abnormalities of cortical lamination and the presence of characteristic microcolumns in the occipital cortex consistent with FCD type Ia (Fig. 2C).

**Discussion**

In this study, we report the finding of mMCDs in patients with SHE due to pathogenic variants in KCNT1. Together with the GATOR1-encoding genes, this study emphasizes the genetic continuum between apparently nonlesional and lesional focal epilepsies. Our four patients present with electro-clinical features compatible with SHE, and FCD type I or PNH. All patients suffered from severe refractory focal seizures, intellectual disability, and psychiatric disturbances consistent with the phenotype previously reported in KCNT1-related SHE. In three unrelated patients, epilepsy surgery failed to achieve seizure freedom, although epilepsy severity markedly improved in one (Engel Class II). Neuropathological analysis detected dyslamination compatible with FCD type I, a type of FCD often associated with unsatisfactory seizure control after surgery, with less than 50% of patients achieving seizure freedom, probably due to the more diffuse nature of FCD type I as compared to FCD type II, which has a better postseizure outcome. The focal onset of seizures documented by intracranial EEG recordings in patients with FCD type I is likely to represent only a portion of a more diffuse epileptogenic network, which probably involves a larger amount of abnormal cortex. Nevertheless, the transient seizure-free period followed by a change of seizure semiology observed in patients A.III.1 and B.III.2 indicates that the surgical intervention indeed impinged on the epileptogenic network, although the resection probably was not large enough to yield seizure freedom.

A role of KCNT1 in provoking widespread epileptogenesis that sustains seizure propensity after epilepsy surgery is also possible. Structural and molecular alterations (such as ion channel expression/function) in the perilesional region, may also contribute to the epileptogenicity of focal MCDs. The coexistence of two highly epileptogenic substrates (i.e., MCD and KCNT1 mutation) could underlie the epilepsy severity in these patients. Indeed, KCNT1 mutations and MCDs might reciprocally
Table 1. Electroclinical features, MRI, and pathological findings in patients with KCNT1-related SHE and MCD.

<table>
<thead>
<tr>
<th>Case</th>
<th>Mutation</th>
<th>Age/sex/Ethnic backgr.</th>
<th>Seizure semiology</th>
<th>Epilepsy syndr.</th>
<th>Interictal scalp EEG findings</th>
<th>MRI Findings (Tesla)</th>
<th>Intracranial/scale video-EEG findings</th>
<th>Surgery/Age at surgery</th>
<th>Surgery outcome/follow-up/seiz. semiology</th>
<th>Histo pathol</th>
<th>Current TRT</th>
<th>Comorbidities</th>
</tr>
</thead>
<tbody>
<tr>
<td>A.II.1</td>
<td>c.2849G&gt;A; p.Arg950Glu</td>
<td>23 y/M/Danish</td>
<td>Sleep-related HMS</td>
<td>ADHSHE</td>
<td>Bilateral sharp waves and bitemporal asynchronous sharp waves</td>
<td>Unremarkable (3 T)</td>
<td>Sleep-related HEMS (asymmetric posturing, choking, retching, distressed breathing) IEEG: R mesial frontal-central onset</td>
<td>Resection of the R mesial frontal-parietal regions/14 y</td>
<td>Engel Class IV</td>
<td>FCD type Ib</td>
<td>CBZ, TPM, LCS, CNZ</td>
<td>Behavioral disturbances (impulsivity, attention deficit), poor executive functions</td>
</tr>
<tr>
<td>B.III.1</td>
<td>c.2386T&gt;C; p.Tyr796His</td>
<td>37 y/F/Italian</td>
<td>Sleep-related focal seizures with tonic posturing</td>
<td>ADHSHE</td>
<td>L Parietocentric nodular heterotopia with transmantlesigns (1.5 T)</td>
<td>Not performed</td>
<td>Not performed</td>
<td>–</td>
<td>–</td>
<td>CBZ, TPM</td>
<td>Severe psychosis, learning disabilities, precocious puberty</td>
<td></td>
</tr>
<tr>
<td>B.III.2</td>
<td>c.2386T&gt;C; p.Tyr796His</td>
<td>35 y/M/Italian</td>
<td>Sleep-related HMS, nocturnal wandering</td>
<td>ADHSHE</td>
<td>R fronto-temporal theta activity</td>
<td>Unremarkable (1.5 T)</td>
<td>Sleep-related HEMS (pelvic thrusting, tonic posturing, pedaling) IEEG: R ant. cingular onset with spread to R mesial superior frontal gyrus, and orbital region</td>
<td>Resection of the R anterior cingular gyrus – lateral frontal cortex (F1,F2, post. F3)21 y</td>
<td>Engel Class II (at present)14 y</td>
<td>FCD type Ib</td>
<td>CBZ, VPA, PB</td>
<td>Severe psychosis, learning disabilities, precocious puberty</td>
</tr>
<tr>
<td>C</td>
<td>c.3100G&gt;A; p.Ala934Thr de novo</td>
<td>27 y/F/French</td>
<td>Sleep-related Tonic motor seizures with consciousness impairment; infrequent daytime episodes with R lower limb atonia, sudden fall, consciousness impairment.</td>
<td>SHE</td>
<td>Bilateral multifocal spikes</td>
<td>Discrete temporal lobe asymmetry (R &gt; L) (1.5 Tesla Uninformative (sequelae of the previous surgery) (3 Tesla)</td>
<td>(before first surgery): Sleep-related R sided tonic seiz., axial tonic seiz., “agitated” seiz. IEEG: L multifocal epileptogenic zone with centro-parietal predominance (before 2nd surgery): Sleep-related R side grimacing, R sided tonic seiz., R sided clonic jerks Scalp EEG: L multifocal (parieto-post. temporal occipital), R fronto-temporal onset</td>
<td>First surgery: L centro-parietal resection/6 y 2nd surgery: L parieto-occipital (- temporo-occipital junction) resection/25 y</td>
<td>Engel Class IV</td>
<td>FCD type la</td>
<td>CBZ, LEV, PB, VNS</td>
<td>Learning disabilities and delayed psychomotor development since 3 years of age. Warning of the cognitive status and appearance of autistic features over the years.</td>
</tr>
</tbody>
</table>

HMS, hypermotor seizures; IEEG, intracranial EEG; CBZ, carbamazepine; LTG, lamotrigine; TPM, topiramate; LCS, lacosamide; CNZ, clonazepam; LEV, levetiracetam; CLB, clobazam; PB, phenobarbital; VPA, valproic acid; VNS, vagus nerve stimulator; pentent., periventricular; ant., anterior; backgr, background; SE, status epilepticus; y, years; mo, months; seiz., seizure; R, right; L, left.
influence each other in developing the pathophysiological mechanisms that generate seizures, as suggested in patients with MCD carrying SCN1A mutations. An alternative hypothesis is the existence of a second-hit somatic mutation that would support the focal cortical lesion, as previously demonstrated in DEPDC5-related focal epilepsy, a hypothesis that could not be verified here because of the lack of resected epileptogenic cortex.

Given the poor epilepsy surgery outcome, our findings raise the issue whether surgery is an effective treatment in KCNT1-related SHE, and, in a broader perspective, whether genetic testing should be included in the presurgical assessment of patients who are considered as possible candidates for epilepsy surgery.

Interestingly, in the fourth nonoperated patient with ADSHE (patient B.III.1), MRI showed unilateral PNH, a type of MCD that has been shown to be associated with FCD type I in the cortex overlying the nodules. Negative MRI in three patients (A.III.2, A.II.3 and B.II.1) might depend on the limited sensitivity of current neuroimaging techniques to detect subtle abnormalities, especially FCD type I or, alternatively, it might suggest that KCNT1 variants can be associated with lesional and nonlesional epilepsies even within the same family.

The contribution of ion channels in the pathogenesis of MCDs has previously been reported. For instance, dysplastic neurons present in FCDs display strong Kv4.2 expression. Recently, neuropathological investigations in a child diagnosed with a severe KCNQ2-related neonatal epileptic encephalopathy, revealed the presence of heterotopic neurons in the deep white matter. In a knockdown mouse model, KCNK potassium channels dysfunction, resulting in increased frequency of spontaneous calcium influx, impeded physiological neuronal migration in the developing cerebral cortex, possibly playing a role in the genesis of MCD via an activity-dependent mechanism. Lastly, MCDs in patients with SCN1A and SCL35A2 mutations have been documented.

Our study supports an emerging view in which subtle structural brain changes (MRI-undetectable) can occur in epilepsies caused by mutations in ion channel genes and further emphasizes the need for repeated and careful review of high-resolution imaging for subtle

---

Figure 2. Neuropathological findings. (A) In individual A.III.1, NeuN staining highlights areas of underdeveloped or focal losses of cortical layer 2 and scattered malpositioned pyramidal type neurons in cortical layer 1, consistent with FCD type Ib (original magnification 100X). Scale bar: 250 μm. (B) In individual B.III.2, Nissl-stained section shows a laminar disorganization of the cortex indicative of FCD type Ib. Scale bar: 250 μm. (C) In individual C, NeuN staining section shows an abnormal cortical radial lamination and neurons with microcolumnar disposition consistent with FCD type Ia. No dysmorphic neurons or balloon cells were observed. Scale bar 200 μm. (D) MRI of individual B.III.1 showing deep nodular heterotopia in the left frontal lobe. Axial T2 WI (first panel from the left) and sagittal 3D T1WI (second panel from the left) show a left periventricular nodular heterotopia (in the red dashed ellipse). Coronal IR T1WI (third panel from the left) and magnification of the area included in the red square (fourth panel from the left) show a single heterotopic nodule in the deep-periventricular left frontal white matter with the same signal intensity of the grey matter. The nodule is connected by a radial band (arrow) with the normal-appearing overlying cortex. R = right, L = left.
abnormalities, that may not be immediately appreciated, in patients with KCNT1-related focal epilepsies.

Acknowledgments

We thank Roberto Sprefaco and Nadia Colombo for their helpful comments and suggestions on the manuscript. We are indebted to the patients and their families for their collaboration in performing this study. This work was supported by the European Research Council (ERC 682345 to SB).

Author contributions

G.R. and R.S.M: concept and study design, data acquisition and analysis, drafting of the manuscript and figures; S.B.: concept and study design, drafting of the manuscript and figures; G.P., F.P., L.N., E.H., J.C., R.A.P., J.B, M.B., P.K., L.M.D., E.G.: data acquisition and analysis.

Conflict of Interest

None of the authors has any conflict of interest to disclose. We confirm that we have read the Journal’s position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

References