Spectrum of Phenotypic, Genetic, and Functional Characteristics in Patients With Epilepsy With KCNC2 Pathogenic Variants

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Abstract

Background and Objectives
KCNC2 encodes Kv3.2, a member of the Shaw-related (Kv3) voltage-gated potassium channel subfamily, which is important for sustained high-frequency firing and optimized energy efficiency of action potentials in the brain. The objective of this study was to analyze the clinical phenotype, genetic background, and biophysical function of disease-associated Kv3.2 variants.

Methods
Individuals with KCNC2 variants detected by exome sequencing were selected for clinical, further genetic, and functional analysis. Cases were referred through clinical and research collaborations. Selected de novo variants were examined electrophysiologically in Xenopus laevis oocytes.

Results
We identified novel KCNC2 variants in 18 patients with various forms of epilepsy, including genetic generalized epilepsy (GGE), developmental and epileptic encephalopathy (DEE).

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including early-onset absence epilepsy, focal epilepsy, and myoclonic-atonic epilepsy. Of the 18 variants, 10 were de novo and 8 were classified as modifying variants. Eight drug-responsive patients became seizure-free using valproic acid as monotherapy or in combination, including severe DEE cases. Functional analysis of 4 variants demonstrated gain of function in 3 severely affected DEE cases and loss of function in 1 case with a milder phenotype (GGE) as the underlying pathomechanisms.

Discussion

These findings implicate KCNC2 as a novel causative gene for epilepsy and emphasize the critical role of Kv3.2 in the regulation of brain excitability.

Epilepsy is one of the most prevalent neurologic diseases, with ≈50 million people affected worldwide. Particularly in children and young adults, epilepsy represents a substantial disease burden relative to other neurologic conditions. The identification of epilepsy-associated genes in the past decade has dramatically improved the understanding of epileptogenesis. More than 10 epilepsy-associated genes coding for potassium channels have been identified. Based on this knowledge, new precision medicine approaches like the treatment with the potassium channel blocker 4-aminopyridine in KCNA2-related developmental and epileptic encephalopathies (DEE) were developed. Based on the original description, potassium channels are divided into Shaker, Shal, Shab, and Shaw subtypes. The Shaw-related potassium channel family (Kv3) plays a pivotal role in the excitability of the CNS by regulating action potential duration and firing pattern of neurons as well as neurotransmitter release. So far, only KCNC1 and KCNC3 as members of this potassium channel gene family have been implicated in human neurologic diseases (progressive myoclonus epilepsy and spinocerebellar ataxia). KCNC2 codes for the potassium channel Kv3.2 (an additional member of the Kv3 subfamily) mainly expressed in the brain in the interneurons of cortex, thalamus, hippocampus, and basal ganglia.

We identified 16 different heterozygous variants in KCNC2 in 18 unrelated individuals with DEE including early-onset absence epilepsy (EOAE) and other, more mild epilepsy syndromes such as genetic generalized epilepsy (GGE), epilepsy with focal epilepsy (FE), and myoclonic-atonic epilepsy (MAE), and provide a detailed phenotypical, genetic, and functional analysis emphasizing the role of KCNC2 as a novel disease gene in human epilepsies.

Methods

Patients

Participating patients and relatives were identified through clinical and research collaborations in our study, including individuals contributing through the Epi25 consortium. Recruitment was performed by the local centers. The data collection included age at onset, sex, seizure types, epilepsy syndrome, result of neurologic examination, cognitive state and development, EEG, brain MRI results, treatment, and outcome. The epilepsy syndromes were classified using the actual International League Against Epilepsy criteria by trained neurologists, epileptologists, and neuroepidemiologists. The clinical, neuroimaging, and electrophysiologic data were reviewed in detail by 2 experienced clinicians. The epilepsy syndromes were only subclassified (e.g., juvenile myoclonic epilepsy [JME]) as a subtype of GGE if the clinical aspects met the above mentioned criteria; if not, the term GGE was chosen. Individuals with KCNC2 variants were selected and variants were identified by whole exome sequencing in single or trio setting. Segregation analysis was performed when possible. No other relevant variants were detected in these cases based on the classification criteria by the American College of Medical Genetics.

Standard Protocol Approvals, Registrations, and Patient Consents

Written informed consent was obtained from all participants in the study or by their legal representatives. The study was approved by each of the following local ethics committees: Universities of Aachen, Bonn, Frankfurt, and Kiel, Germany; Epilepsy Centre Heemstede, the Netherlands; Epilepsiecentrum Bodensee, Weissenau, Germany; Danish Epilepsy Centre, Dianalund, Denmark; IRCCS Istituti delle Scienze Neurologiche di Bologna, Italy; Cooper Medical School of Rowan University, Camden, NJ; Royal College of Surgeons in Ireland, Dublin; University of Florence, Italy; The Cyprus Institute of Neurology and Genetics, Nicosia; Duke University, Durham, NC; University Medical Center Utrecht, the Netherlands; Sheba Medical Center, Tel Hashomer, Israel; Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan, Italy; Tel-Aviv University, Israel; University of Genova, Italy; Philipps-University Marburg, Germany; and Cleveland Clinic, OH.
Methods

Sanger Sequencing Analysis
We performed bidirectional Sanger sequencing of the respective areas of KCNC2 (NM_139137) using the BigDye Terminator v3.1 Cycle Sequencing kit on an ABI3730XL DNA Analyzer to confirm the described mutations and define the inheritance model (Applied Biosystems; primer sequences available upon request).

The presented KCNC2 variants were grouped into 2 different categories. The first category (group 1, pathogenic) includes patients with de novo variants. We also acquired variants absent in large population databases (except D128E, once described in gnomAD) that were either inherited from an unaffected parent or variants where a positive family history could be determined without knowledge available about the inheritance model. We defined these as likely pathogenic or modifying variants (group 2) if a minimum of 3/4 prediction scores indicate them as potentially damaging and the CADD score was high (>20) (eTable 1, links.lww.com/WNL/B901).

Protein Structure Analysis
No experimentally solved protein structure was available for the human Kv3.2 channel. We generated a model of the human KV3.2 channel. We generated a model of the No experimentally solved protein structure was available for

Functional Investigations
Four variants were selected for functional analysis according to the associated phenotype, the location, and the predicted effect on the structure of the protein.

Backbone and RNA Preparation
Vector pcDNA3.1 (+) + insert KCNC2 wild-type (WT) and the mutant clones (NM_139137: c.375C > G/p.Cys125Trp/C125W; c.404A > G/p.Glu135Gly/E135G; c.656T > C/p.Phe219Ser/F219S; c.1309A > G/p.Thr437Ala/T437A) were acquired from GenScript USA Inc. WT and mutant cDNA sequences were fully resequenced before being used in experiments to confirm the variant and exclude the presence of any additional sequence alterations. cRNA was prepared using the SP6 mMessage kit from Ambion according to the manufacturer’s instructions.

Electrophysiology
Collagenase-treated Xenopus laevis oocytes were acquired from Ecocyte Bioscience (1 mg/mL type CLS II collagenase [Biocrom] in OR-2 solution [in mM] 82.5 NaCl, 2.5 KCl, 1 MgCl2, and 5 HEPES, pH 7.5), washed 3 times, and stored at 16°C in Barth solution ([in mM] 88 NaCl, 2.4 NaHCO3, 1 KCl, 0.33 Ca[NO3]2, 0.41 CaCl2, 0.82 MgSO4, and 5 Tris-HCl, pH 7.4 with NaOH) supplemented with 50 μg/mL gentamicin (Biocrom). To compare current amplitudes of WT and mutant channels, 70 nL of cRNA encoding WT or mutant KCNC2 cRNA (c = 1 μg/μL) were injected on the same day using the same batch of oocytes. For recordings of homozygous conditions of the WT or mutant Kv3.2 channels, 70 nL of cRNA (1.0) were injected. To be able to record the heterozygous condition, 35 nL of the WT and 35 nL of the related mutant KCNC2 cRNA were injected. Injection was performed with the automated Roboinject system (Multi Channel Systems) and oocytes were incubated for 5 days (at 17°C) before the experiments were performed. Potassium currents in oocytes were recorded at room temperature (20–22°C) using Roboocyte2 (Multi Channel Systems). For 2-electrode voltage-clamp (TEVC) recordings, oocytes were impaled with 2 glass electrodes (resistance of 0.4–1 MΩ) containing a solution of 1 M KCl and 1.5 M potassium acetate and clamped at a holding potential of ~80 mV. Oocytes were perfused with an ND96 bath solution containing (in mM) 93.5 NaCl, 2 KCl, 1.8 CaCl2, 2 MgCl2, and 5 HEPES (pH 7.6). Currents were sampled at 2 kHz.

Western Blot Analysis
For protein blotting, injected Xenopus laevis oocytes were lysed in a buffer containing 20 mM Tris HCl (pH7.6), 100 M NaCl, 1% Triton X-100, and 1X complete protease inhibitors (Roche). After measuring the protein concentrations (BCA Systems [Thermo Fisher Scientific]), 50 μg of protein were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis on 8% polyacrylamide gels. Proteins were transferred onto polyvinylidene fluoride membranes (Pall Corporation) and protein blotting was performed using a monoclonal mouse antibody (S410-17) against Kv3.2 (KCNC2) (MAS5-27683 [Thermo Fisher]) with a concentration of 1:500. Water-injected oocytes were used as negative control.

Data and Statistical Analysis
Data analysis and graphical illustrations were achieved using Roboocyte2+ (Multi Channel Systems), Excel (Microsoft), and GraphPad Prism Software. Normality was tested using the Shapiro-Wilk test and statistical evaluation for multiple comparisons was conducted using 1-way analysis of variance on ranks with Dunn post hoc test. Statistical testing was performed with SigmaPlot 12.0 (Systat Software, Inc.) and differences between groups were considered significant with p < 0.05. Data are reported as mean ± SEM.
| Patient ID | Protein variant | Inheritance mode, FH | F/M Syndrome | Age at onset (current age) | Seizure types | Neurologic examination | Intellectual disability | EEG | Brain MRI | Treatments | Outcome (last treatment) |
|------------|-----------------|---------------------|-------------|--------------------------|--------------|-----------------------|-----------------------|-----|-----------|-----------|------------------------|
| 1          | C125W           | DN                  | M           | DEE (EOAE)               | Absence, Myo, GTCS | Facial dysmorphism, hypotonia, ataxia, ASD | Mild–moderate         | G (PSW) | N         | CBZ, CZP, LEV, TPM, VPA | SF (VPA, CZP) |
| 2          | E135G           | DN                  | M           | DEE                      | 1 y (7 y) | Myo, atonic           | N         | Mild         | G         | ESM, LEV, VPA         | DR         |
| 3          | D167Y^15        | DN                  | F           | DEE (WS to LGS)          | 1 mo (10 y) | IS, atypical absence, Myo, focal unaware, GTCS | Ataxia, speech disturbance, macrocephaly | Mild–moderate | Hypsarrhythmia, later MF | CLB, LEV, VPA, LTG, PER, CBV, VGB, ST, OXC, TPM, LCM, VNS | DR |
| 4          | F219S           | DN^2                | F           | GGE                      | 18 y (34 y) | GTCS                  | N         | No          | G (PSW)   | NA                     | DR         |
| 5          | R351K           | DN                  | M           | DEE (CSWS)               | 7 mo (15 y) | GTCS, Myo, focal unaware | N         | Mild         | CSWS      | VPA, LTG, STM, LEV, ST, CLB, TPM | SF (VPA) |
| 6          | R351K           | DN^3                | M           | DEE (CSWS)               | 8 mo (30 y) | GTCS, Myo, absence, focal unaware | ASD, hyperactivity, impaired sleep, aphasia | Severe       | CSWS      | VPA, CLB, ST, ESM, VGB, LEV, CZP, TPM, LTG, CBZ, DZP, PB | DR |
| 7          | F382C           | DN                  | M           | MAE                      | NA (4 y) | Myo, atonic, clonic | N         | No          | G (PSW)   | Venous anomaly        | STM       | DR         |
| 8          | T437A           | DN                  | M           | DEE (EOAE)               | 8 mo (8 y) | Absence, tonic       | Regression in language development, cannot follow commands | Severe       | G         | KD, CZP, CLB, DZP, LEV, PER, VPA, ZNS | DR         |
| 9          | T437N           | DN                  | F           | DEE                      | 11 mo (15 y) | FS, Myo              | N         | Moderate     | G         | N                     | VPA, CNZ, RUF | DR |
| 10         | T437N           | DN                  | M           | DEE                      | 3 mo (12 y) | FS, Myo, absence    | Hyperactivity | Moderate   | G         | PB, VPA, LEV, CNZ, CLB, ESM, LTG | DR |
| 11         | T32A            | Mc (neg)            | F           | GGE (JAE)                | 10 y (21 y) | GTCS, absence       | N         | No          | N         | NA                     | VPA       | SF (VPA) |
| 12         | D128E           | NA (neg)            | F           | GGE (JAE)                | 14 y (67 y) | Absence, GTCS      | N         | No          | G (PSW)   | Generalized brain atrophy | VPA, TPM, ESM | SF (VPA, ESM) |
| 13         | D144E           | NA^4                | M           | GGE (JAE)                | 10 y (29 y) | GTCS, absence       | N         | No          | G (3 Hz spike wave discharges) | NA, LEV, VPA | DR (SF 2 y VPA) |
| 14         | V330M           | Mc (neg)            | F           | GGE                      | 3 y (11 y) | GTCS                | N         | Mild         | G (PSW)   | N                     | VPA       | SF (VPA) |
| 15         | S333T           | Mc (neg)            | F           | Dravet-like     (DEE) | 2 y (20 y) | GTCS, Myo, absence | N         | Mild         | G         | N                     | VPA       | SF (VPA) |
| 16         | I485V           | NA^5                | M           | FE                       | 13 mo (NA) | FS, SE, GTCS, focal aware and unaware | NA        | Na          | T-B       | 15 AEDs tried, details NA, VNS | DR |

Continued
### Table 1
Clinical and Genetic Information on the Analyzed Cohort (continued)

| Patient ID | Protein variant | Inheritance model | M. Syndrome | Age at onset (current age) | Seizure types | Neurologic examination | Intellectual disability | EEG | Brain MRI | Treatments | Outcome last treatment |
|------------|-----------------|-------------------|-------------|---------------------------|---------------|------------------------|------------------------|-----|-----------|------------|------------------------|
| 17         | N530H           | NA                | F (current age) | 8 y (45 y) | GGE (JME) | Depression and anxiety | No | N | VPA | GTCS, Myo, Depression and anxiety | No G (3–4 Hz spike wave discharges) |
| 18         | S656F           | Mc                 | F (45 y)     | 12/4 (24 y) | Focal aware, GTCS | Depressed and anxiety | No | N | VPA | VPA |

Abbreviations: ASD = autism spectrum disorder; BRV = brivaracetam; CAE = childhood absence epilepsy; CBZ = carbamazepine; CLB = clobazam; CSWS = continuous spikes and waves during slow sleep; CTP = childhood Tremors and Paroxysmal Sleep Dysrhythmia; CWS = continuous waves; CSW/S = continuous sharp waves; CTB = chronic tonic bilateral; DBS = deep brain stimulation; DEE = developmental and epileptic encephalopathies; DTP = de novo tonic; DZP = diazepam; EOAE = early-onset absence epilepsy; ES = early-onset seizures; ESM = ethosuximide; EEG = electroencephalogram; EMG = electromyography; F = female; FBC = frequent bilateral clonic; FE = focal epilepsy; FH = family history; FC = fever; FGE = familial generalized epilepsy; G = generalized; GBP = gabapentin; GGS = genetic generalized epilepsy; GGE = genetic generalized epilepsy; GTCS = generalized tonic-clonic seizures; HB = hemiparesis; HK = hereditary; IL = infantile; IS = infantile spasms; JAE = juvenile absence epilepsy; JME = juvenile myoclonic epilepsy; KD = ketogenic diet; LCM = lacosamide; LEV = levetiracetam; LGS = Lennox-Gastaut syndrome; MAE = myoclonic-atonic epilepsy; MCD = mental retardation, cerebral dysgenesis; MD = multiple dysmorphic features; MND = myoclonic, atonic, and dyskinetic seizures; Myo = myoclonic seizures; NA = not available; Neg = negative; OXC = oxcarbazepine; P = posterior; PB = phenobarbital; PE = paroxysmal epilepsy; PHT = phenytoin; PSW = polyspike wave discharges; RUF = rufinamide; SE = status epilepticus; SF = seizure-free; ST = steroids; STM = sulthiame; T-B = temporal bilateral; T-L = temporal left; TLE = temporal lobe epilepsy; TPM = topiramate; V = vagus nerve stimulation; VGB = vigabatrin; VPA = valproic acid; VPS = vagus nerve stimulation; W = West syndrome; ZNS = zonisamide.

Affected relatives of patients were not available for genetic testing unless noted otherwise. Variants in italic were functionally measured. Subclassifications of syndromes (for example, JME for GGE) are only given if classical aspects are present. More detailed EEG information is only given if applicable.

#### Data Availability
Anonymized data not published within this article will be made available by request from any qualified investigator.

#### Results

##### Patients
We identified 18 patients carrying 16 different KCNC2 variants (Table 1), 10 male and 8 female, and separated the patients into 2 categories regarding the potential pathogenicity of their respective KCNC2 variants. Within these groups, we could describe the following phenotypes.

##### Pathogenic Variants (de novo, Group 1): 10/18 Patients

**Myoclonic-Atonic Epilepsy**
One of the 10 patients presented with myoclonic, atonic, and generalized tonic-clonic seizures (exact onset not applicable but before 4 years of age), which were drug-resistant. EEG recordings showed polyspike-wave discharges. The neurologic examination and the mental presentation were normal. On brain MRI, the patient had a developmental venous anomaly, which is a nonspecific finding for epilepsy.

**Genetic Generalized Epilepsy**
One of the 10 patients presented a drug-resistant subtype of GGE featuring generalized tonic-clonic seizures with onset at 18 years of age. EEG recordings showed typical generalized epileptic discharges with polyspike-waves. The neurologic examination and the brain MRI results were normal.

**Developmental and Epileptic Encephalopathy**
Six of the 10 patients with a DEE could be identified carrying a de novo KCNC2 variant. The age at onset ranged from the first month of life to 1 year. Moderate to severe intellectual disability was present in all patients. Further neuropsychiatric findings were present in 3/6 patients: 1 patient demonstrated an ataxic gait, hypotonia, speech disturbance, and macrocephaly; a second patient had autism spectrum disorder, impaired sleep, hyperactivity, and aphasia; and the third patient presented with isolated hyperactivity. Depending on the syndrome, EEG showed continuous spikes and waves during sleep (CSWS) (2 patients), hypsarrhythmia and later multifocal spikes (1 case), or bilateral/generalized discharges (3 cases). The brain MRI was normal in 5 patients; 1 patient showed an arachnoid cyst combined with brain atrophy. Only 1 patient achieved seizure freedom, using valproic acid (VPA).

##### Early-Onset Absence Epilepsy
Two of the 10 patients had EOE, with an onset between 1.5 and 8 months. The EOE syndrome also can be defined as a subtype of DEE but we preferred to describe these patients separately. Both had normal MRI and generalized epileptiform discharges on EEG. They had mild intellectual disability and demonstrated additional abnormalities including facial dysmorphism (more detailed information was...
not available), hypotonia, ataxia, and autism spectrum disorder. One of the patients showed a regression in language development. The other patient achieved seizure freedom using a combination of VPA and clobazam.

**Likely Pathogenic Variants (Group 2)—8/18 Patients**

**Genetic Generalized Epilepsy**

Five of the 8 patients presented with subtypes of GGE featuring absences, myoclonic, and generalized tonic-clonic seizures (onset age 3–14 years). EEG recordings showed 3–4 Hz spike-wave complexes, polyspike waves, or normal interictal results. The neurologic examination was normal in all patients. One patient with GGE had psychiatric symptoms with depression and anxiety, while another showed mild intellectual disability. GGE subclassification was possible in 4/5 patients with GGE of this subgroup of variants—3 with juvenile absence epilepsy and 1 with JME. On the available brain MRI, 1 patient had generalized brain atrophy, which is a nonspecific finding for epilepsy. Four of the 5 patients with GGE were seizure-free (1 lasting >2 years) using VPA in monotherapy or in combination.

**Focal Epilepsies**

Two of the 8 patients had FE. Neurologic examination and brain MRI were normal in 1 patient and not available in the other. Neither had intellectual disability. EEG demonstrated bilateral temporal and generalized discharges. One patient achieved seizure freedom using VPA.

**Developmental and Epileptic Encephalopathy**

One of the 8 patients had DEE. The age at onset was 2 years. This patient had mild intellectual disability but no other neuropsychiatric findings. The EEG showed generalized discharges and the brain MRI was normal. The patient received seizure freedom using VPA.

**Genetic Findings**

We present 16 unique heterozygous missense variants identified in 18 patients including a first initial case published already by our group. Fifteen of the 16 variants were not found in control cohorts previously. The remaining variant was found once in the control cohort gnomAD (D128E). The presented KCNC2 variants were grouped into 2 different categories (see also Table 1 and Methods). Pathogenic variants (group 1) were found in 10/18 cases. Modifying variants...
(group 2) were detected in 8/18 cases, either inherited from an unaffected parent (n = 4) or variants where a positive family history could be determined without knowledge available about the inheritance model (n = 4).

Interestingly, 2 variants were identified in our cohort with a similar clinical picture with regard to seizure onset, seizure types, and developmental delay. Both patients (2 cases) with the variant R351K presented with a CSWS phenotype and all 3 patients carrying the T437N/A variant had severe DEE with onset between 3 and 11 months of age, dominant absences and myoclonic seizures, moderate to profound intellectual impairment, and multiple drug-resistant epilepsy.

Protein Structure Analysis

Based on knowledge of potassium channel structure, we located the KCNC2 variants across the 6 transmembrane segments of the Kv3.2 subunit as well as in the long cytoplasmic N- and C-terminal regions18 (Figure 1A). The fourth transmembrane domain of the Kv3.2 subunit builds a voltage sensor and the extracellular loop between the fifth and the sixth transmembrane domain serves as a selectivity filter for potassium ions. The identified variants were located in the N- and C-terminal portion of the protein as well as in the last 4 transmembrane domains, but the localization of each variant did not correlate with a specific phenotype. Because there was no crystal structure available, we modeled the structure of the Kv3.2 subunit and mapped the identified variants onto our model (Figure 1B). By combining the evidence of both scores used, we detected critical regions (>5 consecutively amino acids) characterized by paralog conservation (Para_zscore > 0) and depletion of population variants (MTR < 0.459). A total of 9 of 18 patient variants (F219S, V330M, S333T, R351K [2 patients], F382C, T437A/N [3 patients]) are located within these special regions. These regions/variants were all located close to or within the transmembrane region of the protein. Interestingly, one further variant (I465V) was located in the transmembrane region. All others were localized in the cytoplasmic regions. Three variants were de novo cytosolic N-terminal variants (C125W, E135G, D167Y) within or close to the structured N-terminal cytoplasmic region, the so-called T1 domain, which is important for the tetramerization of the protein.19

We could not detect a strong phenotype–localization correlation in the predicted structure of the Kv3.2 subunit, but most of the identified variants are located in specific relevant and conserved areas of the channel.

Figure 2: Electrophysiologic Analysis of the p.F219S KCNC2 Variant

(A) Representative traces of Kv3.2 currents in Xenopus laevis oocytes expressing WT, F219S, or a 1:1 mixture of both in response to the voltage steps from −70 mV to +30 mV. (B) Immunoblot analysis for lysates of X laevis oocytes injected with cRNA for Kv3.2 WT, F219S, equal amounts of WT + F219S, or water. All channels showed a band at about 90 kDa. (C) Mean current amplitudes of analyzed oocytes injected with WT (n = 101), F219S (n = 39), equal amounts of WT + F219S (n = 29), or water (n = 44). (D) Resting membrane potentials of oocytes injected with WT (n = 101), F219S (n = 39), equal amounts of WT + F219S (n = 29), or water (n = 44). Shown are means ± SEM. Statistically significant differences between WT channels and the tested groups were verified by analysis of variance on ranks (indicated by asterisks).
**Functional Analysis**

We analyzed 4 different variants, which were selected according to the location of the variant within the channel structure as well as the phenotype of the patient (C125W-EOAE, E135G-DEE, F219S-GGE, and T437A-EOAE; see also Figure 1). All of them showed a de novo inheritance pattern and none of them was found in control cohorts (gnomAD). C125W and E135G are located within the T1 domain, which is important for the tetramerization of the channel (see eFigure 1, links.lww.com/WNL/B901). The Zn²⁺ coordinating motif is also located in this region (Hx₅Cx₂₀CC), which is important in bridging the interaction interface between 2
proteins and stabilizing the tetrameric protein structure. The Zn$^{2+}$ location within the Shaw and Shaker family is different, suggesting that zinc may play a role in differentiating Shaw from Shaker T1 in assembly.19 F219S is located shortly before the first transmembrane domain and T437A is located within the P-domain (TxxT/SxGY/FG), which acts as the K$^+$ selectivity filter, and affects the second important amino acid of the P-domain motif.18

The functionally analyzed variant F219S-GGE showed a complete loss of function in the homozygous and heterozygous state with current amplitudes comparable to the ones obtained from water-injected control oocytes (Figure 2A). Thus, this variant causes a dominant negative effect on WT channels (Figure 2, A and C). T437A-EOAE led to a significant reduction of the current amplitude, while C125W-EOAE showed a significant increase in the current amplitude. The analysis of E135G-DEE could not demonstrate any differences compared with oocytes injected with the WT subunit (Figure 3, A and B). To make sure that all variants have been expressed in the injected oocytes, we performed a Western blot analysis. This analysis showed a band at about 90 kDa in all protein lysates except for the water-injected control oocytes. As loading control, the housekeeping gene $\beta$-actin was used, and all protein lysates showed a band at about 40 kDa. Thus, all variants showed expression in oocytes (Figures 2B and 3D). The analysis of the channel kinetics of the variants C125W-EOAE, E135G-DEE, and T437A-EOAE showed a significant shift of the activation curve to more hyperpolarized potentials (Figure 3E) and in total a slower deactivation compared with oocytes expressing WT channels (Figure 3F). E135G-DEE showed a significantly slower deactivation time constant compared with cells injected with RNA encoding only WT subunits at all recorded voltages except for 0 mV. For C125W-EOAE and T437A-EOAE, the deactivation time constant was significantly reduced between −50 mV and −20 mV or −30 mV, respectively, in comparison with oocytes expressing WT channels (Figure 3F). Thus, analysis of the variants C125W, E135G, and T437A in total demonstrates a gain of function in the channel kinetics. The membrane potential of the variants C125W, F219S, and T437A was significantly shifted to more hyperpolarized potentials (Figures 2D and 3C).

Taken together, the variant T437A-EOAE showed a mixed effect on the channel function, with a gain of function based on changes in the channel kinetics and a loss of function based on a decrease in the normalized current amplitude. For the other 2 variants (C125W-EOAE and E135G-DEE), the gain of function was the predominant effect.

Overall, we could detect 18 patients carrying a KCNC2 variant, of which 10/18 were defined as pathogenic and 8/18 as modifying, with a variable phenotype including GGE (6/18 cases in total: 1 with pathogenic variants, 5 modifying), DEE (7/18 cases in total: 6 with pathogenic variants, 1 modifying), EOAE (2/18 cases in total: 2 pathogenic), FE (2/18 cases in total: 2 modifying), and MAE (1/18: pathogenic). The 10 pathogenic variants are mainly located in the T1 domain (responsible for tetramer formation), voltage sensor, and P domain (K$^+$ selectivity filter; see also eFigure 1, links.lww.com/WNL/B901). Three of the 8 modifying variants are located in the T1 domain; others, in nongeneric specific areas. The functional analysis demonstrated in the more severe phenotypes a clear gain-of-function effect (DEE and EOAE), whereas the milder phenotype F219S-GGE causes a dramatic dominant negative loss-of-function effect.

Discussion

We describe pathogenic variants in KCNC2 as a novel genetic etiology for human epilepsies. Phenotypes range from mild generalized epilepsies to severe DEEs related to specific functional changes.

The variants collected ranged from pathogenic to modifying. Therefore, we divided these into 2 different categories. The first category includes only de novo variants that are pathogenic (10/18 variants). The second category includes patients with variants in KCNC2 that are likely to be disease-causing or to have only a modifying effect because the prediction scores indicated pathogenicity but the variants were inherited by unaffected family members or the inheritance mode was unclear (8/18). The clinical spectrum observed in KCNC2-related disorders is very broad concerning the extent of severity. DEE was the main phenotype (39% in total cohort, including EOAE: 50%), but closely followed by GGE (34% in total cohort), FE (11% in total cohort), and 1 case with MAE (5% in total cohort). Nevertheless, the recurrent variants had a recognizable homogenous clinical picture with a CSWS syndrome (R351K) and similar onset, seizure types, mental status, and drug resistance (T437N/A), hinting at potential unique variant-specific genotype–phenotype correlation.

In general, the KCNC2 cohort showed a reduced drug response, as only 8/18 patients were seizure-free (4/8 patients with GGE). In addition, the GGE-KCNC2 drug-responsive patients seem to have a poor prognosis as only 66% were drug-responsive compared with approximately 90% in the general GGE population.20 Nevertheless, it is interesting that all drug-responsive patients became seizure-free using VPA as monotherapy or in combination, including with severe DEE. The patients responding to VPA carry variants that mainly cluster in 2 regions. One is the intracellular N-terminal part including the variants (T32A, D128E, D144E) and the other one the extracellular region of the third and fourth domain including the variants V330M, S333T, and R351K. VPA is an antiepileptic drug with a broad spectrum of mechanisms of action. VPA has been demonstrated to limit high-frequency repetitive firing in cultured neurons.21 This effect is linked to the modulation of sodium, calcium, and potassium channels, especially with a use-dependent decrease in inward sodium currents. VPA increases the amplitude of the late potassium outward currents, further increasing the threshold for
epileptiform activity, which might explain the special effect of this drug in our cohort. However, the cohort is small and further studies are needed to prove this hypothesis.



KCNC2 has previously been proposed as a modifying factor and to play a role in other neuropsychiatric diseases such as ataxia, schizophrenia, bipolar disorder, and other DEEs. Our patients also displayed additional neurologic features including facial dysmorphism, ataxia, speech disturbance, depression, hyperactivity, and autism spectrum disorder, with additional support to this hypothesis. We could demonstrate a much broader clinical spectrum including GGE, EOAIE, FE, and MAE.

The virtual structure of Kv3.2 identified very important regions characterized by paralog conservation (Paraz score) and depletion of population variants (MTR score). We could not find a strong phenotype–structure association but the variants of our patients were concentrated at the C-terminus, N-terminus, and transmembrane segments 3 to 6, indicating high relevance of these regions to channel function.

We selected 4 variants for functional analysis based on the phenotype as well as the location within the channel. The functional results demonstrated a gain of function in the more severe phenotypes DEE and EOAIE, whereas in the GGE-associated variant, a dramatic loss-of-function effect was observed. Among all Kv channels, channels of the Kv3 family in particular play a crucial role in the rapid repolarization of action potentials and therefore dictate action potential duration. The Kv3.2 subunit is predominantly expressed in inhibitory interneurons in neocortex as well as somatostatin-expressing GABAergic interneurons in deep cortical layers and therefore may be responsible for the modulation of excitation. Furthermore, functional analysis demonstrated that voltage-gated potassium channels are important for the regulation of firing, action potential duration, and neurotransmitter release. The Kv3.2 subunit and the Kv3 family in general have some unusual electrophysiologic properties compared with other potassium channels such as the fast rate of depolarization so that balanced cortical operations can occur. As described for other variants in potassium channel genes associated with epilepsy, the recorded Kv3.2 variants demonstrate gain- and loss-of-function effects.

KCNC2 is a novel and important gene for a broad spectrum of epilepsy syndromes with an intriguing phenotype–pathophysiologic correlation.

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Appendix

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Spectrum of Phenotypic, Genetic, and Functional Characteristics in Patients With Epilepsy With KCNC2 Pathogenic Variants

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