MUTATION UPDATE

Human Mutation | HGVS HUMAN GENOME VARIATION SOCIETY | WILEY

Expanding the genetic and phenotypic relevance of *KCNB1* variants in developmental and epileptic encephalopathies: 27 new patients and overview of the literature

Claire Bar[1,2,3] | Giulia Barcia[2,3,4] | Mélanie Jennesson[5] | Gwenaël Le Guyader[6,7] | Amy Schneider[8] | Cyril Mignot[9,10] | Gaetan Lesca[11,12] | Delphine Breuillard[1] | Martino Montomoli[13] | Boris Keren[10] | Diane Doummar[14] | Thierry Billette de Villemeur[14] | Alexandra Afenjar[15] | Isabelle Marey[10] | Marion Gerard[16] | Hervé Isnard[17] | Alice Poisson[18] | Sophie Dupont[9,19] | Patrick Berquin[20] | Pierre Meyer[21,22] | David Genevieve[23] | Anne De Saint Martin[24] | Salima El Chehadeh[25] | Jamel Chelly[25] | Agnès Guët[26] | Emmanuel Scalais[27] | Nathalie Dorison[28] | Candace T. Myers[29] | Heather C. Mefford[30] | Katherine B. Howell[31,32] | Carla Marini[13] | Jeremy L. Freeman[31,32] | Anca Nica[33] | Gaetano Terrone[34] | Tayeb Sekhara[35] | Anne-Sophie Lebre[36] | Sylvie Odent[37,38] | Lynette G. Sadleir[39] | Arnold Munnich[3,4] | Renzo Guerrini[13] | Ingrid E. Scheffer[8,31,40] | Edor Kabashi[2,3] | Rima Nabbout[1,2,3]

[1]Department of Pediatric Neurology, Reference Centre for Rare Epilepsies, Hôpital Necker-Enfants Malades, Paris, France

[2]Imagine institute, laboratory of Translational Research for Neurological Disorders, INSERM UMR 1163, Imagine Institute, Paris, France

[3]Université Paris Descartes-Sorbonne Paris Cité, Paris, France

[4]Department of genetics, Necker Enfants Malades hospital, Assistance Publique-Hôpitaux de Paris, Paris, France

[5]Department of Pediatrics, American Memorial Hospital, Reims, France

[6]Department of genetics, University hospital Poitiers, Poitiers Cedex, France

[7]EA3808-NEUVACOD Unité Neurovasculaire et Troubles Cognitifs, Pôle Biologie Santé, Université de Poitiers, Poitiers, France

[8]Department of Medicine, Epilepsy Research Centre, Austin Health, The University of Melbourne, Heidelberg, Victoria, Australia

[9]Institut du Cerveau et de la Moelle épinière, INSERM, U 1127, CNRS UMR 7225, Sorbonne Universités, UPMC Univ Paris 06 UMR S 1127, Paris, France

[10]Département de Génétique et de Cytogénétique, Centre de Reference Déficience Intellectuelle de Causes Rares, APHP, Hôpital Pitié-Salpêtrière, GRC UPMC (Déficience Intellectuelle et Autisme), Paris, France

[11]Department of genetics, Hospices Civils de Lyon, Lyon, France

[12]Neurosciences centre of Lyon, INSERM U1028, UMR CNRS 5292, Université Claude Bernard Lyon 1, Bron Cedex, France

[13]Pediatric Neurology, Neurogenetics and Neurobiology Unit and Laboratories, Department of Neuroscience, A Meyer Children's Hospital, University of Florence, Florence, Italy

[14]Department of Pediatric Neurology, Hôpital Armand Trousseau, AP-HP, Paris, France

[15]Département de Génétique et Embryologie Médicale, Pathologies Congénitales du Cervelet-LeucoDystrophies, Centre de Référence déficiences intellectuelles de causes rares, AP-HP, Hôpital Armand Trousseau, GRC n°19, Sorbonne Université, Paris, France

[16]Department of genetics, CHU Côte de Nacre, Caen, France

[17]Neurology clinic, Lyon, France

[18]Reference Center for Diagnosis and Management of Genetic Psychiatric Disorders, Centre Hospitalier le Vinatier and EDR-Psy Team, Centre National de la Recherche Scientifique & Lyon 1 Claude Bernard University, Villeurbanne, France

[19]Epileptology and Rehabilitation department, GH Pitie-Salpêtrière-Charles Foix, AP-HP, Paris, France

[20]Department of pediatric neurology Amiens-Picardie university hospital, Université de Picardie Jules Verne, Amiens, France

[21]Department of pediatric neurology, Montpellier university hospital, Montpellier, France

[22]PhyMedExp, U1046 INSERM, UMR9214 CNRS, Montpellier, France

[23]Service de génétique clinique et du Département de Génétique Médicale, Maladies Rares et Médecine Personnalisée, Centre de référence maladies rares anomalies du développement, CHU Montpellier, Montpellier, France

[24]Department of Pediatric Neurology, Strasbourg University Hospital, Strasbourg, France

[25]Department of genetics, Hôpital de Hautepierre, Hôpitaux Universitaires de Strasbourg, Strasbourg, France

[26]Department of Pediatric, Louis-Mourier Hospital, Colombes, France

[27]Department of Pediatric Neurology, Centre Hospitalier de Luxembourg, Luxembourg City, Luxembourg City, Luxembourg

[28]Department of pediatric Neurosurgery, Rothschild Foundation Hospital, Paris, France

[29]Department of Pediatrics, University of Washington, Seattle, Washington

[30]Department of Pediatrics, Division of Genetic Medicine, University of Washington, Seattle, Washington

[31]Departments of Neurology and Paediatrics, Royal Children's Hospital, University of Melbourne, Melbourne, Victoria, Australia

[32]Murdoch Children's Research Institute, Melbourne, Victoria, Australia

[33]Department of Neurology, Center for Clinical Research (CIC 1414), Rennes University Hospital, Rennes, France

[34]Department of Translational Medical Sciences, Section of Pediatrics-Child Neurology Unit, Federico II University, Naples, Italy

[35]Department of Pediatric Neurology, C.H.I.R.E.C, Brussels, Belgium

[36]Department of genetics, Maison Blanche hospital, University hospital, Reims, Reims, France

[37]Reference Centre for Rare Developmental Abnormalities, CLAD-Ouest, CHU Rennes, Rennes, France

[38]Institute of genetics and development, CNRS UMR 6290, Rennes university, Rennes, France

[39]Department of Paediatrics and Child Health, University of Otago, Wellington, New Zealand

[40]The Florey Institute of Neurosciences and Mental Health, Heidelberg, Victoria, Australia

Correspondence: Rima Nabbout, Department of Pediatric Neurology, Reference Centre for Rare Epilepsies, Necker Enfants Malades Hospital, 149 Rue de Sèvres, 75015 Paris, France.
Email: rimanabbout@yahoo.com

Abstract

Developmental and epileptic encephalopathies (DEE) refer to a heterogeneous group of devastating neurodevelopmental disorders. Variants in KCNB1 have been recently reported in patients with early-onset DEE. KCNB1 encodes the α subunit of the delayed rectifier voltage-dependent potassium channel $K_v2.1$. We review the 37 previously reported patients carrying 29 distinct KCNB1 variants and significantly expand the mutational spectrum describing 18 novel variants from 27 unreported patients. Most variants occur de novo and mainly consist of missense variants located on the voltage sensor and the pore domain of $K_v2.1$. We also report the first inherited variant (p.Arg583*). KCNB1-related encephalopathies encompass a wide spectrum of neurodevelopmental disorders with predominant language difficulties and behavioral impairment. Eighty-five percent of patients developed epilepsies with variable syndromes and prognosis. Truncating variants in the C-terminal domain are associated with a less-severe epileptic phenotype. Overall, this report provides an up-to-date review of the mutational and clinical spectrum of KCNB1, strengthening its place as a causal gene in DEEs and emphasizing the need for further functional studies to unravel the underlying mechanisms.

KEYWORDS
developmental and epileptic encephalopathy, epilepsy, KCNB1, potassium channel

1 | BACKGROUND

Developmental encephalopathies constitute a broad and genetically heterogeneous group of neurodevelopmental disorders diagnosed during early childhood and persisting throughout life. The clinical spectrum includes variable degrees of social, cognitive, motor, language, and behavioral impairments. The concept of "developmental and epileptic encephalopathy" (DEE) refers to the frequently associated epileptic activity (seizures and electroencephalogram [EEG] abnormalities) that contributes to developmental impairment and regression (Scheffer et al., 2017). Recent advances in DNA-sequencing methods have highlighted the important role of gene-

encoding ion channels in the pathogenesis of DEEs (Wang et al., 2017). Ion channels are crucial in the generation and modulation of excitability in the nervous system (Wei et al., 2017). "Channelopathies" are associated with a wide phenotypic and genotypic spectrum as one gene is often associated with different phenotypes and variants in several genes might result in the same epilepsy phenotype (McTague, Howell, Cross, Kurian, & Scheffer, 2016; Wei et al., 2017). In particular, gene-related potassium channel dysfunction causes a clinical spectrum of DEEs including epilepsy of infancy with migrating focal seizures (KCNT1), early-onset DEEs with suppression-burst (KCNQ2), and nonspecific DEEs (e.g., KCNA2, KCND2, KCND3, KCNH5, KCNJ2, KCNJ10, KCNMA1, KCNQ3, KCNQ5, KCNT2, and KCNV2; Ambrosini et al., 2014; Barcia et al., 2012; Gururaj et al., 2017; Jorge et al., 2011; Lee, Lin, Kornblum, Papazian, & Nelson, 2014; Lehman et al., 2017; Pena & Coimbra, 2015; Sicca et al., 2011; Soldovieri et al., 2014; Tabarki, AlMajhad, AlHashem, Shaheen, & Alkuraya, 2016; Veeramah et al., 2013; Wang et al., 2019; Weckhuysen et al., 2012).

Torkamani et al. (2014) identified de novo variants in the potassium voltage-gated channel subfamily B member 1 (KCNB1) in three sporadic patients affected by early-onset DEE. This study provided the initial evidence of the deleterious effect of variants p.Ser347Arg, p.Thr374Ile, and p.Gly379Arg on KCNB1 function. Following the initial identification of KCNB1 variants (Torkamani et al., 2014), 29 new KCNB1 variants have been reported in 37 patients detected through next-generation high-throughput sequencing in cohorts of individuals with developmental delay and/or DEE (Allen et al., 2016; Fitzgerald et al., 2015; Saitsu et al., 2015; Soden et al., 2014; Srivastava et al., 2014; Thiffault et al., 2015; Torkamani et al., 2014; Calhoun, Vanoye, Kok, George, & Kearney, 2017; de Kovel et al., 2016, 2017; Latypova et al., 2017; Marini et al., 2017; Miao, Peng, Chen, Gai, & Yin, 2018, 2018; Parrini et al., 2017; Samanta, 2018; Zhu et al., 2017). The majority of these patients had epilepsy, intellectual disability, and behavioral problems (MIM# 616056; epileptic encephalopathy, early infantile, 26).

In this mutation update, we aim to present an exhaustive review of patients carrying KCNB1 variants and discuss the evidence for the pathophysiological relevance of these variants. Furthermore, we expand the variant spectrum of KCNB1 with the description of 27 new unrelated patients carrying 18 novel variants.

2 | STRUCTURE OF K$_v$2.1 CHANNEL

KCNB1 (MIM# 600397) is a potassium channel gene located on chromosome 20q13.3 and has a full-length transcript of 11.879 kb (NM_004975) containing two exons. KCNB1 protein is a 96-kDa core protein of 858 amino acids forming the α subunit of the voltage-gated potassium channel subfamily 2 (K$_v$2.1). K$_v$2.1 channels are expressed across the central nervous system, especially in large clusters on the soma, proximal dendrites, and axonal initial segment of neurons (King,

Manning, & Trimmer, 2014; Trimmer, 1991). Like other voltage-gated potassium channels, they are composed of four α-subunits surrounding the ion conduction pore. Each α-subunit has six transmembrane helices (S1–S6) that include a voltage-sensing domain (S1–S4) and a pore domain (S5–P–S6). The voltage-sensor S4 helix contains a series of positively charged amino acids that senses the change in the membrane potential leading to channel opening and closing. The selectivity filter of the pore is formed by the TVGYG amino acids motif located in the re-entrant pore loop between S5 and S6. In addition, K$_v$2.1 voltage-gated potassium channels have a N-terminal cytoplasmic region that modulates homotetramerization as well as heterotetramerization with other families of channel-forming subunits, such as the silent α-subunits K$_v$6 (KCNG), K$_v$8 (KCNV), and K$_v$9 (KCNS; Bocksteins et al., 2014; Hugnot et al., 1996; Salinas, Duprat, Heurteaux, Hugnot, & Lazdunski, 1997; Xu, Yu, Jan, Jan, & Li, 1995). Although the functional role of the intracellular C-terminal domain is yet not fully understood, it mediates the restricted and clustered proximal localization of K$_v$2.1 channel (Lim, Antonucci, Scannevin, & Trimmer, 2000) and interacts with the N-terminal domain to regulate intracellular trafficking, surface expression, voltage-dependent activation gating, and phosphorylation-dependent modulation of the K$_v$2.1 channel (Ju, Stevens, Leadbitter, & Wray, 2003; Mohapatra, Siino, & Trimmer, 2008). Homotetrameric K$_v$2.1 channels mediate a delayed-rectifier voltage-dependent outward potassium current which is essential for membrane repolarization during high-frequency firing. Functional properties of heterotetrameric channels are more complex, depending on the channel subunit composition (Ottschytsch, Raes, van Hoorick, & Snyders, 2002; Sano et al., 2002).

3 | IDENTIFICATION OF KCNB1 VARIANTS

The database Pubmed was used to search KCNB1 pathogenic variants by combining the terms "KCNB1" and "variants" or "mutations". Articles were reviewed and crossed with KCNB1 variants listed in professional databases (Human Gene Mutation Database [HGMD], Biobase, and Qiagen). We excluded one patient without Sanger confirmation of de novo inheritance and insufficient clinical data available to validate the variant pathogenicity (Zhu et al., 2017).

We collected 27 new unrelated patients with KCNB1 pathogenic variants through the French reference network for rare epilepsies and international collaborations (Belgium, Italy, Luxembourg, New Zealand, and Australia). KCNB1 variants were detected by targeted next-generation sequencing panels for either epilepsy or intellectual disability (n = 16) or by whole-exome sequencing (n = 11), we classified variants according to the international guidelines of the American College of Medical Genetics (ACMG) Laboratory Practice Committee Working Group (Richards et al., 2015; Table S1). Single-nucleotide variants were confirmed by Sanger sequencing and segregation analysis was completed in each family. In silico predictions supporting evidence of pathogenicity for missense variants reported in our 27 new patients are detailed in Table S2. KCNB1 variants were described according to HGVS variant

nomenclature guidelines (http://varnomen.hgvs.org/; Dunnen et al., 2016), using the reference sequence RefSeq NM_004975.2. Variants have been submitted to ClinVar database (https://www.ncbi.nlm.-nih.gov/clinvar/). Clinical and EEG data were obtained for all patients, including developmental, neurological, behavioral, and epilepsy history, EEG, and imaging data when available. All parents or legal guardians gave written informed consent for genetic diagnosis procedures and research participation according to the ethics committee of each institution.

4 | VARIANT SPECTRUM

We identified 29 pathogenic variants from 37 unrelated patients reported in the literature (Allen et al., 2016; Calhoun et al., 2017; de Kovel et al., 2016, 2017; Fitzgerald et al., 2015; Latypova et al., 2017; Marini et al., 2017; Miao et al., 2017, 2018; Parrini et al., 2017; Saitsu et al., 2015; Samanta, 2018; Soden et al., 2014; Srivastava et al., 2014; Thiffault et al., 2015; Torkamani et al., 2014).

In our cohort of 27 unrelated patients, we found 25 distinct *KCNB1* variants including 18 novel variants (Table 1). All patients carried heterozygous missense or truncating variants, arising de novo in 24/27 patients. We report the first inherited variant (p.Arg583*) in Patient 64 and his mother affected by intellectual disability without epilepsy. Study of the maternal grandparents showed that the variant occurred de novo in the proband's mother who carried the variation at the heterozygous state. Segregation could not be completed in both parents for the remaining two variants to confirm their de novo occurrence (Patients 51 and 58), but both were considered pathogenic. The p.Gly395Arg variant in Patient 51 was identified as a de novo variant in Patient 50 in this series. The p.Phe416Leu variant in Patient 58 has been previously reported as likely pathogenic in two patients (Allen et al., 2016; Patient 8; de Kovel et al., 2016; ID-2010D05815).

Overall, including our data and those of the literature, we reviewed data of 47 distinct pathogenic variants identified in 64 unrelated patients (Table 1 and Figure 1). All variants except one were in exon 2. They included 37 missense variants (37/47; 79%), eight nonsense variants (8/47; 17%), and two frameshift variants (2/47; 4%). Ten variants were recurrent.

Most variants (42/47, 89%) were located in the S1 to S6 transmembrane segments of the protein. The K+ selectivity filter located between the S5 and S6 transmembrane segments (amino acid 377–381) had eight distinct missense variants, including three novel variants in our series. Three were at amino acid position 378 (p.Val378Ala; p.Val378Phe; p.Val378Leu), representing a potential variant hotspot. Two recurrent variants affected the voltage sensor domain. The p.Arg306Cys was found in four patients (Patients 12–15) and the p.Arg312His was found in five patients (Patients 17–21). A different amino acid change at this position, p.Arg312Cys, was also found in one patient (Patient 16). Six recurrent variants were localized in the pore domain of the protein (S5–S6). Two were in the S5 transmembrane segment, each one found in two patients

(p.Ser347Arg, Patients 26 and 27; p.Val349Phe, Patients 28 and 29). The p.Val349Phe variant found in Patient 29 resulted from somatic mosaicism (Marini et al., 2017). Three recurrent variants were located in the S6 transmembrane segment. The p.Phe416Leu variant was found in three patients (Patients 56–58), whereas the p.Gly395Arg and p.Gly401Arg variants were found in two patients each (Patients 50, 51, and 53, 54).

Patient 1 had a *KCNB1* variant in the exon 1, affecting the cytoplasmic N-terminal region (p.Glu43Gly). The highly conserved N-terminal domain is a critical determinant for subunit self-association into tetrameric channels and the p.Glu43Gly variant was thus predicted to be damaging (Xu et al., 1995). This patient also carried a de novo variant in *GABRA5* gene (MIM# 137142, p.Thr301Met), which encodes the α5 subunit of the gamma-aminobutyric acid type-A (GABA$_A$) receptor. Recently, Butler et al. (2018) provided functional evidence of the pathogenic effect of a nearby *GABRA5* variant (p.Val294Leu) in a patient with severe DEE. Therefore, both variants were considered to be contributing to the patient's phenotype.

All variants located within the intracellular C-terminal domain of the protein were truncating. The non-sense variant p.Arg583* was found in 3 unrelated patients (Patients 62–64; inherited from the mother in Patient 64). Two other nonsense variants were in the pore helix between S5 and S6 (p.Trp369*; p.Trp370*). The last four truncating variants were in the linkers: S1–S2 linker (p.Gln228*), S3–S4 linker (p.Val286Glyfs*58), S4–S5 linker (p.Tyr328*), and S5–S6 linker (p.Ser363Thrfs*13).

5 | FUNCTIONAL RELEVANCE AND CHARACTERIZATION OF *KCNB1* VARIANTS

To date, seven *KCNB1* missense variants have been functionally characterized using different cellular models (Calhoun et al., 2017; Saitsu et al., 2015; Thiffault et al., 2015; Torkamani et al., 2014). Distinct functional effects have been described among studies. The p.Ile199Phe variant, located in the S1 transmembrane segment of the voltage-sensing domain, reduces channel availability due to shift in the voltage dependence of activation compared to the wild-type (WT) channel (Calhoun et al., 2017). A variant in the voltage sensor domain S4, p.Arg306Cys, induces currents similar to those in the WT channel but disrupts sensitivity and cooperativity of the sensor whereas the p.Gly401Arg variant, located in the pore domain S6, has a dominant-negative effect on WT channels and abolishes endogenous currents (Saitsu et al., 2015). Both variants inhibit repetitive neuronal firing by preventing the production of sufficiently deep interspike voltages (Saitsu et al., 2015). Other *KCNB1* variants affecting the pore domain result in loss of ion selectivity and gain of inward cation conductance of K$_v$2.1 channels (Thiffault et al., 2015; Torkamani et al., 2014). Since these mutants also induce reduced current density at more depolarized voltages, they were predicted to result in depolarized resting membrane potential and impaired membrane repolarization leading to increased cellular excitability

TABLE 1 KCNB1 genotype and clinical features of patients in our cohort and the literature

Patient ID	Nucleotide change	Amino acid change	Protein effect	Inheritance	Age (years)/Gender	DD	Walk	Verbal skills	Behavioral disorders	Epilepsy/Age at seizure onset (months)	References
1	c.128A>G	p.Glu43Gly[a]	Missense	De novo	29/M	Yes	Yes	Nonverbal	No	Yes/16	This study
2	c.586A>T	p.Ile196Phe	Missense	De novo	12/F	Yes	NA	Nonverbal	Yes	Yes/18	Marini et al. (2017; ID-4)
3	c.595A>T	p.Ile199Phe pore domain of the protein	Missense	De novo	NA/M	Yes	NA	NA	Yes	Yes/NA	Calhoun, Vanoye, Kok, George, andKearney (2017)
4	c.605C>T	p.Ser202Phe	Missense	De novo	7/M	Yes	Yes	Sentences	Yes	Yes/24	de Kovel et al. (2017; Patient 26)
5	c.629C>G	p.Thr210Arg	Missense	De novo	11/M	Yes	Yes	Words	Yes	Yes/48	This study
6	c.629C>T	p.Thr210Met	Missense	De novo	4/F	NA	NA	NA	NA	No	de Kovel et al. (2017; Patient 24)
7	c.629C>T	p.Thr210Met	Missense	De novo	8/F	Yes	NA	Nonverbal	Yes	Yes/12	Marini et al. (2017; ID-5)
8	c.629C>A	p.Thr210Lys	Missense	De novo	7/M	Yes	Yes	Sentences	Yes	Yes/4	de Kovel et al. (2017; Patient 25)
9	c.632T>C	p.Leu211Pro	Missense	De novo	7/F	Yes	NA	NA	No	Yes/11	de Kovel et al. (2017; Patient 23)
10	c.682C>T	p.Gln228*	Nonsense	De novo	3,5/F	Yes	Yes	NA	Yes	Yes/10	de Kovel et al. (2017; Patient 22)
11	c.857del	p.Val286Glyfs*58	Frameshift	De novo	2/M	Yes	Yes	Sentences	No	Yes/5	This study
12	c.916C>T	p.Arg306Cys	Missense	De novo	8/F	Yes	Yes	Sentences	Yes	Yes/48	de Kovel et al. (2017; Patient 21)
13	c.916C>T	p.Arg306Cys	Missense	De novo	22/F	Yes	Yes	Sentences	No	Yes/6	Marini et al. (2017; ID-3)
14	c.916C>T	p.Arg306Cys	Missense	De novo	7/M	Yes	Yes	Words	Yes	Yes/12	Saitsu et al. (2015; Patient 2) and de Kovel et al. (2017; Patient 20)
15	c.916C>T	p.Arg306Cys	Missense	De novo	9/M	Yes	NA	NA	Yes	Yes/12	de Kovel et al. (2017; Patient 21)
16	c.934C>T	p.Arg312Cys	Missense	De novo	23/M	Yes	Yes	Words	Yes	No	This study
17	c.935G>A	p.Arg312His	Missense	De novo	9/M	Yes	Yes	Nonverbal	Yes	Yes/10	de Kovel et al. (2017; Patient 19)
18	c.935G>A	p.Arg312His	Missense	De novo	1,2/F	Yes	No	Nonverbal	NA	Yes/14	Samanta (2018)
19	c.935G>A	p.Arg312His	Missense	NA	11/M	Yes	No	Nonverbal	Yes	Yes/14	de Kovel et al. (2016; ID-KIEL20) and de Kovel et al. (2017; Patient 18)
20	c.935G>A	p.Arg312His	Missense	De novo	33/M	Yes	Yes	Nonverbal	Yes	Yes/18	This study
21	c.935G>A	p.Arg312His	Missense	De novo	13/M	Yes	Yes	Nonverbal	Yes	Yes/60	This study
22	c.968C>T	p.Thr323Ile	Missense	De novo	3/M	Yes	Yes	Nonverbal	Yes	Yes/21	This study
23	c.984C>G	p.Tyr328*	Nonsense	De novo	4,5/F	Yes	Yes	Words	Yes	Yes/7	This study
24	c.990G>C	p.Glu330Asp	Missense	De novo	3,6/F	Yes	NA	NA	NA	Yes/18	Miao et al. (2018)
25	c.1001T>C	p.leu334Pro	Missense	De novo	5/F	Yes	No	Nonverbal	Yes	Yes/24	This study
26	c.1041C>G	p.Ser347Arg	Missense	De novo	10/M	Yes	Yes	Sentences	Yes	Yes/18	This study
27	c.1041C>A	p.Ser347Arg	Missense	De novo	9/F	Yes	Yes	NA	NA	Yes/48	Torkamani et al. (2014; ID-9) and de Kovel et al. (2017; Patient 17)
28	c.1045G>T	p.Val349Phe	Missense	De novo	1,6/F	Yes	No	Nonverbal	Yes	Yes/9	This study

(Continues)

TABLE 1 (Continued)

| Patient ID	Nucleotide change	Amino acid change	Protein effect	Inheritance	Age (years)/ Gender	DD	Walk	Verbal skills	Behavioral disorders	Epilepsy/Age at seizure onset (months)	References
29	c.1045G>T	p.Val349Phe	Missense	De novo	17/M	Yes	NA	Words	Yes	Yes/11	Marini et al. (2017; ID-6)
30	c.1088delG	p.Ser363Thrfs*13	Frameshift	De novo	2/M	Yes	NA	Nonverbal	No	Yes/14	de Kovel et al. (2017; Patient 16)
31	c.1105T>C	p.Trp369Arg	Missense	De novo	14/M	Yes	No	Nonverbal	Yes	Yes/10	This study
32	c.1107G>A	p.Trp369*	Nonsense	De novo	5/M	Yes	NA	NA	Yes	Yes/18	de Kovel et al. (2017; Patient 15)
33	c.1109G>A	p.Trp370*	Nonsense	De novo	7/M	Yes	NA	Words	No	Yes/9	Parrini et al. (2017) and Marini et al. (2017; ID-1)
34	c.1115C>T	p.Thr372Ile	Missense	De novo	8/M	Yes	Yes	Sentences	No	No	This study
35	c.1115C>A	p.Thr372Asn	Missense	De novo	12/F	Yes	Yes	Sentences	Yes	No	This study
36	c.1121C>T	p.Thr374Ile	Missense	De novo	5/F	Yes	NA	NA	NA	Yes/6	Allen et al. (2016; ND27062, Torkamani et al. (2014; Patient 3), and de Kovel et al. (2017; Patient 13)
37	c.1121C>T	p.Thr374Ile	Missense	De novo	11/F	Yes	With aids	Nonverbal	Yes	Yes/13	de Kovel et al. (2017; Patient 14)
38	c.1130C>A	p.Thr377Asn	Missense	De novo	2/M	Yes	No	Nonverbal	No	Yes/6	This study
39	c.1132G>C	p.Val378Leu	Missense	De novo	10/F	Yes	Yes	Words	Yes	No	Latypova et al. (2017)
40	c.1132G>T	p.Val378Phe	Missense	De novo	8/M	Yes	No	Nonverbal	NA	Yes/14	This study
41	c.1133T>C	p.Val378Ala	Missense	De novo	3/F	Yes	No	Nonverbal	NA	Yes/13	Soden et al. (2014), Thiffault et al. (2015), and de Kovel et al. (2017; Patient 12)
42	c.1135G>A	p.Gly379Arg	Missense	De novo	7/M	Yes	Yes	Nonverbal	Yes	Yes/8	Torkamani et al. (2014; Individual 2), Srivastava et al. (2014), and de Kovel et al. (2017; Patient 11)
43	c.1136G>T	p.Gly379Val	Missense	De novo	2/ND	Yes	NA	NA	NA	Yes/24	Miao et al. (2017)
44	c.1139A>G	p.Tyr380Cys	Missense	De novo	5,5/F	Yes	Yes	Words	No	Yes/6	This study
45	c.1141G>A	p.Gly381Arg	Missense	De novo	7/M	Yes	No	NA	NA	Yes/3	Allen et al. (2016; Patient 7) and de Kovel et al. (2017; Patient 10)
46	c.1144G>A	p.Asp382Asn	Missense	De novo	6/F	Yes	Yes	Nonverbal	No	Yes/8	This study
47	c.1153C>A	p.Pro385Thr	Missense	De novo	17/M	Yes	With aids	Nonverbal	Yes	Yes/13	de Kovel et al. (2017; Patient 9)
48	c.1173A>C	p.Lys391Asn	Missense	De novo	NA	NA	NA	NA	NA	NA	Fitzgerald et al. (2015) and de Kovel et al. (2017; Patient 8)
49	c.1180G>A	p.Gly394Arg	Missense	De novo	9/F	Yes	Yes	Nonverbal	Yes	Yes/3,5	This study
50	c.1183G>A	p.Gly395Arg	Missense	De novo	9.5/F	Yes	Yes	Nonverbal	Yes	Yes/12	This study
51	c.1183G>A	p.Gly395Arg	Missense	NA	14/M	Yes	No	Nonverbal	Yes	Yes/8	This study

(Continues)

TABLE 1 (Continued)

Patient ID	Nucleotide change	Amino acid change	Protein effect	Inheritance	Age (years)/ Gender	DD	Walk	Verbal skills	Behavioral disorders	Epilepsy/Age at seizure onset (months)	References
52	c.1190G>T	p.Cys397Phe	Missense	De novo	22/M	Yes	NA	NA	NA	Yes/10	de Kovel et al. (2017; Patient 7)
53	c.1201G>A	p.Gly401Arg	Missense	De novo	4/M	Yes	No	Nonverbal	NA	Yes/17	Saitsu et al. (2015; Patient 1) and de Kovel et al. (2017; Patient 6)
54	c.1201G>A	p.Gly401Arg	Missense	De novo	9/M	Yes	No	Nonverbal	NA	Yes/8	This study
55	**c.1226T>C**	**p.Ile409Thr**	**Missense**	**De novo**	**8/M**	**Yes**	**Yes**	**Sentences**	**Yes**	**No**	**This study**
56	c.1248C>G	p.Phe416Leu	Missense	De novo	15/F	Yes	NA	NA	Yes	Yes/14	Allen et al. (2016; Patient 8) and de Kovel et al. (2017; Patient 5)
57	c.1248C>G	p.Phe416Leu	Missense	De novo	18/F	Yes	NA	NA	NA	Yes/42	de Kovel et al. (2016; ID-2010D05815) and de Kovel et al. (2017; Patient 4)
58	c.1248C>A	p.Phe416Leu	Missense	NA	14/F	Yes	Yes	Nonverbal	No	Yes/10 days	This study
59	**c.1489G>T**	**p.Glu497***	**Nonsense**	**De novo**	**20/M**	**Yes**	**Yes**	**Sentences**	**Yes**	**No**	**This study**
60	c.1503dup	p.Lys502*	Nonsense	De novo	10/M	Yes	NA	NA	Yes	No	Fitzgerald et al. (2015) and de Kovel et al. (2017; Patient 3)
61	c.1599C>A	p.Tyr533*	Nonsense	De novo	32/M	Yes	Yes	NA	NA	Yes/5	de Kovel et al. (2016; ID-EP1852) and de Kovel et al. (2017; Patient 2)
62	c.1747C>T	p.Arg583*	Nonsense	De novo	8/F	Yes	NA	Nonverbal	No	Yes/5	Marini et al. (2017; ID-2)
63	c.1747C>T	p.Arg583*	Nonsense	De novo	11/F	Yes	Yes	Sentences	Yes	No	de Kovel et al. (2017; Patient 1)
64	c.1747C>T	p.Arg583*	Nonsense	Maternal	12/F	Yes	Yes	Nonverbal	Yes	No	This study

Note: Numbering is according to the complementary DNA sequence (RefSeq NM_004975.2). Novel mutations are indicated in bold.

Abbreviation: NA, not available.

[a]This patient also carries a de novo variant in GABRA5 (NM_000810:c.902C>T, p.Thr301Met).
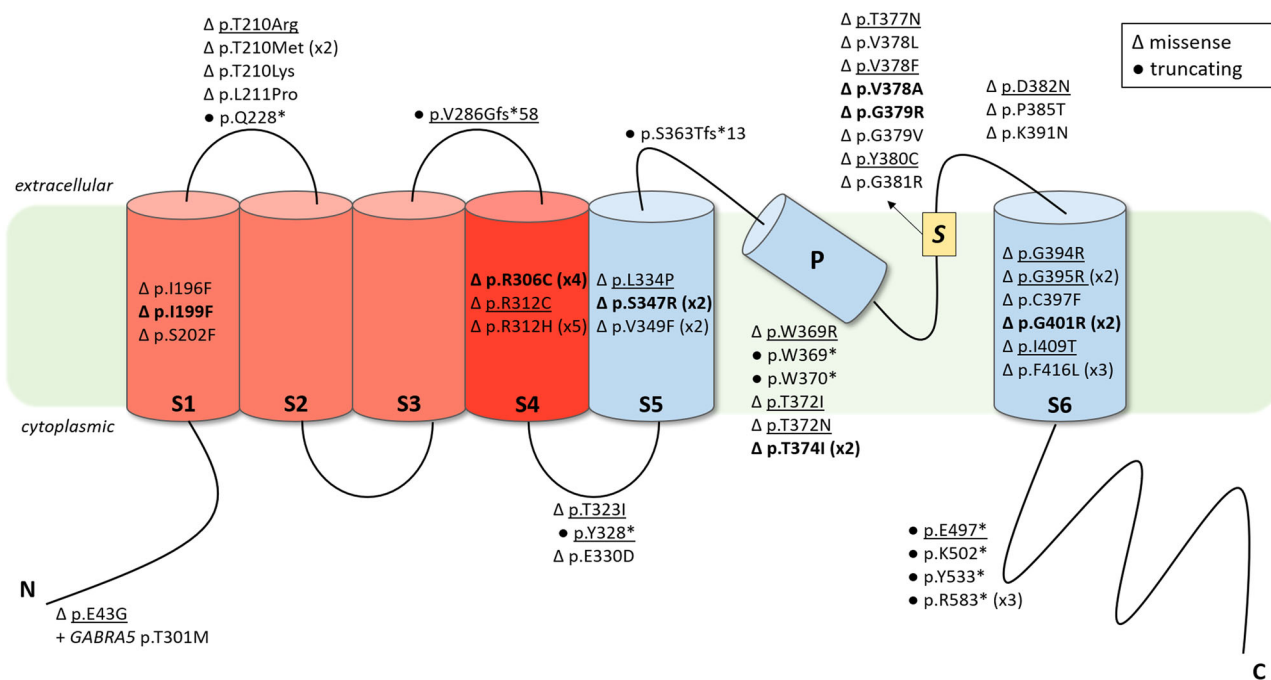
FIGURE 1 Schematic representation of K$_v$2.1 protein structure and location of *KCNB1* previously described and novel variants. Variants are displayed as changes at protein level (p). Underlined variants correspond to novel variants reported for the first time in this report. For recurrent variants, the number of reported patients is indicated between parentheses next to the variant. Triangles and black circles respectively indicate missense variants and truncating variants (frameshift or nonsense variants). Variants, where functional studies have been previously conducted, are indicated in bold. Functional domains are represented by N: N-terminal domain (residues 1–186), S1: Segment S1 (residues 187–208), S2: Segment S2 (residues 229–250), S3: Segment S3 (residues 260–280), S4: Segment S4 (residues 295–316), S5: Segment S5 (residues 331–351), P: pore helix (residues 365–376), S: Selectivity filter (residues 377–381), S6: Segment S6 (residues 392–420), C: C-terminal domain (residues 421–858)

(Torkamani et al., 2014). Changes in channel expression and localization were also suggested to contribute to the pathophysiology of K$_v$2.1 pore variants (Thiffault et al., 2015). Therefore, given the biophysical properties of these pathogenic variants on K$_v$2.1, they should be considered as loss-of-function rather than gain-of-function.

Finally, delayed-rectifier potassium current is diminished in hippocampal neurons cultured from *Kcnb1*$^{-/-}$ (K$_v$2.1$^{-/-}$) mice (Speca et al., 2014). Interestingly, mice lacking K$_v$2.1 have no spontaneous seizures but display increased seizure susceptibility in response to proconvulsant drugs and exhibit a range of behavioral disorders associated with marked hyperactivity (Speca et al., 2014).

6 | EXPANDING THE PHENOTYPIC SPECTRUM OF *KCNB1* VARIANTS

The cohort of 64 patients included 34 males and 28 females (sex ratio M/F 1.2; data available for *n* = 62/64) with pathogenic *KCNB1* variants, aged from 1.2 to 33 years (median age at study, 8 years; data available for *n* = 62/64).

We report here six new patients aged from 8 to 23 years who did not develop seizures (Patients 16, 34, 35, 55, 59, 64).

Overall, 53/63 patients (85%) developed epilepsy with a median age at seizure onset of 12 months (range: 10 days-5 years; mean, 15 months; data available for *n* = 52/53). All patients had developmental delay before

seizure onset. Thirty-seven patients (70%) exhibited several seizure types during follow-up, including generalized tonic–clonic seizures (*n* = 28), focal seizures (*n* = 24), epileptic spasms (*n* = 21), tonic seizures (*n* = 14), myoclonic seizures (*n* = 14), atypical absences (*n* = 13), atonic seizures (*n* = 9), and clonic seizures (*n* = 5). Nine patients (18%) developed only epileptic spasms at a mean age of 12 months (range, 5-21 months), consistent with the syndrome of infantile spasms (Pavone, Striano, Falsaperla, Pavone, & Ruggieri, 2014). Behavioral issues occurred in 37/49 patients with available data (76%), including autism spectrum disorder in 26 (53%), aggression in 20 (41%), and hyperactivity in 12 (24%).

Developmental delay was reported in all patients with available data (*n* = 62/64). A severe expressive language disorder was found in all 42 patients aged 3 years or older in whom some language data was available. Twenty-four patients (57%) were nonverbal whereas the remaining were able to speak some words or short sentences. Data on ambulation was available for 43 out of 61 patients aged 2 years or older. Thirty-one patients (72%) achieved independent walking. Two patients (5%) aged 11 and 17 years walked with assistance and 10 patients (23%) were nonambulatory. The median age of walking reported for 22/31 patients was 24 months (range, 18-54 months). Neurological examination data were available for 52 patients. Hypotonia was the most frequently reported sign (*n* = 25/52, 48%), followed by spasticity (*n* = 11, 21%), ataxia (*n* = 10, 19%), extrapyramidal symptoms including dystonia and choreiform movements (*n* = 8, 15%) and hyperlaxity (*n* = 8, 15%).

Electroencephalographic (EEG) data were available for 51 patients with epilepsy. EEG recordings were characterized by slow background activity, with a combination of multifocal (*n* = 30), focal (*n* = 12) or generalized spikes and/or spikes and waves (*n* = 23). Hypsarrhythmia was reported in eight children. Sleep activation of EEG abnormalities was found in 13 patients and photosensitivity in four patients. Thirty-eight patients (72%) had pharmacoresistant epilepsy, whereas 13 patients (26%) responded to antiepileptic drugs. In particular, six out of nine patients who developed only infantile spasms became seizure-free with treatment.

Data on brain magnetic resonance imaging (MRI) was available for 54/64 patients. MRI was normal in 42 of the patients carrying *KCNB1* variants (78%). One patient had a normal computed tomography scan. Mild atrophy was reported in seven patients and documented as progressive by serial MRIs in three of them (Patients 47, 53, and 54). Nonspecific periventricular white matter abnormalities were reported in two patients (Patients 37 and 52). One patient had two areas of focal cortical dysplasia associated with focal seizures, multifocal spikes on EEG and responded to carbamazepine (Patient 4). Another patient had small bilateral periventricular heterotopias (Patient 34).

7 | GENOTYPE-PHENOTYPE CORRELATION

Patients with *KCNB1* variants have a wide phenotypic spectrum including intellectual disability, behavioral disorders, and frequent epilepsy. We examined whether the localization or the type of variant correlated with the phenotype.

We reported the first pathogenic variant in the N-terminal domain in a patient who have a DEE with early developmental delay and a seizure onset at 16 months, evolving into severe cognitive impairment and intractable focal and generalized seizures (Patient 1). Interestingly, a pathogenic variant in *GABRA5* was also identified in this patient, located in the pore-forming M2 transmembrane domain of the GABA receptor (p.Thr301Met). A nearby pathogenic *GABRA5* variant (p.Val294Leu) was reported in a patient with severe developmental delay who differs from our patient as that patient had earlier seizure onset, autistic behavior and spastic quadriparesis (Butler et al., 2018). As both variants (*KCNB1* and *GABRA5*) were predicted to be damaging, a double hit mechanism may be responsible for our patient's phenotype.

In a previous review of 26 patients with a *KCNB1* variant, missense variants in the voltage sensor domain or the pore region of the protein were thought to correlate with a more severe phenotype of epilepsy and global developmental delay (de Kovel et al., 2017). Our results show that nonambulatory patients were more frequent in those with a variant in the S4 to S6 crucial domains (*n* = 10/31) compared with those with a variant in other regions of the protein (*n* = 0/10; *p* = .048, unilateral Fisher's exact test). However, there was no significant difference in terms of epilepsy severity, language acquisition or behavioral issues. In particular, we report here four new variants in the S4 to S6 domains in

patients with no epilepsy and a moderate developmental delay (Patients 16, 34, 35, and 55). These findings do not support a clear correlation of mutations in these domains to the most severe phenotype (de Kovel et al., 2017). The epilepsy phenotype was highly variable, as illustrated in patients carrying the same variant. For instance, the recurrent variant p.Arg312His in the voltage sensor domain was associated with infantile spasms (Patients 17 and 18), infantile-onset focal seizures (Patient 19), pharmacoresponsive late-onset focal seizures (Patient 21), and intractable generalized seizures (Patient 20). In addition, a patient carrying a different amino acid change at the same position (p.Arg312Cys, Patient 16) did not develop epilepsy but had a developmental encephalopathy with intellectual disability and behavioral issues. Therefore, no genotype–phenotype correlations can be established based on the localization of the missense variants on the protein domains and more experimental studies are needed to understand the underlying pathophysiology of this phenotype heterogeneity.

Interestingly, all four variants found in the C-terminal part of the protein were truncating. Though these patients had severe developmental delay and behavioral disorders, four out of six did not develop seizures (Patients 59, 60, 63, and 64) and both patients that developed epilepsy had pharmacoresponsive infantile spasms syndrome (Patients 61). As all these truncating variants occur within the last exon, they are not anticipated to result in a nonsense-mediated messenger RNA (mRNA) decay but rather to produce a truncated protein with a potential dominant-negative effect on channel function (Khajavi, Inoue, & Lupski, 2006). C-terminal truncation of $K_v2.1$ channel has been shown to impact surface expression, voltage-dependent gating function, and phosphorylation-dependent modulation of the channel (Jensen et al., 2017; Mohapatra et al., 2008). Truncated $K_v2.1$ channel could thus impact trafficking of tetrameric $K_v2.1$ channel to the cell membrane leading to functional consequences on channel properties, as demonstrated in few other channelopathies (Aizawa et al., 2004; Duarri et al., 2015; Mezghrani et al., 2008; Puckerin et al., 2016). Other truncating variants were also in the last exon of the gene but located upstream the C-terminal domain, either in extracellular loops or in the pore helix, with no obvious phenotypic difference compared to missense variants. We speculate that they may also escape nonsense-mediated mRNA decay and result in either a nonfunctional or even deleterious truncated protein missing vital channel domains. Indeed, most functionally studied missense variants displayed a range of dominant-negative effects (Calhoun et al., 2017; Saitsu et al., 2015; Thiffault et al., 2015; Torkamani et al., 2014) that might thus represent the main underlying pathogenic mechanism of *KCNB1* variants, as for some other potassium channelopathies-related DEE (Jorge et al., 2011; Masnada et al., 2017; Orhan et al., 2014; Smets et al., 2015). The phenotype severity might be related to the extent of mutation-induced functional $K_v2.1$ channel impairment, in addition to other (genetic or environmental) modulating factors that could interact with the mutation during development shaping the phenotype. More electrophysiological studies and animal models are now needed to confirm the pathogenic mechanism of truncating *KCNB1* variants.

8 | CLINICAL AND DIAGNOSTIC RELEVANCE

Even though targeted therapy is not yet available for *KCNB1* encephalopathies, genetic diagnosis is important in clinical practice to stop unnecessary diagnostic tests, to correctly inform parents about prognosis and allow accurate genetic counseling.

All the *KCNB1* variants identified occurred de novo except in one patient who inherited her p.Arg583* variant from her affected mother. Her mother had an intellectual disability with delayed language skills, no reading and writing abilities but could live independently. She did not develop epilepsy. Her 12-year-old daughter did not have seizures but had a severe neurodevelopmental disorder with no language acquisition, autism spectrum disorder and behavioral disorders. We thus report the first inherited *KCNB1* variant, associated with intrafamilial variable expressivity. This observation that individuals harboring a *KCNB1* variant with a "less-severe" phenotype can transmit a severe disease is important for accurate interpretation of inherited variants, prenatal diagnosis, and genetic counseling.

In addition, Patient 1 carrying *KCNB1* and *GABRA5* variants suggests the co-occurrence of two deleterious variants in the same patient. This 30-year-old patient had a severe phenotype of DEE with social withdrawal, no language acquisition, and daily seizures. Additional data from exome and genome studies will enable us to better understand such double hit genotypes and will help in genetic counseling. In some patients, seizure frequency attenuates and disappears over time with prolonged periods of remission and the possibility of withdrawn antiepileptic drugs (Marini et al., 2017). In this study, we also reported six new patients without epilepsy, further strengthening the importance of *KCNB1* in neurodevelopmental disorders, beyond DEEs.

9 | CONCLUSIONS AND PERSPECTIVES

We review the clinical and molecular spectrum of patients with *KCNB1* variants through the description of 18 unreported pathogenic variants in 27 new unrelated patients and an exhaustive review of the literature increasing the number of patients to 64 and of pathogenic variants to 47. *KCNB1* encephalopathies encompass a wide spectrum of neurodevelopmental disorders, including early-onset global developmental delay with predominant language difficulties and behavioral impairment. Epilepsy is frequent, including the DEEs, but syndrome type and prognosis are variable. Identification of new patients is thus important to fully delineate the phenotypic spectrum of KCNB1 dysfunction. Most variants occur de novo and mainly consist of missense variants with some hotspots located in the voltage sensor and the pore domain of the protein. Few truncating variants are reported with one hotspot in the C-terminal domain associated without epilepsy or with a mild epilepsy. However, available data do not inform further genotype–phenotype correlations, especially in terms of neurodevelopmental outcome. The variety of reported functional effects might contribute to the heterogeneous phenotype. Potential modulation by coexisting pathogenic variants in other genes is

likely to be another modifier of the phenotype. Beyond the variant effects on ion currents, new animal models reproducing *KCNB1* variants are needed to explore more accurately the associated pathophysiological mechanisms of disrupted neurodevelopmental pathways and to develop targeted therapies.

CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

ORCID

Claire Bar http://orcid.org/0000-0003-1489-0211
Rima Nabbout http://orcid.org/0000-0001-5877-4074

REFERENCES

Aizawa, Y., Ueda, K., Wu, L., Inagaki, N., Hayashi, T., Takahashi, M., ... Hiraoka, M. (2004). Truncated KCNQ1 mutant, A178fs/105, forms hetero-multimer channel with wild-type causing a dominant-negative suppression due to trafficking defect. *FEBS Letters*, *574*, 145–150.

Allen, N. M., Conroy, J., Shahwan, A., Lynch, B., Correa, R. G., Pena, S. D. J., ... King, M. D. (2016). Unexplained early onset epileptic encephalopathy: Exome screening and phenotype expansion. *Epilepsia*, *57*, e12–e17.

Ambrosini, E., Sicca, F., Brignone, M. S., D'Adamo, M. C., Napolitano, C., Servettini, I., ... Pessia, M. (2014). Genetically induced dysfunctions of Kir2.1 channels: Implications for short QT3 syndrome and autism-epilepsy phenotype. *Human Molecular Genetics*, *23*, 4875–4886.

Barcia, G., Fleming, M. R., Deligniere, A., Gazula, V.-R., Brown, M. R., Langouet, M., ... Nabbout, R. (2012). De novo gain-of-function KCNT1 channel mutations cause malignant migrating partial seizures of infancy. *Nature Genetics*, *44*, 1255–1259.

Bocksteins, E., Mayeur, E., van Tilborg, A., Regnier, G., Timmermans, J.-P., & Snyders, D. J. (2014). The subfamily-specific interaction between Kv2.1 and Kv6.4 subunits is determined by interactions between the N- and C-termini. *PLOS One*, *9*, e98960.

Butler, K. M., Moody, O. A., Schuler, E., Coryell, J., Alexander, J. J., Jenkins, A., & Escayg, A. (2018). De novo variants in GABRA2 and

GABRA5 alter receptor function and contribute to early-onset epilepsy. *Brain*, *141*, 2392–2405.

Calhoun, J. D., Vanoye, C. G., Kok, F., George, A. L., & Kearney, J. A. (2017). Characterization of a KCNB1 variant associated with autism, intellectual disability, and epilepsy. *Neurology: Genetics*, *3*, e198.

Duarri, A., Lin, M.-C. A., Fokkens, M. R., Meijer, M., Smeets, C. J. L. M., Nibbeling, E. A. R., ... Verbeek, D. S. (2015). Spinocerebellar ataxia type 19/22 mutations alter heterocomplex Kv4.3 channel function and gating in a dominant manner. *Cellular and Molecular Life Science*, *72*, 3387–3399.

den Dunnen, J. T., Dalgleish, R., Maglott, D. R., Hart, R. K., Greenblatt, M. S., Mcgowan-Jordan, J., ... Taschner, P. E. M. (2016). HGVS recommendations for the description of sequence variants: 2016 update. *Human Mutation*, *37*, 564–569.

Fitzgerald, T., Gerety, S., Jones, W., Kogelenberg, M., van King, D., McRae, J., ... Bayzetinova, T. (2015). Large-scale discovery of novel genetic causes of developmental disorders. *Nature*, *519*, 223–228.

Gururaj, S., Palmer, E. E., Sheehan, G. D., Kandula, T., Macintosh, R., Ying, K., ... Bhattacharjee, A. (2017). A de novo mutation in the sodium-activated potassium channel KCNT2 alters ion selectivity and causes epileptic encephalopathy. *Cell Reports*, *21*, 926–933.

Hugnot, J. P., Salinas, M., Lesage, F., Guillemare, E., de Weille, J., Heurteaux, C., ... Lazdunski, M. (1996). Kv8.1, a new neuronal potassium channel subunit with specific inhibitory properties towards Shab and Shaw channels. *The EMBO Journal*, *15*, 3322–3331.

Jensen, C. S., Watanabe, S., Stas, J. I., Klaphaak, J., Yamane, A., Schmitt, N., ... Misonou, H. (2017). Trafficking of Kv2.1 channels to the axon initial segment by a novel nonconventional secretory pathway. *Journal of Neuroscience*, *37*, 11523–11536.

Jorge, B. S., Campbell, C. M., Miller, A. R., Rutter, E. D., Gurnett, C. A., Vanoye, C. G., ... Kearney, J. A. (2011). Voltage-gated potassium channel KCNV2 (Kv8.2) contributes to epilepsy susceptibility. *Proceedings of the National Academy of Sciences of the United States of America*, *108*, 5443–5448.

Ju, M., Stevens, L., Leadbitter, E., & Wray, D. (2003). The roles of N- and C-terminal determinants in the activation of the Kv2.1 potassium channel. *Journal of Biological Chemistry*, *278*, 12769–12778.

Khajavi, M., Inoue, K., & Lupski, J. R. (2006). Nonsense-mediated mRNA decay modulates clinical outcome of genetic disease. *European Journal of Human Genetics*, *14*, 1074–1081.

King, A. N., Manning, C. F., & Trimmer, J. S. (2014). A unique ion channel clustering domain on the axon initial segment of mammalian neurons. *Journal of Comparative Neurology*, *522*, 2594–2608.

de Kovel, C. G. F., Brilstra, E. H., van Kempen, M. J. A., van't Slot, R., Nijman, I. J., Afawi, Z., ... Koeleman, B. P. C. (2016). Targeted sequencing of 351 candidate genes for epileptic encephalopathy in a large cohort of patients. *Molecular Genetics & Genomic Medicine*, *4*, 568–580.

de Kovel, C. G. F., Syrbe, S., Brilstra, E. H., Verbeek, N., Kerr, B., Dubbs, H., ... Koeleman, B. P. C. (2017). Neurodevelopmental disorders caused by de novo variants in KCNB1 genotypes and phenotypes. *JAMA Neurol*, *74*, 1228.

Latypova, X., Matsumoto, N., Vinceslas-Muller, C., Bézieau, S., Isidor, B., & Miyake, N. (2017). Novel KCNB1 mutation associated with non-syndromic intellectual disability. *Journal of Human Genetics*, *62*, 569–573.

Lee, H., Lin, M. A., Kornblum, H. I., Papazian, D. M., & Nelson, S. F. (2014). Exome sequencing identifies de novo gain of function missense mutation in KCND2 in identical twins with autism and seizures that slows potassium channel inactivation. *Human Molecular Genetics*, *23*, 3481–3489.

Lehman, A., Thouta, S., Mancini, G. M. S., Naidu, S., van Slegtenhorst, M., McWalter, K., ... Vercauteren, S. (2017). Loss-of-function and gain-of-function mutations in KCNQ5 cause intellectual disability or epileptic encephalopathy. *The American Journal of Human Genetics*, *101*, 65–74.

Lim, S. T., Antonucci, D. E., Scannevin, R. H., & Trimmer, J. S. (2000). A novel targeting signal for proximal clustering of the Kv2.1 K+ channel in hippocampal neurons. *Neuron*, *25*, 385–397.

Marini, C., Romoli, M., Parrini, E., Costa, C., Mei, D., Mari, F., ... Guerrini, R. (2017). Clinical features and outcome of 6 new patients carrying de novo KCNB1 gene mutations. *Neurology: Genetics*, *3*, e206.

Masnada, S., Hedrich, U. B. S., Gardella, E., Schubert, J., Kaiwar, C., Klee, E. W., ... Rubboli, G. (2017). Clinical spectrum and genotype–phenotype associations of KCNA2-related encephalopathies. *Brain*, *140*, 2337–2354.

McTague, A., Howell, K. B., Cross, J. H., Kurian, M. A., & Scheffer, I. E. (2016). The genetic landscape of the epileptic encephalopathies of infancy and childhood. *The Lancet Neurology*, *15*, 304–316.

Mezghrani, A., Monteil, A., Watschinger, K., Sinnegger-Brauns, M. J., Barrere, C., Bourinet, E., ... Lory, P. (2008). A destructive interaction mechanism accounts for dominant-negative effects of misfolded mutants of voltage-gated calcium channels. *Journal of Neuroscience*, *28*, 4501–4511.

Miao, P., Feng, J., Guo, Y., Wang, J., Xu, X., Wang, Y., ... Cheng, H. (2018). Genotype and phenotype analysis using an epilepsy-associated gene panel in Chinese pediatric epilepsy patients. *Clinical Genetics*, *94*, 512–520.

Miao, P., Peng, J., Chen, C., Gai, N., & Yin, F. (2017). A novel mutation in KCNB1 gene in a child with neuropsychiatric comorbidities with both intellectual disability and epilepsy and review of literature. *Zhonghua Er Ke Za Zhi. Chinese Journal of Pediatrics*, *55*, 115–119.

Mohapatra, D. P., Siino, D. F., & Trimmer, J. S. (2008). Interdomain cytoplasmic interactions govern the intracellular trafficking, gating, and modulation of the Kv2.1 channel. *Journal of Neuroscience*, *28*, 4982–4994.

Orhan, G., Bock, M., Schepers, D., Ilina, E. I., Reichel, S. N., Löffler, H., ... Maljevic, S. (2014). Dominant-negative effects of KCNQ2 mutations are associated with epileptic encephalopathy. *Annals of Neurology*, *75*, 382–394.

Ottschytsch, N., Raes, A., van Hoorick, D., & Snyders, D. J. (2002). Obligatory heterotetramerization of three previously uncharacterized Kv channel subunits identified in the human genome. *Proceedings of the National Academy of Sciences of the United States of America*, *99*, 7986–7991.

Parrini, E., Marini, C., Mei, D., Galuppi, A., Cellini, E., Pucatti, D., ... Guerrini, R. (2017). Diagnostic targeted resequencing in 349 patients with drug-resistant pediatric epilepsies identifies causative mutations in 30 different genes. *Human Mutation*, *38*, 216–225.

Pavone, P., Striano, P., Falsaperla, R., Pavone, L., & Ruggieri, M. (2014). Infantile spasms syndrome, West syndrome and related phenotypes: What we know in 2013. *Brain and Development*, *36*, 739–751.

Pena, S. D. J., & Coimbra, R. L. M. (2015). Ataxia and myoclonic epilepsy due to a heterozygous new mutation in *KCNA2*: Proposal for a new channelopathy:: Aaxia and myoclonic epilepsy due to a heterozygous new mutation. *Clinical Genetics*, *87*, e1–e3.

Puckerin, A., Aromolaran, K. A., Chang, D. D., Zukin, R. S., Colecraft, H. M., Boutjdir, M., & Aromolaran, A. S. (2016). hERG 1a LQT2 C-terminus truncation mutants display hERG 1b-dependent dominant negative mechanisms. *Heart Rhythm: The Official Journal of the Heart Rhythm Society*, *13*, 1121–1130.

Richards, S., Aziz, N., Bale, S., Bick, D., Das, S., Gastier-Foster, J., ... Rehm, H. L. (2015). Standards and guidelines for the interpretation of sequence variants: A joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genetics in Medicine*, *17*, 405–423.

Saitsu, H., Akita, T., Tohyama, J., Goldberg-Stern, H., Kobayashi, Y., Cohen, R., ... Matsumoto, N. (2015). De novo KCNB1 mutations in infantile epilepsy inhibit repetitive neuronal firing. *Scientific Reports*, *5*, 5.

Salinas, M., Duprat, F., Heurteaux, C., Hugnot, J. P., & Lazdunski, M. (1997). New modulatory α subunits for mammalian ShabK+Channels. *Journal of Biological Chemistry*, *272*, 24371–24379.

Samanta, D. (2018). Epileptic spasm and de novo KCNB1 mutation: If it is not one potassium channel, it is another! *Acta Neurologica Belgica*, 1–4. https://doi.org/10.1007/s13760-018-0950-8

Sano, Y., Mochizuki, S., Miyake, A., Kitada, C., Inamura, K., Yokoi, H., … Furuichi, K. (2002). Molecular cloning and characterization of Kv6.3, a novel modulatory subunit for voltage-gated K(+) channel Kv2.1. *FEBS Letters*, *512*, 230–234.

Scheffer, I. E., Berkovic, S., Capovilla, G., Connolly, M. B., French, J., Guilhoto, L., … Perucca, E. (2017). ILAE classification of the epilepsies: Position paper of the ILAE Commission for classification and terminology. *Epilepsia*, *58*, 512–521.

Sicca, F., Imbrici, P., D'Adamo, M. C., Moro, F., Bonatti, F., Brovedani, P., … Pessia, M. (2011). Autism with seizures and intellectual disability: Possible causative role of gain-of-function of the inwardly-rectifying K + channel Kir4.1. *Neurobiology of Disease*, *43*, 239–247.

Smets, K., Duarri, A., Deconinck, T., Ceulemans, B., Warrenburg, B. P., van de Züchner, S., … Verbeek, D. S. (2015). First de novo KCND3 mutation causes severe Kv4.3 channel dysfunction leading to early onset cerebellar ataxia, intellectual disability, oral apraxia and epilepsy. *BMC Medical Genetics*, *16*, 51.

Soden, S. E., Saunders, C. J., Willig, L. K., Farrow, E. G., Smith, L. D., Petrikin, J. E., … Noll, A. (2014). Effectiveness of exome and genome sequencing guided by acuity of illness for diagnosis of neurodevelopmental disorders. *Science Translational Medicine*, *6*, 265ra168.

Soldovieri, M. V., Boutry-Kryza, N., Milh, M., Doummar, D., Heron, B., Bourel, E., … Echenne, B. (2014). Novel KCNQ2 and KCNQ3 mutations in a large cohort of families with benign neonatal epilepsy: First evidence for an altered channel regulation by syntaxin-1A. *Human Mutation*, *35*, 356–367.

Speca, D. J., Ogata, G., Mandikian, D., Bishop, H. I., Wiler, S. W., Eum, K., … Nerbonne, J. M. (2014). Deletion of the Kv2.1 delayed rectifier potassium channel leads to neuronal and behavioral hyperexcitability. *Genes, Brain and Behavior*, *13*, 394–408.

Srivastava, S., Cohen, J. S., Vernon, H., Barañano, K., McClellan, R., Jamal, L., … Fatemi, A. (2014). Clinical whole exome sequencing in child neurology practice. *Annals of Neurology*, *76*, 473–483.

Tabarki, B., AlMajhad, N., AlHashem, A., Shaheen, R., & Alkuraya, F. S. (2016). Homozygous KCNMA1 mutation as a cause of cerebellar atrophy, developmental delay and seizures. *Human Genetics*, *135*, 1295–1298.

Thiffault, I., Speca, D. J., Austin, D. C., Cobb, M. M., Eum, K. S., Safina, N. P., … Trimmer, J. S. (2015). A novel epileptic encephalopathy mutation in KCNB1 disrupts Kv2.1 ion selectivity, expression, and localization. *Journal of General Physiology*, *146*, 399–410.

Torkamani, A., Bersell, K., Jorge, B. S., Bjork, R. L., Friedman, J. R., Bloss, C. S., … Kearney, J. A. (2014). De novo KCNB1 mutations in epileptic encephalopathy. *Annals of Neurology*, *76*, 529–540.

Trimmer, J. S. (1991). Immunological identification and characterization of a delayed rectifier K+ channel polypeptide in rat brain. *Proceedings of the National Academy of Sciences of the United States of America*, *88*, 10764–10768.

Veeramah, K. R., Johnstone, L., Karafet, T. M., Wolf, D., Sprissler, R., Salogiannis, J., … Pazzi, M. (2013). Exome sequencing reveals new causal mutations in children with epileptic encephalopathies. *Epilepsia*, *54*, 1270–1281.

Wang, J., Lin, Z.-J., Liu, L., Xu, H.-Q., Shi, Y.-W., Yi, Y.-H., … Liao, W.-P. (2017). Epilepsy-associated genes. *Seizure*, *44*, 11–20.

Wang, J., Wen, Y., Zhang, Q., Yu, S., Chen, Y., Wu, X., … Bao, X. (2019). Gene mutational analysis in a cohort of Chinese children with unexplained epilepsy: Identification of a new KCND3 phenotype and novel genes causing Dravet syndrome. *Seizure*, *66*, 26–30.

Weckhuysen, S., Mandelstam, S., Suls, A., Audenaert, D., Deconinck, T., Claes, L. R. F., … Ceulemans, B. (2012). KCNQ2 encephalopathy: Emerging phenotype of a neonatal epileptic encephalopathy. *Annals of Neurology*, *71*, 15–25.

Wei, F., Yan, L.-M., Su, T., He, N., Lin, Z.-J., Wang, J., … Liao, W.-P. (2017). Ion channel genes and epilepsy: Functional alteration, pathogenic potential, and mechanism of epilepsy. *Neuroscience Bulletin*, *33*, 455–477.

Xu, J., Yu, W., Jan, Y. N., Jan, L. Y., & Li, M. (1995). Assembly of voltage-gated potassium channels conserved hydrophilic motifs determine subfamily-specific interactions between the α-subunits. *Journal of Biological Chemistry*, *270*, 24761–24768.

Zhu, X., Padmanabhan, R., Copeland, B., Bridgers, J., Ren, Z., Kamalakaran, S., … Guerrini, R. (2017). A case-control collapsing analysis identifies epilepsy genes implicated in trio sequencing studies focused on de novo mutations. *PLOS Genetics*, *13*, e1007104.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.