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

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This is the author manuscript accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the [Version of Record](#). Please cite this article as [doi: 10.1002/humu.23915](https://doi.org/10.1002/humu.23915).

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Funding Information

This work was supported by State funding from the Agence Nationale de la Recherche under “Investissements d’avenir” program (ANR-10-IAHU-01) and the Fondation Bettencourt Schueller (CB and RN), the ERC consolidator grant (EK), the Curekids New Zealand and the Health Research Council of New Zealand (LS, IES), the National Health and Medical Research Council of Australia (IES), the European Commission Seventh Framework Programme under the project DESIRE (grant agreement no. 602531, RG).

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 alpha 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.

Graphical Abstract

KCNB1 encodes the alpha subunit of the delayed-rectifier voltage-dependent potassium channel Kv2.1. In this mutation update, we provide an up-to-date review of the mutational and clinical spectrum of the KCNB1 encephalopathy and report 18 novel variants.

Keywords

KCNB1, developmental and epileptic encephalopathy, epilepsy, 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 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 genes 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 since one gene is often associated with different phenotypes and variants in several genes might result in the same epilepsy phenotype (McTague et al., 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 (eg, *KCNA2*, *KCND2*, *KCND3*, *KCNH5*, *KCNJ2*, *KCNJ10*, *KCNMA1*, *KCNQ3*, *KCNQ5*, *KCNT2*, *KCNV2*) (Jorge et al., 2011; Sicca et al., 2011; Barcia et al., 2012; Weckhuysen et al., 2012; Veeramah et al., 2013; Ambrosini et al., 2014; Lee et al., 2014; Soldovieri et al., 2014; Pena and Coimbra, 2015; Tabarki et al., 2016; Gururaj et al., 2017; Lehman et al., 2017; Wang et al., 2019).

In 2014, Torkamani et al. identified *de novo* variants in the potassium voltage-gated channel subfamily B member 1 (*KCNB1*) in 3 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 (Torkamani et al., 2014). 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 (Soden et al., 2014; Srivastava et al., 2014; Torkamani et al., 2014; Fitzgerald et al., 2015; Saitsu et al., 2015; Thiffault et al., 2015; Allen et al., 2016; de Kovel et al., 2016, 2017; Calhoun et al., 2017; Latypova et al., 2017; Marini et al., 2017; Miao et al., 2017, 2018; Parrini et al., 2017; Zhu et al., 2017; Samanta, 2018). 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_v2.1 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 2 exons. *KCNB1* protein is a 96 kDa core protein of 858 amino acids forming the alpha subunit of the voltage-gated potassium channel subfamily 2 (K_v2.1). K_v2.1 channels are expressed across the central nervous system, especially in large clusters on the soma, proximal dendrites and axonal initial segment of neurons (Trimmer, 1991; King et al., 2014). Like other voltage-gated potassium channels, they are composed of four alpha subunits surrounding the ion conduction pore. Each alpha subunit has 6 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_v2.1 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 alpha subunits K_v6 (*KCNG*), K_v8 (*KCNV*) and K_v9 (*KCNS*) (Xu et al., 1995; Hugnot et al., 1996; Salinas et al.,

1997; Bocksteins et al., 2014). 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_v2.1 channel (Lim et al., 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_v2.1 channel (Ju et al., 2003; Mohapatra et al., 2008). Homotetrameric K_v2.1 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 (Ottshytsch et al., 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, 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 (NGS) panels for either epilepsy or intellectual disability (n=16) or by Whole Exome Sequencing (WES) (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; Supp. Table S1). Single nucleotide variants (SNVs) 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 Supp. Table S2. *KCNBI* 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, electroencephalogram (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 (Soden et al., 2014; Srivastava et al., 2014; Torkamani et al., 2014; Fitzgerald et al., 2015; Saitsu et al., 2015; Thiffault et al., 2015; Allen et al., 2016; de Kovel et al., 2016, 2017; Calhoun et al., 2017; Latypova et al., 2017; Marini et al., 2017; Miao et al., 2017, 2018; Parrini et al., 2017; Samanta, 2018).

In our cohort of 27 unrelated patients, we found 25 distinct *KCNBI* 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 2 variants in order to confirm their *de novo* occurrence (patients 51, 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%), 8 nonsense variants (8/47; 17%) and 2 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 to 381) had 8 distinct missense variants, including 3 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 4 patients (Patients 12 to 15) and the p.Arg312His was found in 5 patients (Patients 17 to 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 to 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 3 patients (Patients 56 to 58) while the p.Gly395Arg and p.Gly401Arg variants were found in 2 patients each (Patients 50, 51 and 53,54).

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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 alpha5 subunit of the gamma-aminobutyric acid type-A (GABA_A) receptor. Recently, Butler et al. provided functional evidence of the pathogenic effect of a nearby *GABRA5* variant (p.Val294Leu) in a patient with severe DEE (Butler et al., 2018). 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 to 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 4 truncating variants were in the linkers: S1 to S2 linker (p.Gln228*), S3 to S4 linker (p.Val286Glyfs*58), S4 to S5 linker (p.Tyr328*) and S5 to 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 (Torkamani et al., 2014; Saitsu et al., 2015; Thiffault et al., 2015; Calhoun et al., 2017). 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 while the p.Gly401Arg variant, located in the pore domain S6, has a dominant-negative effect on WT channels and abolishes endogenous currents (Saito et al., 2015). Both variants inhibit repetitive neuronal firing by preventing the production of sufficiently deep interspike voltages (Saito et al., 2015). Other *KCNB1* variants affecting the pore domain result in loss of ion selectivity and gain of inward cation conductance of K_v2.1 channels (Torkamani et al., 2014; Thiffault et al., 2015). 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 (Torkamani et al., 2014). Changes in channel expression and localization were also suggested to contribute to the pathophysiology of K_v2.1 pore variants (Thiffault et al., 2015). Therefore, given the biophysical properties of these pathogenic variants on K_v2.1, 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 *Kcnbl*^{-/-} (K_v2.1^{-/-}) mice (Specia et al., 2014). Interestingly, mice lacking K_v2.1 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 (Specia 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 6 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 prior to 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 et al., 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 non-verbal while 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 non-ambulatory. 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 8 children. Sleep activation of EEG abnormalities was found in 13 patients and photosensitivity in 4 patients. Thirty-eight patients (72%) had pharmacoresistant epilepsy while 13 patients (26%) responded to antiepileptic drugs. In particular, 6 out of 9 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 (CT) scan. Mild atrophy was reported in 7 patients and documented as progressive by serial MRIs in 3 of them (Patients 47, 53, 54). Nonspecific periventricular white matter abnormalities were reported in 2 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 *KCNBI* 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 (*KCNBI* 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 *KCNBI* 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 non-ambulatory patients were more frequent in those with a variant in the S4 to S6 crucial domains (n=10/31) compared to those with a variant in other regions of the protein (n=0/10, $p = 0,048$, 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 4 new variants in the S4 to S6 domains in patients with no epilepsy and a moderate developmental delay (Patients 16, 34, 35, 55). This findings does 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 (Patient 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 4 variants found in the C-terminal part of the protein were truncating. While these patients had severe developmental delay and behavioral disorders, 4 out of 6 did not develop seizures (Patients 59, 60, 63, 64) and both patients that developed epilepsy had pharmacoresponsive infantile spasms syndrome (Patients 61). Since all these truncating variants occur within the last exon, they are not anticipated to result in a nonsense-mediated mRNA decay but rather to produce a truncated protein with a potential dominant-negative effect on channel function (Khajavi et al., 2006). C-terminal truncation of K_v2.1 channel have been shown to impact surface expression, voltage-dependent gating function and phosphorylation-dependent modulation of the channel (Mohapatra et al., 2008; Jensen et al., 2017). 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 non-functional or even deleterious truncated protein missing vital channel domains. Indeed, most functionally studied missense variants displayed a range of dominant-negative effects (Torkamani et al., 2014; Saitsu et al., 2015; Thiffault et al., 2015; Calhoun et al., 2017) that might thus represent the main underlying pathogenic mechanism of *KCNBI* variants, as for some other potassium channelopathies-related DEE (Jorge et al., 2011; Orhan et al., 2014; Smets et al., 2015; Masnada et al., 2017). 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 *KCNBI* variants.

8. CLINICAL AND DIAGNOSTIC RELEVANCE

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

All the *KCNBI* variants identified occurred *de novo* except in one patient who inherited her p.Arg583* variant from her affected mother. Her mother had 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 *KCNBI* variant, associated with intrafamilial variable expressivity. This observation that individuals harboring a *KCNBI*

variant with a “less-severe” phenotype can transmit a severe disease is important for accurate interpretation of inherited variants, prenatal diagnosis and genetic counselling.

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 counselling. In some patients, seizure frequency attenuates and disappears over time with prolonged periods of remission and the possibility of withdrawn AEDs (Marini et al., 2017). In this study, we also reported 6 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.

ACKNOWLEDGMENTS

The authors thank the association “KCNB1 France” as well as patients and their families for their participation in this study.

DISCLOSURE STATEMENT

The authors declare no conflict of interest.

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Figure

Figure 1

Schematic representation of K_v2.1 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).

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.

Table 1. *KCNB1* genotype and clinical features of patients in our cohort and the literature.

Pat ien t ID	nucl eotid e chan ge	ami no- aci d cha nge	Pr ote in eff ect	In her ita nce	Ag e (y) / Sex	D D	W al k	Ve rba l skil ls	Be ha vio ral dis or de rs	Epi lep sy / Ag e at sz ons et (m)	References
1	c.12 8A> G	p.Glu 43Gly ₊	mi sse ns e	de no vo	29 / M	Y e	Y es	No n- ver bal	No	Yes / 16	This study
2	c.58 6A> T	p.Ile19 6Phe	mi sse ns e	de nov o	12 / F	Y e	N A	No n- ver bal	Ye s	Yes / 18	Marini et al. 2017 (ID-4)
3	c.59 5A> T	p.Ile19 9Phe	mi sse ns e	de nov o	NA / M	Y e	N A	NA	Ye s	Yes / NA	Calhoun et al. 2017
4	c.60 5C> T	p.Ser2 02Phe	mi sse ns e	de nov o	7 / M	Y e	Y es	Sen ten ces	Ye s	Yes / 24	De Kovel et al. 2017 (patient 26)
5	c.62 9C> G	p.Thr 210Ar g	mi sse ns e	de no vo	11 / M	Y e	Y es	Wo rds	Ye s	Yes / 48	This study
6	c.62 9C> T	p.Thr2 10Met	mi sse ns e	de nov o	4 / F	N A	N A	NA	N A	No	De Kovel et al. 2017 (patient 24)
7	c.62 9C> T	p.Thr2 10Met	mi sse ns e	de nov o	8 / F	Y e	N A	No n- ver bal	Ye s	Yes / 12	Marini et al. 2017 (ID-5)

8	c.62 9C> A	p.Thr2 10Lys	mi sse ns e	de nov o	7 / M	Y e s	Y es	Sen ten ces	Ye s	Yes / 4	De Kovel et al. 2017 (patient 25)
9	c.63 2T> C	p.Leu2 11Pro	mi sse ns e	de nov o	7 / F	Y e s	N A	NA	No	Yes / 11	De Kovel et al. 2017 (patient 23)
1 0	c.68 2C> T	p.Gln2 28*	no ns en se	de nov o	3,5 / F	Y e s	Y es	NA	Ye s	Yes / 10	De Kovel et al. 2017 (patient 22)
1 1	c.85 7del	p.Val2 86Gly fs*58	fra me shi ft	de no vo	2 / M	Y e s	Y es	Sen ten ces	No	Yes / 5	This study
1 2	c.91 6C> T	p.Arg3 06Cys	mi sse ns e	de nov o	8 / F	Y e s	Y es	Sen ten ces	Ye s	Yes / 48	This study
1 3	c.91 6C> T	p.Arg3 06Cys	mi sse ns e	de nov o	22 / F	Y e s	Y es	Sen ten ces	No	Yes / 6	Marini et al. 2017 (ID-3)
1 4	c.91 6C> T	p.Arg3 06Cys	mi sse ns e	de nov o	7 / M	Y e s	Y es	Wo rds	Ye s	Yes / 12	Saitsu et al. 2015 (patient 2) De Kovel et al. 2017 (patient 20)
1 5	c.91 6C> T	p.Arg3 06Cys	mi sse ns e	de nov o	9 / M	Y e s	N A	NA	Ye s	Yes / 12	De Kovel et al. 2017 (patient 21)
1 6	c.93 4C> T	p.Arg 312Cy s	mi sse ns e	de no vo	23 / M	Y e s	Y es	Wo rds	Ye s	No	This study
1 7	c.93 5G> A	p.Arg3 12His	mi sse ns e	de nov o	9 / M	Y e s	Y es	No n- ver bal	Ye s	Yes / 10	De Kovel et al. 2017 (patient 19)
1 8	c.93 5G> A	p.Arg3 12His	mi sse ns e	de nov o	1,2 / F	Y e s	N o	No n- ver bal	N A	Yes / 14	Samanta et al. 2018
1 9	c.93 5G> A	p.Arg3 12His	mi sse ns e	NA	11 / M	Y e s	N o	No n- ver bal	Ye s	Yes / 14	De Kovel et al. 2016 (ID-KIEL20), De Kovel et al. 2017 (patient 18)
2 0	c.93 5G>	p.Arg3 12His	mi sse	de nov	33 / M	Y e	Y es	No n-	Ye s	Yes / 18	This study

	A		ns	o	s	ver					
			e			bal					
2	c.93	p.Arg3	mi	de	13 /	Y	Y	No	Ye	Yes	This study
1	5G>	12His	sse	nov	M	e	es	n-	s	/ 60	
	A		ns	o		s		ver			
			e					bal			
2	c.96	p.Thr	mi	de	3 /	Y	Y	No	Ye	Yes	This study
2	8C>	323Ile	sse	no	M	e	es	n-	s	/ 21	
	T		ns	vo		s		ver			
			e					bal			
2	c.98	p.Tyr	no	de	4,5	Y	Y	Wo	Ye	Yes	This study
3	4C>	328*	ns	no	/ F	e	es	rds	s	/ 7	
	G		en	vo		s					
			se								
2	c.99	p.Glu3	mi	de	3,6	Y	N	NA	N	Yes	Miao et al. 2018
4	0G>	30Asp	sse	nov	/ F	e	A		A	/ 18	
	C		ns	o		s					
			e								
2	c.10	p.leu3	mi	de	5 /	Y	N	No	Ye	Yes	This study
5	01T	34Pro	sse	no	F	e	o	n-	s	/ 24	
	>C		ns	vo		s		ver			
			e					bal			
2	c.10	p.Ser3	mi	de	10 /	Y	Y	Sen	Ye	Yes	This study
6	41C	47Arg	sse	nov	M	e	es	ten	s	/ 18	
	>G		ns	o		s		ces			
			e								
2	c.10	p.Ser3	mi	de	9 /	Y	Y	NA	N	Yes	Torkamani et al.
7	41C	47Arg	sse	nov	F	e	es		A	/ 48	2014 (ID-9), De
	>A		ns	o		s					Kovel et al. 2017
			e								(Patient 17)
2	c.10	p.Val3	mi	de	1,6	Y	N	No	Ye	Yes	This study
8	45G	49Phe	sse	nov	/ F	e	o	n-	s	/ 9	
	>T		ns	o		s		ver			
			e					bal			
2	c.10	p.Val3	mi	de	17 /	Y	N	Wo	Ye	Yes	Marini et al. 2017
9	45G	49Phe	sse	nov	M	e	A	rds	s	/ 11	(ID-6)
	>T		ns	o		s					
			e								
3	c.10	p.Ser3	fra	de	2 /	Y	N	No	No	Yes	De Kovel et al. 2017
0	88de	63Thrf	me	nov	M	e	A	n-		/ 14	(patient 16)
	IG	s*13	shi	o		s		ver			
			ft					bal			
3	c.11	p.Trp	mi	de	14 /	Y	N	No	Ye	Yes	This study
1	05T	369Ar	sse	no	M	e	o	n-	s	/ 10	
	>C	g	ns	vo		s		ver			
			e					bal			
3	c.11	p.Trp3	no	de	5 /	Y	N	NA	Ye	Yes	De Kovel et al. 2017
2	07G	69*	ns	nov	M	e	A		s	/ 18	(patient 15)
	>A		en	o		s					
			se								

33	c.1109G>A	p.Trp370*	no nsense	<i>de novo</i>	7 / M	Y es	N A	Words	No	Yes / 9	Parrini et al. 2017 Marini et al. 2017 (ID-1)
34	c.1115C>T	p.Thr372Ile	missense	<i>de novo</i>	8 / M	Y es	Y es	Sentences	No	No	This study
35	c.1115C>A	p.Thr372Asn	missense	<i>de novo</i>	12 / F	Y es	Y es	Sentences	Yes	No	This study
36	c.1121C>T	p.Thr374Ile	missense	<i>de novo</i>	5 / F	Y es	N A	NA	N A	Yes / 6	Allen et al. 2013 (ND27062), Torkamani et al. 2014 (patient 3), De Kovel et al. 2017 (patient 13)
37	c.1121C>T	p.Thr374Ile	missense	<i>de novo</i>	11 / F	Y es	With aids	No n-verbal	Yes	Yes / 13	De Kovel et al. 2017 (patient 14)
38	c.1130C>A	p.Thr377Asn	missense	<i>de novo</i>	2 / M	Y es	N o	No n-verbal	No	Yes / 6	This study
39	c.1132G>C	p.Val378Leu	missense	<i>de novo</i>	10 / F	Y es	Y es	Words	Yes	No	Latypova et al. 2016
40	c.1132G>T	p.Val378Phe	missense	<i>de novo</i>	8 / M	Y es	N o	No n-verbal	N A	Yes / 14	This study
41	c.1133T>C	p.Val378Ala	missense	<i>de novo</i>	3 / F	Y es	N o	No n-verbal	N A	Yes / 13	Soden et al. 2014, Thiffault et al. 2015, De Kovel et al. 2017 (patient 12)
42	c.1135G>A	p.Gly379Arg	missense	<i>de novo</i>	7 / M	Y es	Y es	No n-verbal	Yes	Yes / 8	Torkamani et al. 2014 (individual 2), Srivastava et al. 2014, De Kovel et al. 2017 (patient 11)
43	c.1136G>T	p.Gly379Val	missense	<i>de novo</i>	2 / ND	Y es	N A	NA	N A	Yes / 24	Miao et al. 2017
44	c.1139A>G	p.Tyr380Cys	missense	<i>de novo</i>	5,5 / F	Y es	Y es	Words	No	Yes / 6	This study

4	c.11	p.Gly3	mi	de	7 /	Y	N	NA	N	Yes	Allen et al. 2016
5	41G	81Arg	sse	nov	M	e	o		A	/ 3	(patient 7), De Kovel et al. 2017 (patient 10)
	>A		ns	o		s					
			e								
4	c.11	p.Asp	mi	de	6 /	Y	Y	No	No	Yes	This study
6	44G	382As	sse	no	F	e	es	n-		/ 8	
	>A	n	ns	vo		s		ver			
			e					bal			
4	c.11	p.Pro3	mi	de	17 /	Y	W	No	Ye	Yes	De Kovel et al. 2017
7	53C	85Thr	sse	nov	M	e	ith	n-	s	/ 13	(patient 9)
	>A		ns	o		s	ai	ver			
			e				ds	bal			
4	c.11	p.Lys3	mi	de	NA	N	N	NA	N	NA	Fitzgerald et al.
8	73A	91Asn	sse	nov		A	A		A		2015, De Kovel et al.
	>C		ns	o							2017 (patient 8)
			e								
4	c.11	p.Gly3	mi	de	9 /	Y	Y	No	Ye	Yes	This study
9	80G	94Arg	sse	no	F	e	es	n-	s	/	
	>A		ns	vo		s		ver		3,5	
			e					bal			
5	c.11	p.Gly3	mi	de	9.5	Y	Y	No	Ye	Yes	This study
0	83G	95Arg	sse	no	/ F	e	es	n-	s	/ 12	
	>A		ns	vo		s		ver			
			e					bal			
5	c.11	p.Gly3	mi	NA	14 /	Y	N	No	Ye	Yes	This study
1	83G	95Arg	sse		M	e	o	n-	s	/ 8	
	>A		ns			s		ver			
			e					bal			
5	c.11	p.Cys3	mi	de	22 /	Y	N	NA	N	Yes	De Kovel et al. 2017
2	90G	97Phe	sse	nov	M	e	A		A	/ 10	(patient 7)
	>T		ns	o		s					
			e								
5	c.12	p.Gly4	mi	de	4 /	Y	N	No	N	Yes	Saitsu et al. 2015
3	01G	01Arg	sse	nov	M	e	o	n-	A	/ 17	(patient 1), De Kovel et al. 2017 (patient 6)
	>A		ns	o		s		ver			
			e					bal			
5	c.12	p.Gly4	mi	de	9 /	Y	N	No	N	Yes	This study
4	01G	01Arg	sse	nov	M	e	o	n-	A	/ 8	
	>A		ns	o		s		ver			
			e					bal			
5	c.12	p.Ile4	mi	de	8 /	Y	Y	Sen	Ye	No	This study
5	26T	09Thr	sse	no	M	e	es	ten	s		
	>C		ns	vo		s		ces			
			e								
5	c.12	p.Phe4	mi	de	15 /	Y	N	NA	Ye	Yes	Allen et al. 2016
6	48C	16Leu	sse	nov	F	e	A		s	/ 14	(patient 8), De Kovel et al. 2017 (patient 5)
	>G		ns	o		s					
			e								

57	c.12 48C >G	p.Phe4 16Leu	mi sse ns e	<i>de nov o</i>	18 / F	Y e s	N A	NA	N A	Yes / 42	De Kovel et al. 2016 (ID-2010D05815), De Kovel et al. 2017 (patient 4)
58	c.12 48C >A	p.Phe4 16Leu	mi sse ns e	NA	14 / F	Y e s	Y es	No n- ver bal	No	Yes / 10 day s	This study
59	c.14 89G >T	p.Glu 497*	no ns en se	<i>de nov o</i>	20 / M	Y e s	Y es	Sen ten ces	Ye s	No	This study
60	c.15 03dup	p.Lys5 02*	no ns en se	<i>de nov o</i>	10 / M	Y e s	N A	NA	Ye s	No	Fitzgerald et al. 2015, De Kovel et al. 2017 (patient 3)
61	c.15 99C >A	p.Tyr5 33*	no ns en se	<i>de nov o</i>	32 / M	Y e s	Y es	NA	N A	Yes / 5	De Kovel et al. 2016 (ID-EP1852), De Kovel et al. 2017 (patient 2)
62	c.17 47C >T	p.Arg5 83*	no ns en se	<i>de nov o</i>	8 / F	Y e s	N A	No n- ver bal	No	Yes / 5	Marini et al. 2017 (ID-2)
63	c.17 47C >T	p.Arg5 83*	no ns en se	<i>de nov o</i>	11 / F	Y e s	Y es	Sen ten ces	Ye s	No	De Kovel et al. 2017 (patient 1)
64	c.17 47C >T	p.Arg5 83*	no ns en se	ma ter nal	12 / F	Y e s	Y es	No n- ver bal	Ye s	No	This study

Numbering is according to the cDNA sequence (RefSeq NM_004975.2).

Novel mutations are indicated in bold.

y: years, m: months, DD: developmental delay, sz: seizures, NA: Not available

† This patient also carries a *de novo* variant in *GABRA5* (NM_000810:c.902C>T, p.Thr301Met).

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Title:

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

Date:

2020-01-01

Citation:

Bar, C., Barcia, G., Jennesson, M., Le Guyader, G., Schneider, A., Mignot, C., Lesca, G., Breuillard, D., Montomoli, M., Keren, B., Doummar, D., de Villemeur, T. B., Afenjar, A., Marey, I., Gerard, M., Isnard, H., Poisson, A., Dupont, S., Berquin, P. ,... Nabbout, R. (2020). Expanding the genetic and phenotypic relevance of KCNB1 variants in developmental and epileptic encephalopathies: 27 new patients and overview of the literature. HUMAN MUTATION, 41 (1), pp.69-80. <https://doi.org/10.1002/humu.23915>.

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