

SHORT COMMUNICATION

Novel *KCNB1* mutation associated with non-syndromic intellectual disability

This article has been corrected since Advance Online Publication, and a corrigendum is also printed in this issue.

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Potassium voltage-gated channel subfamily B member 1 (*KCNB1*) encodes Kv2.1 potassium channel of crucial role in hippocampal neuron excitation homeostasis. *KCNB1* mutations are known to cause early-onset infantile epilepsy. To date, 10 *KCNB1* mutations have been described in 11 patients. Using whole-exome sequencing, we identified a novel *de novo* missense (c.1132G>C, p.V378L) *KCNB1* mutation in a patient with global developmental delay, intellectual disability, severe speech impairment, but no episode of epilepsy until the lastly examined age of 6 years old. Furthermore, she showed neuropsychiatric symptoms including hyperactivity with irritability, heteroaggressiveness, psychomotor instability and agitation. Our observation might expand the phenotypic spectrum of *KCNB1*-related phenotypes and raises the issue of the occurrence of the epileptic phenotype. *Journal of Human Genetics* (2017) 62, 569–573; doi:10.1038/jhg.2016.154; published online 8 December 2016

INTRODUCTION

Potassium channel-related neurological disorders are typically associated with epileptic features.¹ Multiple pathophysiologic basis of intellectual disability (ID) also include neuronal excitability disturbance. Among the genes encoding the components of potassium channels, potassium voltage-gated channel subfamily B member 1 (*KCNB1*, MIM600397, NM_004975) encodes α subunit of voltage-gated potassium Kv2.1 channel² that consists of a 858 amino acid protein containing 6 transmembrane segments (S1–S6) and locates on the plasma membrane. Kv2.1 is a subunit of Kv2 complex, including Kv2.1 and Kv2.2.³ Basic architecture of Kv channels include a selectivity filter, voltage sensing and gating elements, mostly intramembrane.⁴ *KCNB1* is expressed in the central nervous system with particularly abundant expression in somatodendritic compartment of neocortical and pyramidal neurons.⁵ *Kcnb1*^{-/-} mice model showed neuronal hyperexcitability and confirmed the critical role of Kv2.1 in hippocampal neuronal network homeostatic regulation.⁶

To date, only 10 *KCNB1* mutations have been described, exclusively in patients with epileptic encephalopathy.^{7–11} Here, we report on a 6-year-old patient with a novel missense mutation in *KCNB1* presenting global developmental delay, ID and severe speech impairment, but no episode of epilepsy.

CASE REPORT

The presenting female patient was born as a second child of non-consanguineous healthy parents after uneventful pregnancy

and delivery, except the gestational diabetes of the mother. The patient was referred to genetic consultation because of motor difficulties and weakness at 5 months. The patient held sitting after 14 months and walked at 2 years. Persistent hyperlaxity was noticed since the first year of life. Her language development was significantly delayed: she spoke 2 words at 17 month, 4 words at 3 years and 10 words at 4 years. Auditory evoked potentials excluded hearing impairment at the age of 10 months. Additional behavioral traits included hyperactivity with irritability, heteroaggressiveness, psychomotor instability and agitation, requiring antipsychotic treatment. During the first months of life, epileptic seizures have been suspected by patient's relatives, but never confirmed by clinical assessment. At the age of 8 months, electroencephalogram (EEG) findings showed slow rhythm without paroxysmal patterns and polysomnogram revealed apneas in paradoxical sleep. EEG performed at the age of 6 years showed bilateral centro-parietal spike-wave discharges (Figure 1). Brain MRI performed at a few months of life was normal. Metabolic origin was ruled out after extended workup including urinary guanidinoacetate and creatine levels, plasma amino acid chromatography, acylcarnitine profile and protein glycosylation analysis. Molecular explorations, array comparative genomic hybridization, *DMPK* locus analysis by TP-PCR, and the next-generation sequencing for *CDKL5*, *IQSEC2*, *MBD5*, *MEF2C*, *SLC9A6*, *STXBPI*, *TCF4*, *UBE3A*, *ZEB2* were negative.

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Received 12 September 2016; revised 22 October 2016; accepted 3 November 2016; published online 8 December 2016

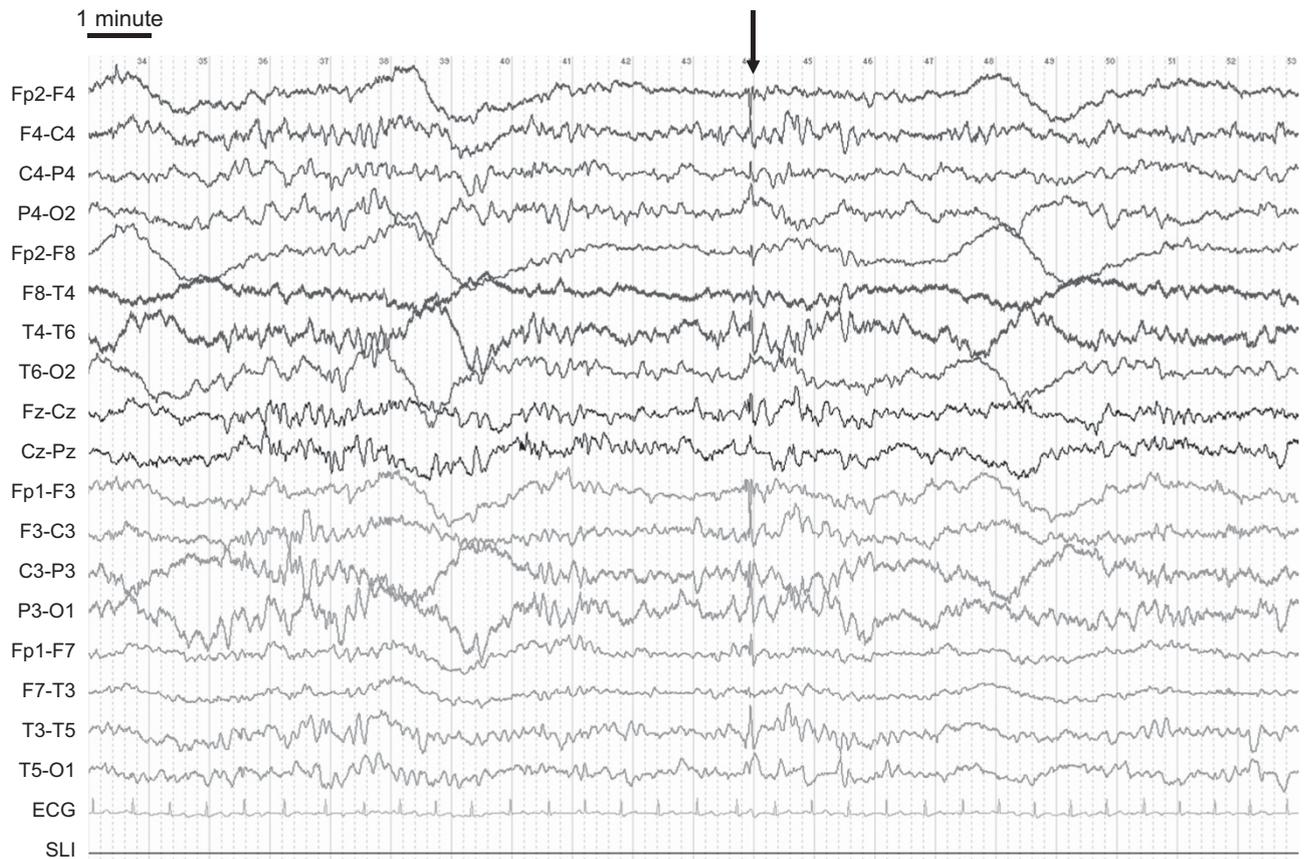


Figure 1 Electroencephalogram at the age of 6 years old. Spike and spike wave (indicated by arrow), and a slow basal activity were observed. A full color version of this figure is available at the *Journal of Human Genetics* journal online.

MATERIALS AND METHODS

Whole-exome sequencing (WES) was performed for trio samples (a patient and her parents) of this family as previously described.¹² In brief, three micrograms of genomic DNA was sheared by Covaris S2 system (Covaris, Woburn, MA, USA) and used for library preparation with SureSelect All Human Exon V5 kit (Agilent, Santa Clara, CA, USA). The prepared samples were run on HiSeq2500 (Illumina, San Diego, CA, USA). Fastq data were aligned to human genome hg19 with Novoalign 3.02.07 (NovoCraft, Selangor, Malaysia). The variants were called by Genome Analysis Tool kit 3.2-0 (GATK, Broad Institute, Cambridge, MA, USA) and annotated with ANNOVAR (Center for Applied Genomics, Children's Hospital of Philadelphia, Philadelphia, PA, USA). The candidate variants were validated by Sanger sequencing. RaptorX online tool was used for secondary structure prediction¹³ for Kv2.1 protein (UniProtKB ID: Q14721).

RESULTS AND DISCUSSION

WES data covered the coding region at 77~100× read depth on average (Supplementary Table S1). The 91.5~94% of the coding regions were covered by 20 reads or more (Supplementary Table S1). As the proband was born to the non-consanguineous healthy parents and no family history of neurological disease, we hypothesized that this condition was caused by a *de novo* mutation. After filtering out the variants registered in dbSNP137, Exome Aggregation Consortium browser, Exome Variant Server, Human Genetic Variation Database, or our in-house exome data ($n=575$) and all the synonymous variants, only one missense mutation of *KCNB1* (NM_004975; c.1132G>C, p.V378L) remained. This variant was confirmed to be

de novo by Sanger sequencing. This variant was predicted as pathogenic by at least two softwares, PolyPhen-2 (Harvard University, Cambridge, MA, USA, probably damaging, HumVar score = 0.987) and MutationTaster (Department of Neuropediatrics, Charité-Universitätsmedizin Berlin, Berlin, Germany, disease causing). The altered residue was evolutionally conserved from lamprey to human (Figure 2a). By the SMART program (<http://smart.embl-heidelberg.de/>), this amino acid residue was located within the ion channel pore domain (Figure 2b).

By reviewing the previously reported 11 patients with *KCNB1* mutations and our patient, the common clinical findings included developmental delay, ID, severe language difficulty and motor delay (Table 1). Interestingly, the previously reported patients with *KCNB1* mutations had severe refractory epilepsy with their onset age at 0 months–4 years (Table 1). However, our patient has not shown epileptic episode until the current age of 6 years old. Our patient rather showed neuropsychiatric symptoms. There is possibility that she will develop seizure later, so the clinical course should be carefully observed.

The altered residue in our patient, p.V378L, is located within the K⁺ selectivity filter, in the loop between transmembrane domain S5 and S6 (Figure 2b). Interestingly, the identical amino acid position but different altered residue, p.V378A has been previously described.¹⁰ Whole-cell patch clamp recordings showed that p.V378A currents were unselective regarding to monovalent cations.¹⁰

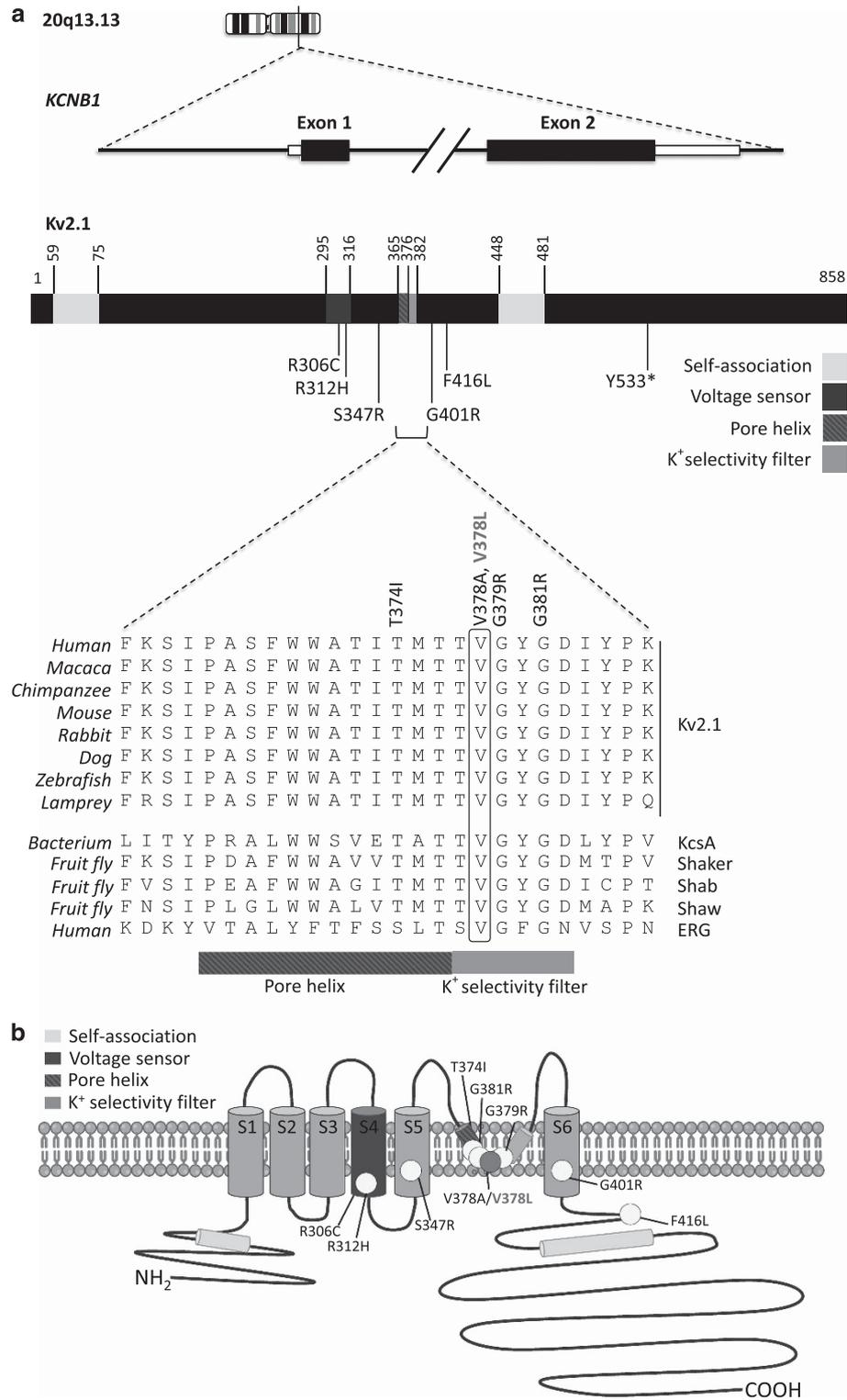


Figure 2 *KCNB1* and Kv2.1 potassium channel. (a) *KCNB1*, located at 20q13.13, contains two coding exons (NM_004975). Previously described *KCNB1* mutations are mainly located within K⁺ selectivity filter region. Alignment of Kv2.1 amino acid sequence with its orthologs and other K⁺ channels shows a high degree of evolutionary conservation. (b) Kv2.1 functional domains. The yellow and red circles denote the position of the previously reported and presenting *KCNB1* mutations, respectively. S347R, T374I, G379R: Torkamani *et al.*,¹¹ V378A: Thiffault *et al.*,¹⁰ R306C, G401R: Saitou *et al.*,⁷ G381R: Allen *et al.*,⁹ R312H, Y533*: Kovel *et al.*,¹⁴ 2016, V378L: this report. ERG: Ether-à-go-go-related gene K⁺ channel. A full color version of this figure is available at the *Journal of Human Genetics* journal online.

Table 1 Summary of the phenotype and *KCNB1* mutations

Author	Torkamani et al.	Torkamani et al.	Torkamani et al.	Thiffault et al.	Saitou et al.	Allen et al.	Allen et al.	Kovel et al.	Kovel et al.	Kovel et al.	Kovel et al.	This report
Year	2014	2014	2014	2015	2015	2016	2016	2016	2016	2016	2016	2016
Patient	Patient 1-ID9	Patient 2	Patient 3	Patient 1	Patient 2							Patient 1
Mutation	c.1041C>A	c.1135G>A	c.1121C>T	c.1133T>C	c.916C>T	c.1141G>A	c.1248C>G	c.1248C>G	c.1248C>G	c.935G>A	c.1599C>A	c.1132G>C
Amino acid change	p.S347R	p.G379R	p.T374I	p.V378A	p.R306C	p.G381R	p.F416L	p.F416L	p.F416L	p.R312H	p.Y533*	p.V378L
Affected domain	S5	K+ SF	Pore helix	K+ SF	Voltage sensing	K+ SF	S6	S6	S6	Voltage sensing	Cytoplasmic	K+ SF
Developmental delay	+	+	+	+	+	+	+	+	+	+	+	+
Intellectual disability	+	+	ND	ND	+	+	+	+	+	+	+	+
Language delay	+	+	ND	No words	No words	ND	ND	ND	ND	ND	ND	+
Motor delay	+	+	ND	+	ND	ND	ND	ND	ND	ND	ND	+
Epilepsy	+	+	+	+	+	+	+	+	+	+	+	-
Type of seizures	Tonic-clonic; tonic; atonic; focal with generalization	Tonic-clonic; atonic; focal; infantile spasms	Tonic-clonic; atonic; atypical absence; spasms	ND	Tonic-clonic; focal	Startle; spasm-like; tonic; focal motor	Tonic-clonic	Tonic-clonic	+	Electrical status epilepticus in sleep	+	-
Age at seizures onset	4 years	8 months	First year	13 months	17 months	3 months	14 months	ND	ND	ND	ND	-
Neuropsychiatric symptoms	+	+	ND	-	-	-	-	ND	ND	ND	ND	+

Abbreviations: ND, no data; K+ SF, K+ selectivity filter; +, present; -, absent.

In conclusion, we report a new, likely pathogenic, *de novo* missense variant of *KCNB1* in a patient with ID, major speech delay and neuropsychiatric symptoms, but no seizure. This case report would expand the clinical spectrum of *KCNB1* mutations, as it has been exclusively linked to infantile epilepsy.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

We are grateful to the patient and her family for their participation in the study. This work was supported in part by a grant for Research on Measures for Intractable Diseases, a grant for Comprehensive Research on Disability Health and Welfare, the Strategic Research Program for Brain Science, a grant for Initiative on Rare and Undiagnosed Diseases in Pediatrics from Japan Agency for Medical Research and Development; a grant-in-aid for scientific research on innovative areas (transcription cycle) from the Ministry of Education, Culture, Sports, Science and Technology of Japan (MEXT); grants-in-aid for scientific research (B) from the Japan Society for the Promotion of Science; the fund for Creation of Innovation Centers for Advanced Interdisciplinary Research Areas Program in the Project for Developing Innovation Systems from the Japan Science and Technology Agency (JST); and the Takeda Science Foundation.

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Supplementary Information accompanies the paper on Journal of Human Genetics website (<http://www.nature.com/jhg>)